Determination of Imipramine in Plasma by High Pressure Liquid Chromatography and Field Ionization Mass Spectrometry: Increased Sensitivity in Comparison with Gas Chromatography Mass Spectrometry†

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A quantitative method is reported for the determination of imipramine in plasma samples in the low nanogram and subnanogram range. The sensitivity and precision of the technique, which involves high pressure liquid chromatography and direct probe field ionization mass spectrometry, are approximately an order of magnitude greater than are offered by gas chromatography mass spectrometry with selected ion monitoring using deuterated or other types of internal standards. [$^2H_6$]Imipramine, labeled in the ethylene bridge and in the aromatic rings, serves as the isotopic diluent. The method has been used for the determination of the comparative bioavailabilities of two different commercial preparations of imipramine. In these tests, subjects ingested a 25 mg tablet of one or the other drug preparation together with a solution containing an equivalent amount of imipramine deuterated in the ethylene bridge ([$^2H_2$]imipramine). The latter served as an internal check for intrasubject variability in absorption of the imipramine tablets. Typical results from one of the subjects are presented.

INTRODUCTION

Imipramine {IP: 5-[3-(dimethylamino-propyl)]-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine-hydrochloride}, the active ingredient in Tofranil® (Ciba–Geigy), is widely prescribed for the treatment of depression. IP is the most thoroughly investigated member of a class of drugs having similar structures and similar pharmacological effects. Such drugs include, among others, nortriptyline, amitriptyline, protriptyline and chlorimipramine. IP is readily absorbed from the gut and is extensively metabolized, a major product being the monomethylated derivative, desipramine (DIP), which also is pharmacologically active.[1-3]

For therapeutic purposes, imipramine is usually administered to depressive patients on a daily basis over a period of several weeks. Under such conditions, a very wide range of plasma concentrations has been found.[4] Analytical techniques that have been used for patient monitoring include thin-layer chromatography,[5] spectrofluorometry,[4,6] gas chromatography,[7] and gas chromatography mass spectrometry.[8-13] Patient recovery is reported to be highly correlated with total plasma levels of IP and DIP that exceed 180 ng ml^{-1}.[4]

The present study was undertaken to compare the bioavailabilities of two commercial imipramine preparations, Tofranil® and generic IP (Biocraft Laboratories, Elmwood Park, New Jersey). The tablets differed in total mass (93 ± 4 (SD) mg and 176 ± 8 (SD) mg for Tofranil® and generic IP, respectively), but both contained identical amounts of imipramine hydrochloride (25 ± 2 mg), as determined by isotope dilution analysis. One tablet of each kind was ingested by healthy male volunteers in each leg of a cross-over study. As an internal check for intrasubject variability in absorption of IP, the subjects simultaneously drank one-half cup of water that contained 25 mg of dissolved imipramine hydrochloride that was deuterated in the ethylene bridge ([$^2H_2$]IP). Plasma samples were obtained at selected times for the next 48 h.

Following these small oral doses, the maximum plasma concentrations of unlabeled or deuterated IP reached shortly after ingestion are much less than the steady-state levels attained during chronic oral administration of the drug. Significantly greater sensitivity and precision were required of the analytical technique than were offered by GCMS with selected ion monitoring using deuterated or other types of internal standards.[8-13] This paper describes the analytical procedure that was developed for this bioavailability study. The detailed results of the bioavailability comparison will be presented elsewhere.

† Abbreviations used: IP = imipramine; DIP = desipramine.

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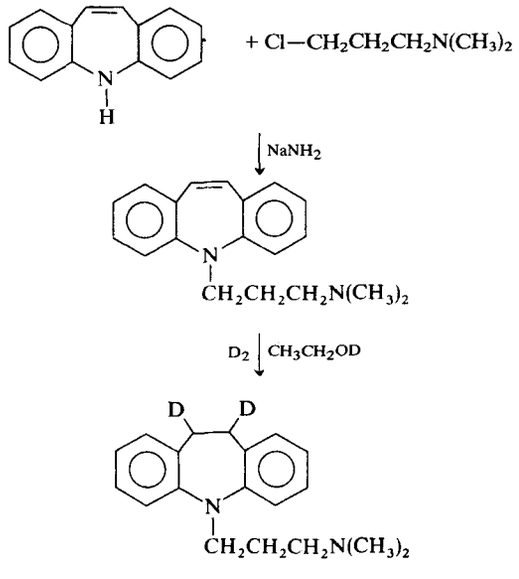
EXPERIMENTAL

Chemicals

Deionized water was distilled from alkaline permanganate before use. Solvents used in high pressure liquid chromatography (HPLC) were usually purified by chemical pretreatment and/or distillation. Methylene chloride (Mallinckrodt reagent grade) was shaken with concentrated H_2SO_4, then it was washed several times with water until the acidity of the washes was slight. The solvent was dried over $CaCl_2$ then was distilled. Methylene chloride (Burdick and Jackson) was also used without purification, and, although it was slightly less free of impurities in the mass region of interest than the pretreated solvent, its purity was judged to be satisfactory. *n*-Propylamine (Eastman) was heated to reflux over BaO for several hours, then was distilled. Iso-propanol (Merck, spectrophotometric grade) was purified by distillation. Hexane (Burdick and Jackson, UV grade) was shaken with 20% NaOH, then with concentrated H_2SO_4, and finally was washed several times with water until neutral. After drying with $CaCl_2$, the hexane was distilled. Methanol (Burdick and Jackson) was used without purification.

Syntheses of deuterated IP

$[^2H_2]$IP was prepared by the routes indicated in Scheme 1. The precursor, 5-(8-dimethylaminopropyl)-iminostilbene, was prepared[14] by mixing equal molar amounts of iminostilbene, sodium amide and chloropropyl-dimethylamine in benzene + toluene, and refluxing for 18 h. The reaction mixture was quenched with water, and the organic phase was extracted with 2 M acetic acid. After neutralization of the acidic solution, the compound was extracted with ether, dried over $MgSO_4$, and solvent was removed by distillation. The residue was distilled under high vacuum at 163–165 °C (0.006 mm), providing a 42% yield of the product.



Scheme 1

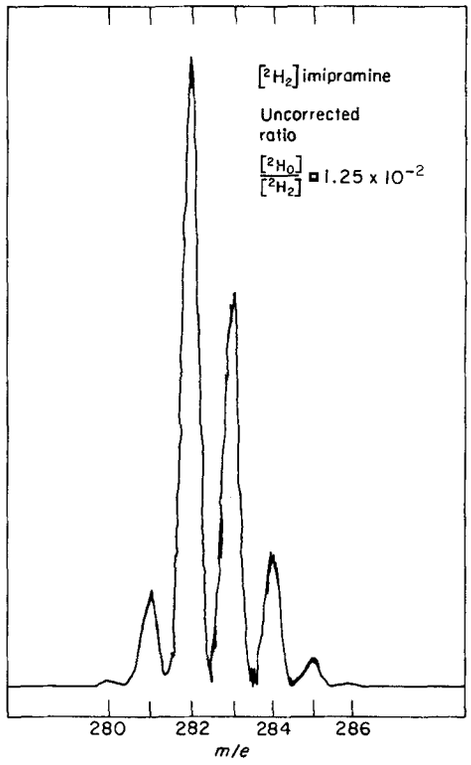
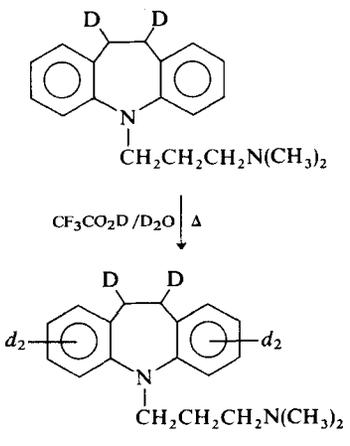


Figure 1. FI mass spectrum of $[^2H_2]$IP.

$[^2H_2]$IP was prepared[15] by dissolving dimethylaminopropyliminostilbene in $[^2H_1]$ethanol and treating with 20 p.s.i.g. deuterium (99.5 mol %) in the presence of Adams catalyst (PtO_2) for 3 days at ambient temperature. The reaction gave approximately 100% conversion of the iminostilbene derivative to $[^2H_2]$IP (free base). The crude product was purified by forming the hydrochloride salt, recrystallizing from acetone, and finally subjecting to gradient sublimation in a Scientific Instruments Accessories thermal gradient sublimer. The field ionization mass spectrum of $[^2H_2]$IP is shown in Fig. 1.

$[^2H_6]$IP was synthesized from $[^2H_2]$IP by heating in deuterated trifluoroacetic acid and deuterium oxide for 48 h (Scheme 2). The $[^2H_6]$IP product was purified in the same manner as was used for $[^2H_2]$IP. The field ionization mass spectrum of $[^2H_6]$IP is shown in Fig. 2. This material was used for all isotope dilution analyses.



Scheme 2

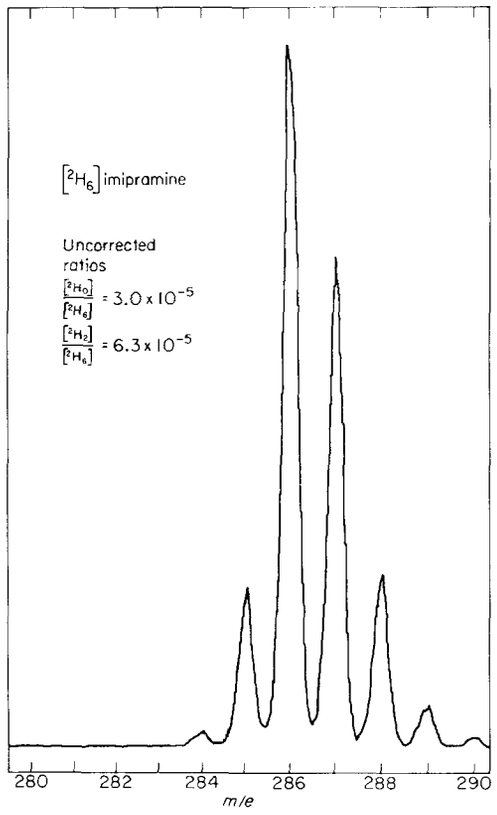


Figure 2. FI mass spectrum of [$^2H_6$]IP.

Two other deuterated imipramines, [$^2H_4$]IP (deuterated in the aromatic rings) and [$^2H_{10}$]IP (deuterated in the rings and in the methyl groups of the dimethylaminopropyl sidechain), were also prepared for this study. The latter material, however, exhibited a significant isotope effect when subjected to chromatographic purification.[16] [$^2H_4$]IP did not provide a sufficiently wide range of isotopic dilution in the [M+2] region, and therefore, was rejected for use as an isotopic diluent.

Glassware and instrumentation

Glassware used for extraction and clean-up of imipramine samples was routinely washed in Alconox, rinsed thoroughly with deionized water, and baked in air in a glassblower's annealing oven at approximately 600 °C. Glass centrifuge tubes used for storage of imipramine extracts before HPLC and for collection of imipramine peaks from the high performance liquid chromatography apparatus were washed only in reagent grade acetone, baked at 600 °C, and silanized using a vapor phase silylation procedure.[17] The high performance liquid chromatography system was a Waters Model 6000A equipped with a $\frac{1}{4}$ in × 2.5 in performance liquid chromatography system was a gel adsorbent (HC Pellosil: Reeve Angel) and a 4.6 × 250 mm column (Altex) packed with totally porous silica gel (LiChrosorb Si60, 5 μ particle size). A Waters Model 440 absorbance detector operating at 254 nm was used for effluent monitoring at a full scale sensitivity of 0.02 absorbance unit.

The mass spectrometer was a 90° magnetic sector instrument that was constructed in this laboratory. The spectrometer is equipped with an electrostatic energy analyzer that provides double focusing capability. The energy analyzer was required to eliminate interference from metastable fragment ions that significantly added to the background when imipramine concentrations were measured in the low nanogram range.

Molecular ions were produced by field ionization using a grid-to-point potential in the glass-lined SRI multipoint source[18] of 2.0 kV. The accelerating voltage was 5.0 kV. The source temperature was operated at 190 °C, and periodic bakeouts of the source to 300 °C with voltage applied were used to maintain a low instrumental background.

Extraction of IP from plasma

To a 4 ml sample of plasma in a Teflon-lined screw-capped centrifuge tube is added 0.02 ml of a stock solution of [$^2H_6$]IP hydrochloride in water. The amount of labeled [$^2H_6$]IP hydrochloride added is 363 ng. (The concentration of the stock solution was determined spectrophotometrically, using an extinction coefficient at 250 nm of 25.5 ml mg^{-1} of imipramine hydrochloride. The absorbance of aqueous solutions of imipramine hydrochloride was shown to be proportional to the concentration over a range of 0.00203–0.0405 mg ml^{-1}.) After addition of the carrier, 2 ml of 1.5 M Na_2CO_3 and 2.5 ml of hexane/iso-amyl alcohol (95/5, v/v) are placed in the tube. The suspension is shaken for 90 s on a Kraft automatic shaker, then is centrifuged for 15 min at approximately 1500 **g** using a refrigerated centrifuge (Beckman Model TJ-6R).

Emulsions are always produced during the extraction process. Centrifugation causes a partial break-up of the emulsion and yields a clear, slightly yellow organic layer as the supernatant. This layer is transferred to a second screw-capped centrifuge tube, and the extraction process is repeated twice, each time using 2 ml of hexane + iso-amyl alcohol.

Purification of IP

To the combined extracts are added 1.2 ml of 0.01 M HCl. The tube is shaken for 30 s, then is centrifuged for 10 min. The lower, aqueous phase is transferred to a glass-stoppered centrifuge tube using a Pasteur pipet. Emulsions are frequently produced during the back extraction, probably due to the presence of dissolved lipids and/or lipoproteins in the hexane + iso-amyl alcohol. When emulsification occurs, a satisfactory recovery of the aqueous phase can be obtained by repeated Vortex mixing and centrifugation of the sample.

The impure extract in 0.01 M HCl is mixed with 1 ml of hexane, and after brief centrifugation, the hexane layer is discarded. Aliquots of 1.5 M Na_2CO_3 (1 ml) and of hexane (1.5 ml) are then added to the aqueous layer, and the phases are mixed on a Vortex mixer. After centrifugation, the hexane layer is withdrawn and is added to a second glass-stoppered centrifuge tube containing 1 ml of 0.01 M HCl. This suspension is

mixed and centrifuged, and the lower aqueous layer is transferred to a third glass-stoppered tube that contains 1 ml of 1.5 M Na_2CO_3 and 1.5 ml of hexane. Mixing and centrifugation are repeated, and the organic layer is transferred to a silanized centrifuge tube for storage.

In the usual protocol, twenty samples are handled simultaneously, and the total clean-up process requires approximately 3 h. Losses are minor as determined by the areas of HPLC peaks (see below). The average overall yield was found to be 72±11 (SD)%. This represents the highest extraction efficiency for imipramine that has been reported.[7,13] Significantly lower efficiencies were found using either hexane alone[13] or hexane+ether (90/10, v/v) for extraction. The difficulty of extracting imipramine quantitatively from plasma is probably caused by its strong affinity for plasma proteins.[19–22]

Purification of the extract by HPLC is performed by a modification of the method of Detaevernier *et al.*[23] Hexane is evaporated from the extract under a stream of N_2, and the residue is dissolved in about 0.15 ml of the HPLC solvent (methylene chloride containing 0.2% iso-propanol and 0.45% *n*-propylamine). The total sample is loaded into the high performance liquid chromatograph, and chromatography is performed at a flow rate of 1 ml min^{-1}. Imipramine emerges as a sharp peak at about 6 min post-injection, and the total peak is collected for subsequent mass spectrometric analysis.

In the development of the above purification scheme, several other chromatographic methods were tested, including TLC, preparative GC, and HPLC using both reversed-phase and ion-exchange columns. The TLC method failed because detection of the IP spot was not possible using $[^2H_6]$IP carrier levels below 1 μg. Preparative GC was unsatisfactory owing to irreversible column adsorption and to difficulties of collection. Reversed phase HPLC of imipramine resulted in poor chromatographic peak shapes and extensive tailing; this was observed by others in the chromatography of other basic drugs on reversed phase columns.[24] High pressure ion exchange chromatography using a microparticulate cation exchange column (Partisil SCX: Reeve Angel) gave satisfactory separations, but the column deteriorated rapidly, even in an acetic acid+ammonium acetate buffer (pH 5). The life expectancy of Partisil SCX columns was not considered adequate for handling large numbers of samples routinely. Similar criticisms have been made by Twitchett *et al.*[25] Thus, chromatography on silica was the only technique of those attempted that gave efficient and reproducible separations of IP from interfering components of the plasma extract.

Mass spectrometric analysis

Samples collected from the high performance liquid chromatograph are evaporated to dryness with pure N_2 and are resuspended in 15 μl of a solution of purified hexane saturated with methanol. The sample is applied to the outside of a sealed glass capillary. After evaporation of solvent, the latter is placed in a larger capillary, and this is dropped into the tip of a direct inlet probe. The probe is cooled to 0 °C with a freon aerosol to prevent sample evaporation, then it is inserted through a vacuum lock into the mass spectrometer. The probe tip is gradually heated from 0 to 110 °C.

While volatilization of the sample is occurring, the magnet is scanned repeatedly over the mass region *m/e* 278–292. Ions are detected using an electron multiplier, and the counts are stored in a multichannel analyzer. Counting is begun as soon as possible after sample insertion and is continued until the sample is exhausted, which occurs at about 110 °C. The multiscanned mass spectrum is displayed, and the counts at each atomic mass unit are integrated and recorded. The mass spectrometric analysis time is approximately 20 min. Typical FI mass spectra from plasma blanks and from plasma spiked with unlabeled IP and $[^2H_2]$IP are shown in Fig. 3.

Human subjects

Eight healthy adult males, ranging in age from 18 to 57 years and in weight from 62 to 80 kg, participated as volunteers in the study. The bioavailability tests began at 8 a.m. Food or liquids other than water had not been consumed for the previous 12 h. Initial blood samples were drawn into heparinized tubes, then the subjects were given a '25 mg' tablet of imipramine (either Tofranil® or generic IP). The source of the IP was not disclosed to the subjects.

To assist in swallowing the tablet and to receive simultaneously an internal deuterated standard, the subjects drank 120 ml of water that contained 25 mg of $[^2H_2]$IP hydrochloride. Blood samples were drawn at 0.5, 1, 1.5, 2, 3, 4, 5, 7, 10, 24, 34 and 48 h after ingestion. The samples were centrifuged as soon as possible after drawing, and the plasma was frozen. Normal feeding (except for water) was not resumed until 4 h after the imipramine was ingested.

One week after the study was begun, the same subjects reassembled and were given a new tablet of

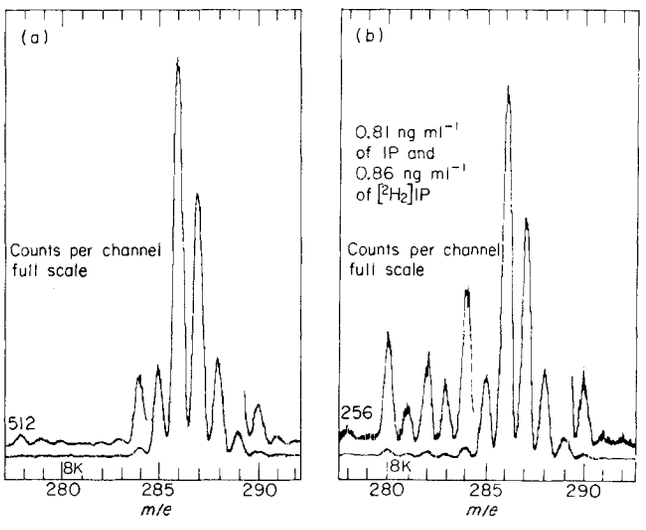


Figure 3. FI mass spectra of imipramine after extraction from plasma and purification by HPLC: (a) plasma blank spiked with 91 ng ml^{-1} of $[^2H_6]$IP hydrochloride; (b) plasma spiked with 0.81 ng ml^{-1} of IP and 0.86 ng ml^{-1} of $[^2H_2]$IP hydrochlorides and with 91 ng ml^{-1} of $[^2H_6]$IP hydrochloride.

imipramine. Those subjects who received Tofranil® in the first trial received generic IP in the second, and *vice versa*. The same procedures were followed in both trials with respect to blood sampling.

RESULTS

Determination of IP in spiked plasma samples

Calibration curves were obtained by spiking 4 ml plasma samples with known amount of IP, $[^2H_2]$IP and $[^2H_6]$IP. The concentrations of IP and $[^2H_2]$IP in the plasma samples covered the range 0.4–8.6 ng ml^{-1}. The additions of the latter compounds were made from accurately diluted stock solutions of the hydrochlorides in methanol (approximately 1.8 μg ml^{-1}) using microliter pipets. [Erroneous results were obtained when imipramine was added from dilute aqueous solutions (approximately 0.2 μg ml^{-1}) using conventional laboratory pipets, owing to adsorption of IP on the pipet walls.] $[^2H_6]$IP hydrochloride was added from the concentrated aqueous solution described previously.

Quantitative calculations of the concentrations of IP and $[^2H_2]$IP in the samples were based on the observed number of counts at the major masses, *m/e* 280, 282 and 286 for IP, $[^2H_2]$IP and $[^2H_6]$IP, respectively. Observed ratios for the standard mixtures are plotted against total concentrations of IP and $[^2H_2]$IP in Fig. 4,

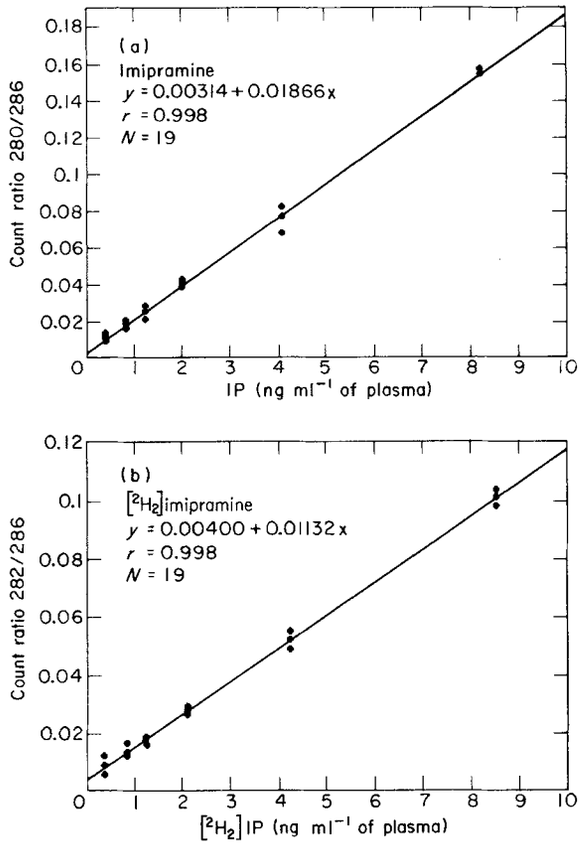


Figure 4. Calibration plots and regression characteristics for IP (a) and for $[^2H_2]$IP (b) quantitation in plasma samples: uncorrected ratios vs concentrations of the hydrochloride salts.

and unweighted linear regression lines are drawn. The data points at each concentration were not obtained by repeated measurements on the same plasma sample, but represent independent determinations on separate days of separate plasma samples spiked individually with the three imipramine isotopic variants. Thus, both random volumetric errors and errors in determining the isotopic ratios contribute to the total variance observed.

Since the ratios in Fig. 4 are uncorrected, the intercepts on the ordinates represent the apparent backgrounds caused by residual impurities from plasma and solvents and from the mass spectrometer. In the absence of chromatographic purification, plasma impurities completely overwhelm the imipramine signal in both raw and partially purified extracts. An exceptionally strong peak was found at *m/e* 278, the origin of which was shown by GCMS to be due to dibutyl phthalate. This material was probably introduced into the plasma samples by storage in plastic packs or by contact of the blood with a plastic syringe.[26]

The precision of the determinations was estimated by carrying out repeated measurements on plasma samples spiked with IP and $[^2H_2]$IP at concentrations of 0.81 and 0.86 ng ml^{-1}, respectively. A total of 11 determinations at these concentrations yielded a standard deviation of 0.066 ng ml^{-1} for IP and 0.13 ng ml^{-1} for $[^2H_2]$IP. The larger standard deviation for $[^2H_2]$IP is due, at least in part, to the smaller signal of the deuterated compound at *m/e* 282 relative to that of IP at *m/e* 280 for a given molar concentration of the two materials. Approximately 78% of the total unprotonated isotopic variants of IP occur at its principal mass, *m/e* 280, whereas only 47% of the $[^2H_2]$IP isotopic variants are found at its major mass, *m/e* 282. [This accounts for the larger slope of Fig. 4(a) relative to that of Fig. 4(b).] Larger fluctuations in the background at *m/e* 282 than at *m/e* 280 could also contribute to the observed standard deviation.

Bioavailability study

Figure 5 illustrates two pharmacokinetic profiles from one of the subjects, following ingestion of either Tofranil® [Fig. 5(a)] or generic IP [Fig. 5(b)]. Several features of these curves are characteristic of results that have also been found with most or all of the other human subjects. These include: (1) an initially high level of $[^2H_2]$IP relative to IP during the first 2–3 h; (2) non-identical maximum concentrations for both the internal standard, $[^2H_2]$IP, and for IP, in the two trials; and (3) evidence of multiple absorption processes, with a peak or shoulder in the profile occurring both at 3 h and at 5 h, and very often at 10 h post-ingestion. The secondary peaks or shoulders at 5 and 10 h post-ingestion seem to coincide with food intake and the consequent increased flow of bile, and therefore suggest enterohepatic cycling.[27]

The intrasubject variations in the maximum concentrations of IP and $[^2H_2]$IP, and the secondary peaks or shoulders in the absorption profiles were greater than can be accounted for by experimental uncertainty. The average intrasubject variations in the concentration maxima were 2.3 ng ml^{-1} for IP and 2.8 ng ml^{-1} for

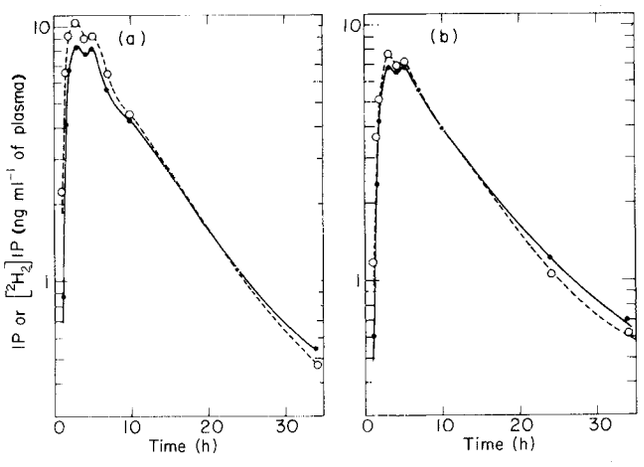


Figure 5. Plasma concentration-time profiles for subject T.A. (age 21 yr, 76 kg) after ingestion of (a) Tofranil®+[$^2H_2$]IP or (b) generic IP+[$^2H_2$]IP. Symbols: unlabeled IP (●); [$^2H_2$]IP (○). Concentrations are in terms of the hydrochloride salts.

[$^2H_2$]IP, which represent about 26% of the average peak concentrations. The coefficient of variation in this concentration range was estimated (three measurements) to be about 1%. A detailed discussion of the kinetics will be presented elsewhere.

DISCUSSION

Comparison with GCMS

Imipramine in plasma has been determined using GCMS by several investigators using both EI and CI modes of ion production.[8-13] The sensitivity of the present HPLC FI mass spectrometry technique can be compared with those results. Such a comparison is presented in Table 1 for the determination of unlabeled imipramine.

Table 1. Comparison of sensitivities for determination of imipramine in plasma by mass spectrometric methods

Estimated lowest concentration on calibration plot (ng ml^{-1})	Standard deviation at or near lowest concentration[a] (ng ml^{-1})	Per cent of total signal due to background at 1 ng ml^{-1} of plasma	Method	Reference
5	NR	95[b]	GCMS	8, 10
5	NR	64.9	GCMS	9
10	NR	93.7	GCMS	11
20	NR	99.7	GCMS	12
13	3.1[c]	90.0	GCMS	13
0.41	0.066[d]	14.4	HPLC FI mass spectrometry	This work

[a] NR signifies values not reported or not calculable from published data.
[b] Calculated from Fig. 8 of Belvedere *et al.*,[10] assuming the reported 'Limit of assay sensitivity' (4 ng ml^{-1}) is equal to the background.
[c] 3 Determinations at 4.1 ng ml^{-1}.
[d] 11 Determinations at 0.81 ng ml^{-1}.

The third column of Table 1 compares the percent of total signal due to background at 1 ng ml^{-1} of plasma. The data were calculated from the ratio $b/(m+b)$, where b is the intercept and m the slope (ml ng^{-1}) in calibration plots. All of the GCMS methods require a substantial background correction for determinations of IP at this concentration.

The residual amount of unlabeled IP in the isotopic diluent is not a major contributor to the background at m/e 280. In one case, where [$^2H_4$]IP was used as the diluent,[13] it can be readily shown that the background contribution caused by residual unlabeled IP in the [$^2H_4$]IP was negligible in comparison to the background obtained with real plasma extracts following GC purification. The same is true of the present technique, in which [$^2H_6$]IP was the isotopic diluent. Thus, a major limitation of both the GCMS and the HPLC FI mass spectrometry methods is caused by impurities in the mass range of interest present in the mass analyzed sample. Other important problems in low level determinations of imipramine by GCMS appear to be variable column adsorption and column bleed.[11] These problems do not occur to a significant extent in HPLC.

A principal advantage of HPLC is the ability to inject an entire sample, which can be in a relatively large volume, without significantly degrading the column efficiency.[28] Since an entire peak can usually be collected without difficulty, the HPLC method when used in conjunction with a direct inlet probe enables a maximum amount of sample to be introduced into the mass spectrometer. The disadvantage of this method is, of course, the greater time required per analysis due to sample handling. For special purposes such as the present study, however, where the sensitivity and precision of GCMS are inadequate, a combination of HPLC and nonfragmenting mass spectrometry using a direct inlet probe can provide the only feasible alternative.

Use of an internal labeled standard in bioavailability studies

The data shown in Fig. 5 clearly indicate the value of using an internal labeled standard in bioavailability comparisons. Without such a standard, it might have been inferred from such data that Tofranil® intrinsically reaches a higher maximum concentration in the plasma than does generic IP. It is obvious, however, that the internal standard also reached a higher plasma level in the Tofranil® trial than it reached in that of the generic drug. In fact, relative to the maximum concentration of the internal standard, the two drug preparations attained almost identical maximum drug levels.

One possible interpretation of these results is that Tofranil® actually has a greater bioavailability than generic IP, and that it somehow causes a greater simultaneous uptake of [$^2H_2$]IP (for example, by a local pH change) at the same time that it is being absorbed. This possibility is discounted, however, by evidence obtained from other human subjects in whom *generic* IP reached a higher level than Tofranil®. In those subjects, the internal standard also reached a higher concentration in the case of the generic IP. Thus, the

variations in the concentration maxima that are observed appear to arise primarily from intrasubject variability in absorption rates. Such intrasubject variations have been observed previously in carefully controlled, repeated measurements on aspirin.[29]

Since bioavailability comparisons involve two or more different formulations of a given drug, intrasubject and intersubject variability usually necessitates that at least eight subjects participate in the study. The present method seems to provide a definitive and simple means of discerning intrasubject variability from a true bioavailability difference, and thus may reduce the number of subjects required, and consequently lower the risk and cost. We fully expect that the methodology of using an orally administered, stable isotope labeled internal standard will become accepted practice in future bioavailability comparisons.

One potential problem that should be borne in mind, however, is the possibility of large kinetic differences in the absorption or elimination of labeled and unlabeled drug molecules. For example, since $[^2H_2]$IP was administered in solution whereas IP was in tablet form, we would expect there to be some difference between the absorption rates of the two isotopic species. Indeed, $[^2H_2]$IP always appears in plasma at a higher level than IP during the initial 2–3 h post-ingestion. This is, of course, precisely what would be predicted if dissolution of the tablet is slower than the rate of stomach emptying. In principle, such data might be used to obtain information about the kinetics of tablet dissolution *in vivo*.

In the case of IP and $[^2H_2]$IP, the peak plasma levels usually occur at the same time, so that although the $[^2H_2]$IP concentration rises more rapidly than that of IP in the initial stages, dissolution of the tablet is fast enough so that the concentration of unlabeled drug very quickly catches up. This might not be the case for drugs that dissolve more slowly.

A second problem that has received considerable attention lately is the possibility of isotopic effects in drug metabolism.[30–32] This can arise particularly in the case of deuterium labeled molecules. Judicious placement of the label at a site that does not undergo extensive metabolism can obviate this problem, even for deuterium labeling. In the present case, IP is hydroxylated at the C−10 (or C−11) position, which is the site of deuterium labeling in $[^2H_2]$IP, but this pathway constitutes a relatively minor fraction of the overall scheme of metabolism.[3] Hence, the elimination kinetics were not significantly affected by deuterium substitution.

In summary, this paper presents a new methodology for studying drug kinetics at low concentrations and for comparing the bioavailabilities of drug preparations. The use of HPLC and direct probe FI mass spectrometry offers significant advantages in terms of sensitivity and precision over that of conventional GCMS with selected ion monitoring when dealing with nanogram and subnanogram concentrations of imipramine in biological samples. We emphasize, in particular, the importance in such low level studies of maximizing the sample extraction efficiency, and of being able to purify and analyze essentially the entire extract. No one aspect of this analytical process can be regarded as more important than any other. Thus, the total analytical scheme, with necessarily includes analysis by direct probe, is responsible for the greater sensitivity and precision of this methodology.

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