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QUANTITATIVE ANALYSIS OF MELPERONE IN HUMAN PLASMA BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY—SELECTED ION MONITORING

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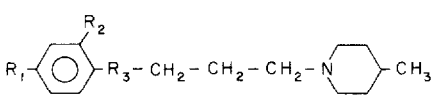
SUMMARY

A sensitive, specific and reliable assay utilizing a combined gas chromatography—mass spectrometry—selected ion monitoring technique is described for the quantitative determination of melperone, a neuroleptic agent, in human plasma. Using a 2-ml plasma sample, the method is sensitive and has acceptable precision in the range of 1 to 100 ng/ml. The method is applied in a limited bioavailability study and found to be adequate. A plasma metabolite was isolated and identified in the clinical samples.

INTRODUCTION

Melperone, 1-(4-fluorophenyl)-4-(4-methyl-1-piperidinyl)-1-butanone (I) (Fig. 1) is an experimental butyrophenone that showed a wide spectrum of neuroleptic properties [1]. Preliminary clinical trials indicated that it is particularly effective in the treatments of senile dementia [2—6]. In order to fa-



melperone (I) $R_1 = F$; $R_2 = H$; $R_3 = C{=}O$

internal standard (II) $R_1 = H$; $R_2 = Cl$; $R_3 = C{=}O$

alcohol metabolite (III) $R_1 = F$; $R_2 = H$; $R_3 = CHOH$

Fig. 1. Chemical structure of melperone, internal standard and alcohol metabolite.

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cilitate the studies of bioavailability and pharmacokinetics of this compound, a sensitive and reliable analytical method is needed to measure plasma concentrations of melperone. Although there have been quite a few published assays for measuring plasma butyrophenone levels [7—9], these methods could not be applied directly to measure melperone due to the fact that there is only one halogen atom in the molecule which affords insufficient sensitivity for the gas chromatographic electron-capture detection. The present paper describes a fast and reliable assay, using a combined gas chromatography—mass spectrometry—selected ion monitoring (GC—MS—SIM) technique and demonstrates sensitivity down to the low nanogram range using 2 ml of plasma sample.

EXPERIMENTAL

Glassware treatment

All glassware used in this assay was cleaned by normal laboratory procedures. They were then rinsed with reagent-grade acetone and air dried prior to use.

Reagents and chemicals

Hexane, methanol and acetone were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Reagent-grade sodium hydroxide and hydrochloric acid were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). Melperone (I), internal standard (II, MDL 17,673) and the alcohol metabolite (III, MDL 18,664) (Fig. 1) were obtained from Merrell Dow Pharmaceuticals (Cincinnati, OH, U.S.A.). [^{14}C]-Melperone was obtained from A.B. Ferrosan (Malmö, Sweden).

Preparation of standard curve

Standard solutions of I were prepared in methanol and an appropriate amount of 0.1 ml was added to 2 ml of drug-free human plasma, capped and frozen until analyzed.

Validation study

To test the precision and accuracy of the assay a four-day validation study was carried out. A nine-point standard curve in duplicate together with ten duplicate unknown spiked plasma samples were analyzed each day.

Feasibility study

To investigate the sensitivity and specificity of the assay it was applied to a human bioavailability study. Four healthy male volunteers were given a single oral dose of melperone. Two volunteers received a 25-mg melperone tablet and the other two received three 25-mg melperone tablets. Predose plasma and twelve post-dose plasma samples were collected from each subject and blind coded before it was sent to the analyst. The analyst was unaware of the dose or sample times. The code was not broken until all samples were analyzed.

Extraction procedure

Standard and unknown plasma samples were carried through the following extraction procedure: In a 25-ml screw cap extraction tube, 0.1 ml (70 ng) internal standard solution in methanol, 0.5 ml 1.5 *N* sodium hydroxide and 11 ml hexane were added to a 2.0-ml sample. The compounds were extracted into the hexane by mixing for 20 min on a horizontal reciprocating shaker. After centrifuging at ca. 600 *g* for 10 min, 10 ml of the hexane phase were transferred to another 25-ml extraction tube containing 2 ml 0.5 *N* hydrochloric acid. The phases were mixed for 20 min then centrifuged for 10 min. The hexane phase was removed by aspiration and discarded. After adding 1 ml 1.5 *N* sodium hydroxide and 11 ml hexane, the phases were mixed for 20 min, then centrifuged for 10 min. A 9-ml aliquot of the hexane phase was transferred to a 15-ml conical tube which was then placed in a heating block set at 50—55°C. The solvent was evaporated to dryness under a slow stream of nitrogen gas. The residue was dissolved in 100 μl of acetone and 5 μl of the resulting solution was taken for GC—MS analysis.

Instrumentation

All analyses were performed on a Finnigan 3300 mass spectrometer coupled with a Finnigan 9500 gas chromatograph. A 1.5 mm × 2 mm I.D. glass column was packed with 5% SE-30 on Chromosorb W HP (80—100 mesh) (Supelco, Bellefonte, PA, U.S.A.). To reduce peak tailing, the column was first base loaded with 10 μl of a 25% tetraethylene pentamine solution in methanol, and one base loading would last about two weeks. The column oven temperature was set at 190°C. The injector port temperature was 230°C. Helium was used as the carrier gas and the flow-rate was adjusted so that the column head pressure gave a reading of 1.7 bar. The mass spectrometer was operated in the electron impact (EI) mode and the ion selected for monitoring was the base peak of both melperone and internal standard II at *m/z* 112 (Fig. 2). The signal was connected to a Hewlett-Packard strip chart recorder, Model 7127A. The recorder sensitivity was set at 0.5 V. Emission current was set at 0.5 mA with an electron energy source of 30 eV. The electron multiplier was set at 20 kV and the preamp sensitivity set at $1 \cdot 10^{-8}$ A for full scale deflection.

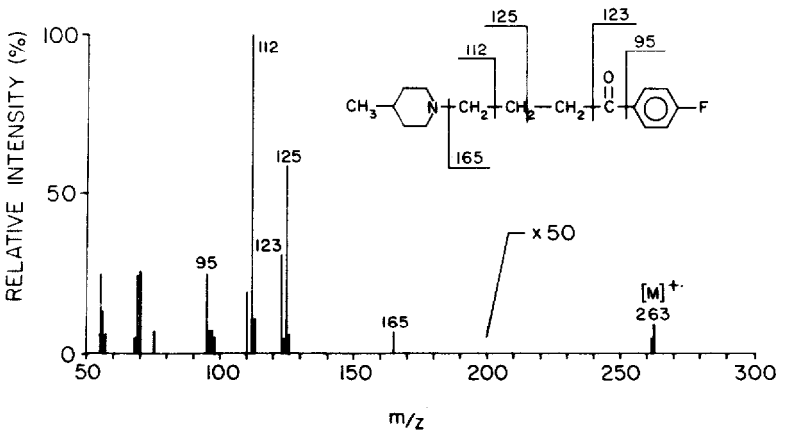

Fig. 2. EI mass spectrum of melperone.

Calibration and calculation

The peak height ratios (expressed as percent) of melperone divided by the internal standard (II) were plotted against melperone concentrations. The peak height ratio of each unknown was applied to this curve to determine the concentration. Alternatively the equation of the line was found by linear regression and the concentration of the unknown calculated.

RESULTS AND DISCUSSION

Extraction efficiency

Using the extraction procedure described above, the extraction efficiency of melperone from plasma at 100 ng/ml was 78.5% by using [^{14}C]-melperone.

GC–MS considerations

Under the GC conditions described above, the retention time for melperone was about 2.4 min and for the internal standard was 4.1 min. Samples could be injected every 6 min. There was no interfering peak extracted from the control drug-free plasma. Fig. 3 shows some typical chromatograms.

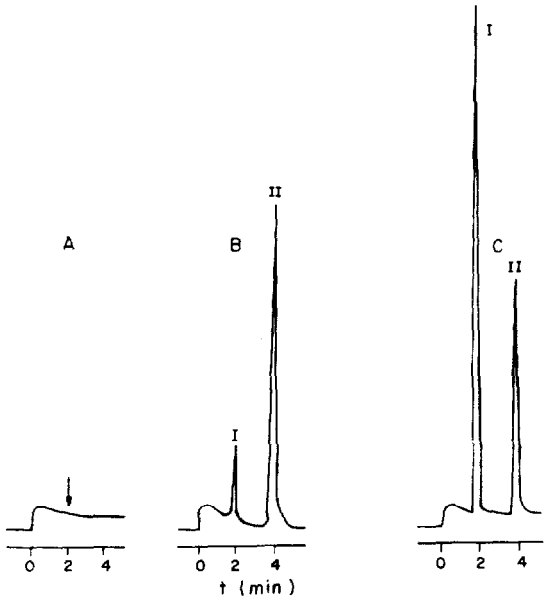

Fig. 3. Chromatograms of extracted plasma standard: (A) blank (↓ is where melperone emerges), (B) 5 ng/ml, (C) 50 ng/ml. Peaks: I = melperone, II = internal standard.

Precision and accuracy

The mean percent peak height ratios for the four-days validation study are tabulated in Table I. The calibration was linear over the entire range, as evidenced by a linear regression analysis of 0.9994 for correlation coefficiency. The slopes of the lines for the four days remained practically constant at 3.259 ± 0.0477 (mean ± standard deviation).

The precision and accuracy of the method were demonstrated by analyzing 40 unknown spiked plasma samples in a random coded fashion. The

TABLE I

PERCENT PEAK HEIGHT RATIO OF MELPERONE/INTERNAL STANDARD

Concentration (ng/ml)	Mean response ± S.D. (n = 8)
1	5.3 ± 1.2
2.5	9.1 ± 1.0
5.0	17.8 ± 1.7
10	34.2 ± 1.3
25	79.1 ± 3.0
50	160.6 ± 8.3
75	244.2 ± 5.5
100	300.0 ± 9.9

TABLE II

ANALYSIS OF CODED UNKNOWN PLASMA SAMPLES

Added	n	Found (ng/ml) (mean ± S.D.)	Recovery (%)
0	4	0 ± 0	—
2.5	6	3.0 ± 0.5	120
10	6	10.7 ± 1.4	107
15	6	15.3 ± 1.1	102
50	6	49.7 ± 2.5	99.4
75	6	73.4 ± 3.7	97.9
100	6	101.8 ± 4.4	101.8

results are shown in Table II. The relative standard deviations ranged between 4.3% (100 ng/ml added) to 17% (2.5 ng/ml added). Recoveries ranged from 97.9% (75 ng/ml added) to 120% (2.5 ng/ml) with a mean recovery of 105% (± 8% S.D.) across all concentrations, excluding 0 ng/ml. Blank samples all gave 0 ng/ml.

Clinical samples

The plasma concentrations of melperone following a single 25- or 75-mg dose to human subjects are plotted in Fig. 4.

Plasma metabolite

During the course of analyzing human clinical samples, it was noticed that there was a drug-related peak that had a retention time of 2.6 min (Fig. 5). The sizes of this peak corresponded closely with the concentrations of melperone. Since detection was based on monitoring the ion at m/z 112, this component must contain an intact piperidine ring; and the GC retention time suggested that the unknown compound was closely related to melperone in molecular weight and structure. Therefore it was assumed that the unknown metabolite could quite possibly be the secondary alcohol that would result from the reduction of the ketone group of melperone. Com-

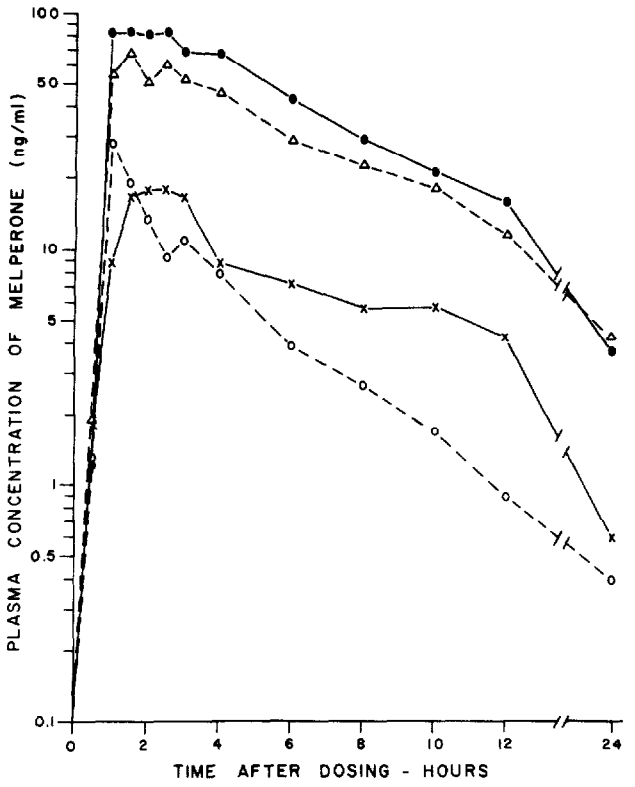

Fig. 4. Plasma concentration of melperone in four subjects following either a 25- or 75-mg oral dose. (●—●, Subject 1 and △— —△, subject 2; 75 mg), (×—×, subject 3 and ○— —○, subject 4; 25 mg).

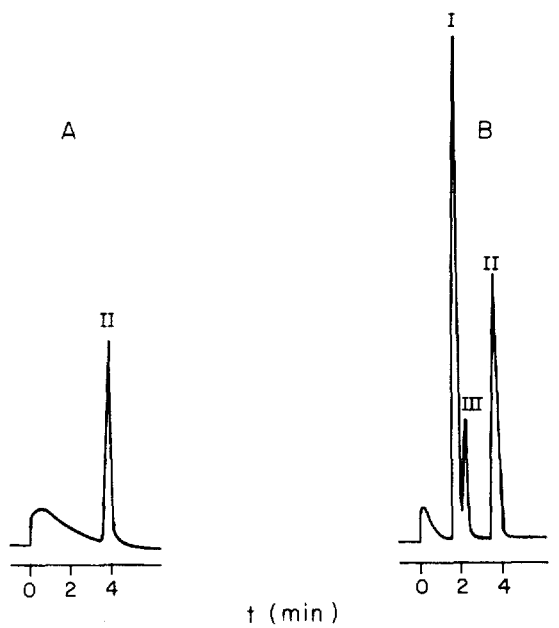

Fig. 5. GC—MS chromatograms from subject 2: (A) predose sample, (B) 2.5-h post-dose sample. Peaks: I = melperone, II = internal standard, III = alcohol metabolite.

paring the reference alcohol (III) with the plasma metabolite confirmed that they had identical GC retention times. By GC—MS—SIM analysis of the ions at *m/z* 112, 125 and 265 it was indicated that the structure of this plasma metabolite was most likely the reduced alcohol of melperone. The alcohol III has been identified as a human urinary metabolite [10].

In conclusion, the results indicated that the GC—MS—SIM assay of melperone in human plasma is a fast, sensitive and reliable method. Besides its use in studying the bioavailability of the parent compound, it could also be used to monitor the alcohol metabolite level in the plasma sample.

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