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Note

Determination of tranylcypromine in plasma using gas chromatography—chemical-ionization mass spectrometry

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Tranylcypromine (*trans*-2-phenylcyclopropylamine, Parnate®) is an irreversible inhibitor of monoamine oxidase (MAO) that is structurally like amphetamine except for the cyclopropyl ring. Despite its use as an antidepressant agent for more than twenty years, there are relatively few methodologies available for determining concentrations of the drug in blood samples of patients administered therapeutic doses. Baselt et al. [1] developed a procedure for tranylcypromine in serum by analyzing the trichloroacetyl derivative of the drug and the internal standard, N-(*n*-propyl)amphetamine, with gas chromatography (GC) and electron-capture detection (ECD). Plasma and urine levels of tranylcypromine have been determined by thin-layer chromatography of either the dansyl [2] or the 4-(7-methoxy-2-oxo-2H-benzopyran-4-yl-methoxy)benzoyl [3] derivative. These latter methods have a lower limit of detection of tranylcypromine in plasma of about 1 ng/ml [3]. Bailey and Barron [4] determined tranylcypromine levels in plasma and urine samples by adding 3-phenylpropylamine as internal standard and forming the heptafluorobutyl derivatives, which were then analyzed by GC using a glass capillary column with a nitrogen-sensitive detector. Calvery et al. [5] described a

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method for the analysis of tranylcypromine in brain tissue by acetylating the drug and then forming the pentafluoropropionyl derivative, which is measured by GC—ECD. Although this method is capable of measuring as little as 5 ng of tranylcypromine, it apparently has not been applied to human plasma samples. In this report, we describe a novel, highly sensitive and specific assay for tranylcypromine in plasma specimens using gas chromatography—mass spectrometry (GC—MS).

EXPERIMENTAL

Reagents

Tranylcypromine (*trans*-2-phenylcyclopropylamine) hydrochloride and phenylpropanolamine hydrochloride (internal standard) were purchased from Sigma (St. Louis, MO, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.), respectively. Pentafluoropropionic anhydride (PFPA) was obtained from Pierce (Rockford, IL, U.S.A.). All other chemicals were obtained from Fisher (Pittsburgh, PA, U.S.A.). Ethyl acetate was redistilled over magnesium sulfate before use.

Patients and blood sample collection

All of the patients were depressed and were being treated with tranylcypromine. No drug was taken for at least 18 h before the study. After an overnight fast, the patients were given an oral dose of 20 mg of tranylcypromine at approximately 10.00 a.m., and blood samples were withdrawn by syringe via an intravenous catheter at various times later (from 0 to 24 h). The catheter was kept patent by slow infusion of normal saline (20 ml/h). The blood samples were then transferred into 7-ml glass Vacutainer® tubes containing EDTA as an anticoagulant (No. 6451, Becton-Dickinson, Rutherford, NJ, U.S.A.). The blood samples were not allowed to come into contact with the stoppers, which were removed from the tubes prior to use. The samples were immediately centrifuged, and the plasma was separated and then stored at −20°C until analyzed.

Extraction and derivatization

Aliquots (0.5 ml) of each plasma sample were added to duplicate 10 × 100 mm silanized glass tubes. Standards were prepared by adding a standard solution of tranylcypromine (1 μg/ml) to 0.5-ml aliquots of pooled plasma (obtained from the remainder of samples having low levels of the drug; e.g., 0-, 12- or 24-h samples) to make the final concentration 0, 35, 70, 105 or 140 ng/ml. Then, 20 μl of the internal standard (1 μg/ml or 20 ng of the free base), 50 μl of 5 *M* potassium hydroxide, 200 mg sodium chloride and 3 ml ethyl acetate were added to each sample and standard and the tubes were shaken 5 min on a Vibrax mixer (Tekmar, Cincinnati, OH, U.S.A.).

After centrifugation (500 *g*, 5 min), each organic layer was transferred with a pasteur pipette to a silanized 15-ml conical centrifuge tube containing 400 μl of 1 *M* hydrochloric acid. The tubes were then shaken for 5 min and centrifuged (500 *g*, 5 min). Each aqueous layer was carefully transferred to a silanized 1-ml Reacti-Vial (Pierce) and evaporated to dryness at 40°C under a stream of nitrogen with a Model 112 N-Evap (Organomation, South Berlin, MA, U.S.A.).

The samples and standards were derivatized in a random order by adding 10 μl ethyl acetate and 10 μl PFPA and allowing the reaction to proceed for 15 min at room temperature.

Gas chromatography—mass spectrometry

Aliquots (3 μl) of reaction mixtures were directly injected onto a 1.8 m × 2 mm silanized glass column packed with 3% OV-1 on 80—100 mesh Supelcoport in a Finnigan Model 3200 quadrupole gas chromatograph—mass spectrometer. The oven was maintained isothermally at 130°C, and the injector was set at 200°C. The flow-rate of the carrier gas, methane (which also served as the reagent gas), was adjusted to provide an ion source pressure of 0.8 Torr. A vacuum diverter was turned on for the 40-sec period following injection to prevent the solvent peak from entering the ion source. The mass spectrometer was operated in the chemical-ionization (CI) mode (electron energy 150 eV). A programmable multiple-ion monitor (PROMIM) was calibrated to focus on the base peaks, which were *m/z* 280 for the PFP derivatives of both tranylcypromine and phenylpropanolamine. To measure low levels of tranylcypromine, a second PROMIM channel was set at the same *m/z* value but the corresponding recorder channel was set to a lower attenuation.

Quantitation

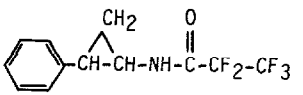
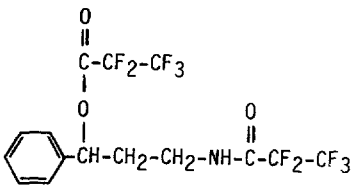
Quantitation was based on the ratio of the peak height of tranylcypromine to the peak height of the internal standard. This ratio for each of the standards was plotted against the concentration of the added drug and the data were fitted to the best straight line by the method of least mean squares. After correcting for the amount of tranylcypromine in the pooled plasma used for preparing the standards (as determined by the *y*-intercept), the standard calibration curve was then used to calculate the concentration of the drug in the unknown plasma samples.

RESULTS

Table I shows that the base peak ions represent the M + 1 ion for tranyl-

TABLE I

PROPOSED STRUCTURE OF THE BASE PEAK IONS OF TRANYLCYPROMINE AND PHENYLPROPANOLAMINE

Compound	Structure of PFP derivative	*m/z* and proposed structure of base peak ion
Tranylcypromine		280 (M + 1)
Phenylpropanolamine		280 (MH^+ — PFPOH)

cypromine and the loss of PFPOH from the M + 1 ion for phenylpropanolamine. Fortuitously, the *m/z* of the base peak for both the drug and this particular internal standard were identical. Therefore, an analysis could be performed with a single PROMIM channel, although we used two channels simply so that the selected-ion current could be monitored simultaneously at two different attenuations.

Typical selected-ion profiles for tranylcypromine and the internal standard in two plasma samples and a standard are depicted in Fig. 1. The retention times were 2.17 min for phenylpropanolamine and 2.95 min for tranylcypromine. In a separate experiment, plasma from a patient not being treated with tranylcypromine was carried through the assay procedure without adding the internal standard. No interfering peaks with retention times similar to those of either the drug or the internal standard were observed.

The recovery of tranylcypromine and phenylpropanolamine for the entire extraction procedure for six separate assays was 79 ± 3% and 98 ± 4%, respectively.

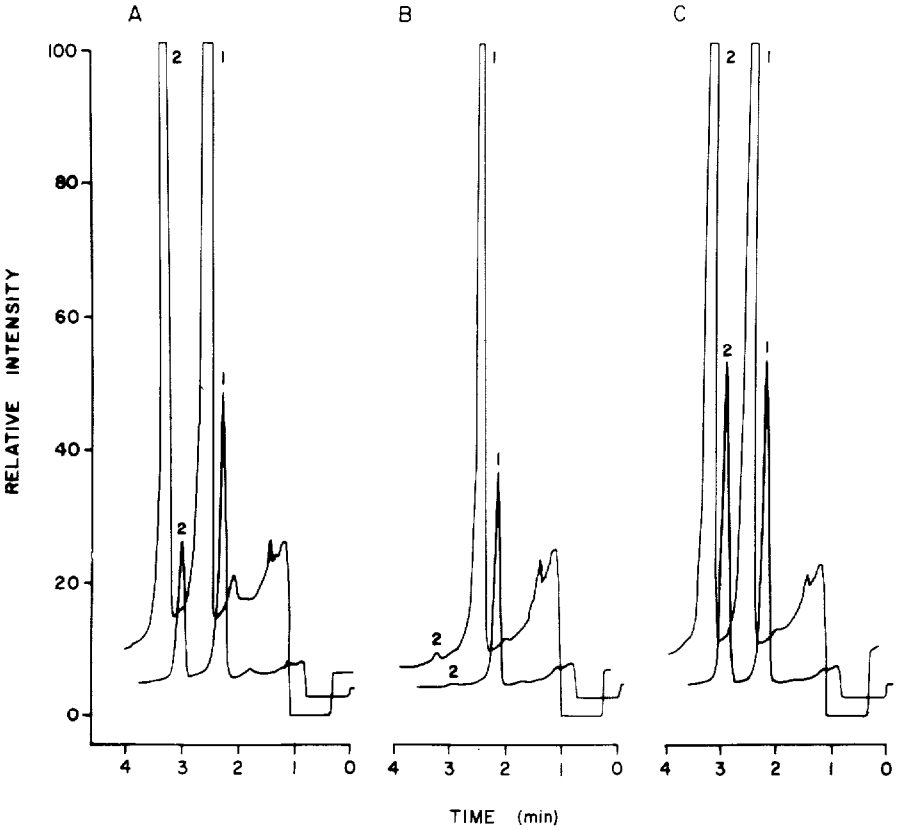


Fig. 1. Selected-ion monitoring for the analysis of tranylcypromine in plasma samples. (A) plasma sample obtained 3 h after the drug was administered (tranylcypromine concentration = 68.7 ng/ml); (B) plasma sample obtained from the same patient (who had been drug-free for at least 18 h) at 0 h (tranylcypromine concentration = 1.1 ng/ml); (C) 140 ng/ml standard. PROMIM channels were set at *m/z* 280; recorder range was 1 V for the upper trace and 5 V for the lower trace. Peaks: 1 = phenylpropanolamine (internal standard); 2 = tranylcypromine.

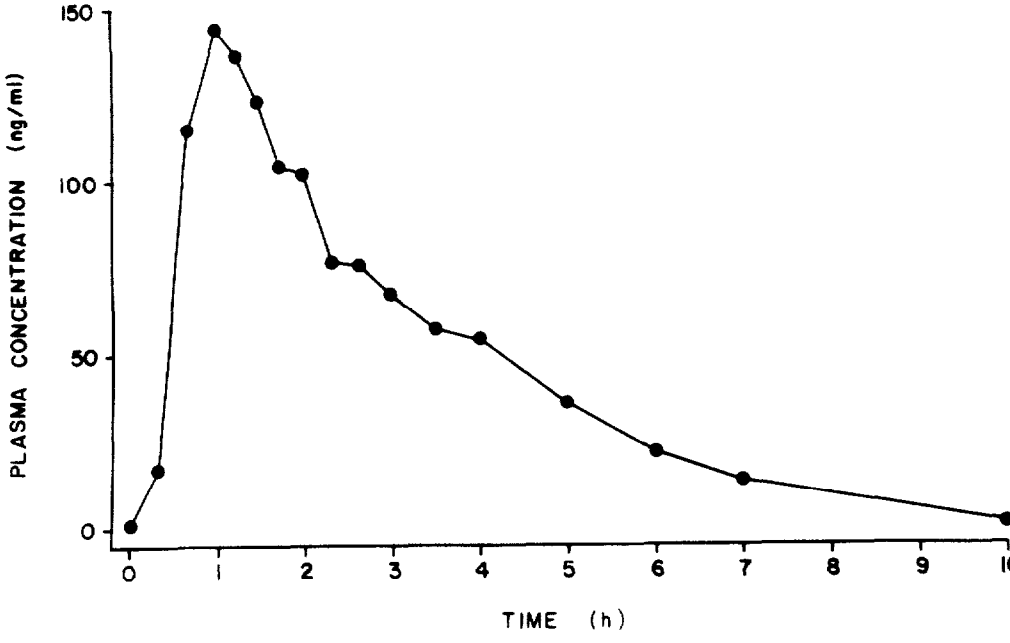


Fig. 2. Pharmacokinetic profile of a patient given a 20-mg dose of tranylcypromine orally at zero time. Each point represents the mean of duplicate determinations. The coefficient of variation of all duplicates in this assay was 4.0%.

The standard calibration curves were linear. The mean slope of the plots of the ratio of tranylcypromine to internal standard versus concentration (ng/ml) for five separate assays (± S.E.) was 0.00703 ± 0.00052; thus, on the average, an internal standard ratio of 1 corresponded to a concentration of 142 ng/ml. The y-intercept ranged from 0 to 0.02 (mean 0.010). The positive intercept was due to the small amounts of tranylcypromine in the pooled plasma used for preparing standards and was thus different in each assay. The correlation coefficient calculated for the five assays ranged from 0.994 to 0.999. The coefficient of variation for the duplicates of 57 samples > 50 ng/ml ($\bar{x}$ = 85.2) was 10.3% and of 40 samples in the range of 10–50 ng/ml ($\bar{x}$ = 30.9) was 8.3%.

Fig. 2 shows the pharmacokinetic curve for plasma tranylcypromine levels in a depressed patient given a dose of 20 mg orally. A peak level of 144 ng/ml was reached 1 h after the drug was administered. The elimination half-life for this subject was calculated to be 1.54 h, as compared to a mean of 2.45 h for the nine patients we have studied so far. Detailed pharmacokinetic data from these patients will be presented elsewhere [6].

DISCUSSION

The present method has several advantages over previously published assays. First, it has excellent sensitivity. This is demonstrated by the fact that despite using only 0.5 ml of plasma for the assay satisfactory results were produced for levels down to 1 ng/ml. In fact, at this level good peaks were produced without any perceptible baseline noise. Clearly, if needed, the assay could be used to

measure tranylcypromine concentrations in either much smaller plasma samples or in samples having much lower levels. In contrast, the thin-layer [3] and GC [4] procedures previously reported require 5-ml and 4-ml samples, respectively.

Another major advantage of the GC—MS procedure is its speed. The extraction procedures easily allow 40—50 samples to be processed in one day. Since the tranylcypromine and internal standard peaks emerge within 3 min and since there are no late-eluting peaks, analysis of that number of samples can be achieved by the gas chromatograph—mass spectrometer in about 2 h.

We have found in our experience that the inclusion of a back-extraction step is an important feature of the extraction procedure. Although Bailey and Barron [4] evaporated the organic phase containing the free amines to dryness and derivatized the resulting residue, we observed low yields and variable results with this approach, presumably due to the volatility of these amines and their losses during the evaporation step. Instead, by converting the amines to salts before the evaporation of solvent and derivatizing the salts rather than the free amines, we greatly improved the recovery and reliability of the assay. This procedure also should be applicable to the extraction and derivatization of other volatile amines, such as amphetamine. Moreover, by using tranylcypromine or a related compound as the internal standard, the procedure could be used to measure levels of phenylpropanolamine with a higher degree of sensitivity than other published methods [7].

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