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Note

Automated gas chromatographic–electron-capture assay for the selective serotonin uptake blocker sertraline

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Sertraline is a potent and selective inhibitor of neuronal serotonin uptake [1] and is currently under development for the treatment of depression and obesity [2] Sertraline is well tolerated in man at clinically effective doses of 50–200 mg per day [3]. Following a single oral dose to male volunteers, the maximum plasma concentration of drug was about 0.22 ng/ml per mg dose or 22 ng/ml for a 100-mg sertraline dose [4]. Plasma concentrations were determined by a capillary gas chromatographic–mass spectrometric (GC–MS) assay, requiring 3 ml plasma for detection of 1.0 ng/ml. This report describes a modified assay that permitted the automated analysis of a few thousand human plasma samples from a number of clinical studies, with a detection limit of 1.0 ng/ml for a 1-ml sample volume. The major changes from the previous assay included a derivatization step with trifluoroacetic anhydride (TFAA) to enhance electron-capture sensitivity and improve peak shape, improvements in sample clean-up to permit quantitation of 1.0 ng/ml by electron-capture detection and alterations in GC conditions to permit direct on-line injection for automated sample analysis The assay sensitivity permitted measurement of drug concentrations in man for a period of three or more half-lives following a single 100-mg oral dose.

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EXPERIMENTAL

Materials

All solvents were organic residue analysis grade (Baker-resi-analyzed, J.T. Baker, Phillipsburg, NJ, U.S.A.) and all reagents were ACS grade and used as received. TFAA was purchased from Eastman Kodak (Rochester, NY, U.S.A.) Sertraline [1*S*,4*S*-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine], desmethylsertraline and the internal standard (I.S.) CP-53,630 [N-methyl-4-(4-bromophenyl)-1,2,3,4-tetrahydro-1-naphthylamine] were from Pfizer The synthesis of sertraline has been previously reported [5] Standard solutions of sertraline and the I.S at concentrations of 1.0 mg/ml were prepared in methanol and stored at 4°C. Diluted stock solutions were prepared by the serial dilution of aliquots of these solutions in methanol (for sertraline) or water (for the I.S.)

Assay

Each extraction step was performed using disposable borosilicate tubes To each 1.0-ml aliquot of plasma, 100 μl of the I.S solution were added (0.1 or 0.3 μg/ml; total added 10 or 30 ng). The plasma was then adjusted to a pH > 10.5 using 100 μl of 1.0 *M* sodium hydroxide. One extraction was made using 5 ml of diethyl ether–hexane (80:20), vortexing for 15 s and centrifuging at 800 *g* for 5 min. The plasma was quick-frozen by placing the tubes in a dry ice–acetone bath, and the organic phase was decanted into a second tube containing 2.0 ml 0.005 *M* sulfuric acid for back extraction into the aqueous phase. Samples were vortexed for 15 s and centrifuged at 800 *g* for 5 min, and the organic phase was aspirated off and discarded The aqueous phase was adjusted to pH > 10.5 with 200 μl of 1.0 *M* sodium hydroxide A second extraction with 5 ml diethyl ether–hexane (80:20) was made, vortexing for 15 s and centrifuging at 800 *g* for 5 min. The organic phase was pipetted into a clean conical tube (Kimax No. 73790-15 centrifuge tube, American Scientific Products, McGaw Park, IL, U.S.A.), and reduced to a volume of about 200 μl under a nitrogen stream.

To prepare the acyl derivative, 50 μl of 10% TFAA (v/v) in diethyl ether–hexane (80:20) were added and samples vortexed a few seconds to mix. The TFAA dilution was prepared fresh prior to use. Samples were placed on the heating block (set at 50°C) of the vortex evaporator (Haake Buchler Instruments, Saddle Brook, NJ, U.S.A.) and heated for 5 min, then taken to dryness by vortex evaporation (approximately 20 min drying time). The residue was dissolved in 50 or 100 μl of ethyl acetate and vortexed for 10 s. Each sample was transferred to a 2-ml crimp top vial containing a 300-μl glass insert (Sun Brokers, Wilmington, NC, U.S.A.). A portion of the sample was injected on column by direct-injection, 'splitless' operation. A 2-μl volume of 50 μl or a

1-μl volume of 100 μl was injected from sample extracts quantified by the low or high standard curve, respectively.

Instrumentation

A Hewlett Packard HP5890 gas chromatograph equipped with a Hewlett Packard HP7673A autosampler, a 12 m$\times$0.32 mm I.D. SE-54 capillary column of 0.25 μm film thickness (J&W Scientific, Folsom, CA, U S A.) and a ^{63}Ni electron-capture detector was employed. The oven temperature was held at 165°C for 0.5 min following the injection, then increased to 210°C at a rate of 50°C/min and held for 12 min at the final temperature. The inlet and detector temperatures were set at 260 and 300°C, respectively. The carrier gas was helium and detector make-up gas was nitrogen, set at flow-rates of 3.0 and 30 ml/min, respectively. Total system flow-rate was 50 ml/min and the septum purge was 4 ml/min. Column head pressure (which determines the column flow-rate) was 50 kPa. The inlet purge was programmed 'OFF' from 0.0 to 0.5 min.

Preparation of standard curves

Standards were prepared by fortifying large quantities of control human plasma (20–50 ml) with known amounts of sertraline. Aliquots (1 ml) were pipetted into culture tubes and stored at −20°C. Duplicate standards for each concentration were thawed on the day of sample assay

Data handling

To obtain drug concentrations, the ratio of sertraline peak height divided by the internal standard peak height was calculated for both standards and unknowns. A multiple-point standard curve was constructed from duplicate standards of five or six concentrations, based on an unweighted least-squares linear regression fit. Linear regression analysis was performed using an IBM PC with Supercalc 3, Release 2 software.

RESULTS AND DISCUSSION

The current limit of quantitation for sertraline was 1.0 ng/ml, based on a 1.0-ml aliquot of plasma The extraction efficiency into diethyl ether-hexane was 75% and the overall recovery of the extraction process was about 40%. Following TFAA derivatization, the evaporated sample is reconstituted in 50 μl, and 2 μl were injected A chromatogram obtained for a spiked standard of 1 0 ng/ml is shown in Fig. 1. Retention times were 10.2 and 8.4 min for sertraline and the I.S., respectively Also shown are a plasma blank (pre-dose sample) and a 72-h plasma sample of an individual administered a single 100-mg capsule of sertraline. The sertraline concentration at 72 h was 1 2 ng/ml. Linearity was maintained over a ten- to fifteen-fold range in concentration. Concentration ranges that were run for assay of samples were typically 1.0–12.5

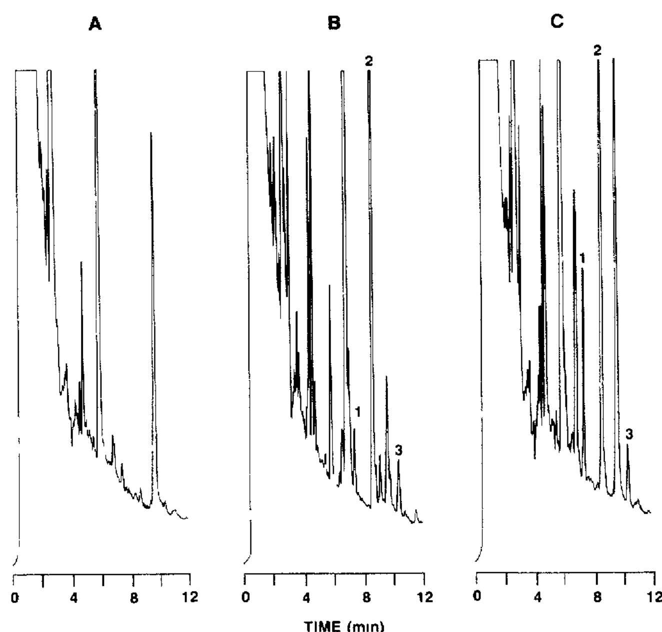

Fig 1 Chromatograms of (A) control human plasma, (B) control human plasma fortified with sertraline, desmethylsertraline and the internal standard at concentrations of 1 0, 1 0 and 10 ng/ml, respectively, and (C) a human plasma sample drawn 72 h after a single 100-mg oral dose of sertraline Peaks 1 = desmethylsertraline, 2 = internal standard, 3 = sertraline

ng/ml (low curve) and 5–50 ng/ml (high curve). For nine standard curves over the range of 1.0–12.5 ng/ml, the mean correlation coefficient was 0 9967 and for seven high curves the mean correlation coefficient was 0 9968

To determine the intra-assay precision at several concentrations, 6-ml portions of control human plasma were spiked with sertraline. Replicate 1 0-ml aliquots (four or five) of each concentration were spiked with I S and assayed The lower limit of quantitation of 1.0 ng/ml had a coefficient of variation (C.V) of 16% and an accuracy of 94% for five replicate determinations (Table I). The upper concentration for the low curve (12 5 ng/ml) had a C.V of 2 0% and accuracy of 102%. In concentrations spanning the high curve (5–50 ng/ml), the range of C V from quadruplicate determinations of each concentration was 2 5–6 4%, while the range in accuracy was 98–106% (Table I)

Inter-assay precision was determined by calculation of the same parameters based on duplicate samples for each concentration in standard curves over a three-month period. For the low curve, data from nine sets were used. The C V at the lower limit of quantitation (1.0 ng/ml) was 12 % and the mean accuracy was 96% (Table II). For the other standard concentrations, the C V ranged from 4.8 to 7.7% and the mean accuracy ranged from 99 to 103%. The slope of the linear regression had a C.V. of 7.0% and the mean *y*-intercept was 24% of

TABLE I

ANALYSIS OF PLASMA STANDARDS CONTAINING KNOWN AMOUNTS OF SERTRALINE PRECISION AND ACCURACY

Concentration added (ng/ml)	Concentration found[a] (mean ± S D) (ng/ml)	Coefficient of variation (%)	Accuracy (mean) (%)
Low curve			
1 0	0 94 ± 0 15	16	94
2 5	2 9 ± 0 18	6 2	115
5 0	4 8 ± 0 14	2 9	96
7 5	7 1 ± 0 42	5 9	95
10 0	9 9 ± 0 72	7 2	99
12 5	12 8 ± 0 25	2 0	102
High curve			
5 0	5 3 ± 0 13	2 5	105
10	10 6 ± 0 33	3 1	106
20	19 6 ± 1 0	5 2	98
50	50 1 ± 3 2	6 4	100

[a]Four (high curve) or five (low curve) plasma aliquots were assayed from each fortified concentration

the 1 0 ng/ml peak-height ratio. The high-curve inter-assay precision was based on data from seven standard curves. The C V ranged from 3 4 to 11%, while the mean accuracy ranged from 94 to 102% (Table II) The slope of the linear regression had a C.V. of 14% and the mean y-intercept was 29% of the 5.0 ng/ml peak-height ratio

Sertraline was found to be stable in frozen plasma for seven months and in plasma at room temperature for one week The TFA derivatives of sertraline and the internal standard were equally stable in ethyl acetate at room temperature for at least three days. However, a noticeable increase in a closely eluting peak to the sertraline-TFA derivative was observed after 48 h.

The assay was specific for sertraline, being without interference from any of the known metabolites in man, rat or dog. A significant metabolite arising from N-demethylation is the primary amine, desmethylsertraline [6] Underivatized desmethylsertraline and its TFA derivative both elute prior to the TFA derivative of the I.S. The TFA derivative of desmethylsertraline appears in the spiked standard and subject plasma sample with a retention time of 7.2 min (Fig 1) Another metabolite that was specifically examined for potential interference was sertraline carbamoyl-O-glucuronide, since loss of the glucuronide moiety creates sertraline carbamic acid, a compound that quickly converts to sertraline and carbon dioxide This metabolite has been identified in human plasma, at concentrations similar to sertraline following a single oral dose of

TABLE II

ANALYSIS OF PLASMA STANDARDS CONTAINING KNOWN AMOUNTS OF SERTRALINE INTER-ASSAY PRECISION AND ACCURACY

Concentration added (ng/ml)	Concentration found[a] (mean ± S D) (ng/ml)	Coefficient of variation (%)	Accuracy (mean) (%)
Low curve			
1 0	0 96 ± 0 11	12	96
2 5	2 6 ± 0 20	7 7	103
5 0	5 1 ± 0 24	4 8	101
7 5	7 6 ± 0 51	6 7	101
10 0	9 9 ± 0 48	4 9	99
12 5	12 4 ± 0 63	5 1	99
High curve			
5 0	4 7 ± 0 51	11	94
10	9 8 ± 0 62	6 3	98
20	20 3 ± 0 89	4 4	102
30	30 4 ± 1 1	3 6	101
40	39 8 ± 1 4	3 4	99
50	49 0 ± 3 0	6 2	98

[a]Nine standard curves, based on duplicate samples for each concentration in the low curve, were run over a three-month period The mean (± S D) slope for the linear regressions was 0 0584 ± 0 0041 and the mean y-intercept was 0 018, or 24% of the peak-height ratio at 1 0 ng/ml For the high curve, seven standard curves were run over the three month period The mean (± S D) slope for the linear regressions was 0 0241 ± 0 0033 and the mean y-intercept was 0 050, or 29% of the peak-height ratio at 5 0 ng/ml

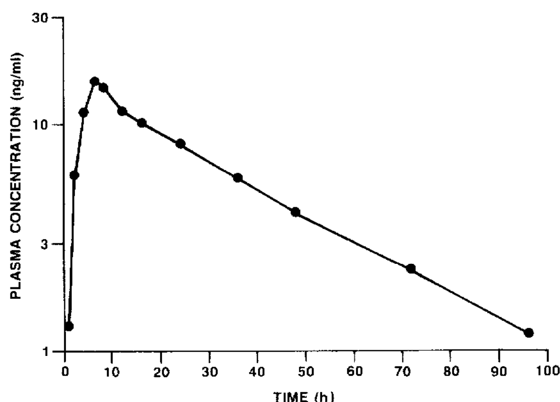

Fig 2 Mean plasma concentrations of sertraline in twenty-four subjects after the administration of single 100-mg oral capsule doses

drug [7]. Sertraline carbamoyl-O-glucuronide was stable in basified plasma (pH 11) at 37°C for greater than 60 min [7], a condition more extreme than that of the extraction procedure. Sertraline and desmethylsertraline both undergo oxidative deamination [6], and the resulting ketone undergoes hydroxylation on the α-carbon prior to excretion. The glucuronide of the α-hydroxyketone is the major excretory metabolite in the rat and dog. Neither it, the aglycone, nor the TFA derivative of the aglycone co-eluted with the TFA derivative of sertraline or the I.S

The mean plasma concentrations of sertraline over a 96-h period in twenty-four fasted subjects, each receiving a single 100-mg capsule dose and 240 ml water, are displayed in Fig. 2. Based on the mean data, the maximum plasma concentration was reached 6 h post-dose and the elimination half-life was 25 h In many subjects, the plasma concentration at 96 h was greater than 1.0 ng/ml. Therefore, plasma concentrations of drug were measurable for three half-lives with a single 100-mg oral dose.

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