CHROMBIO 2925

QUANTITATIVE DETERMINATION OF N-[*trans*-2-(DIMETHYLAMINO)-CYCLOPENTYL]-N-(3',4'-DICHLOROPHENYL)PROPANAMIDE, ITS $^2H_5$-LABELED ANALOGUE AND THEIR N-DEALKYLATED METABOLITES IN DOG SERUM BY CAPILLARY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

DON L THEIS*, GORDON W HALSTEAD, VINCENT J CAPPONI*, BRADEN L ROACH** and RUSSELL H ROBINS

The Upjohn Company, Control Research Laboratories, Kalamazoo, MI 49001 (U S A)

SUMMARY

This paper describes the development of a capillary gas chromatographic—mass spectrometric method for the determination of N-[*trans*-2-(dimethylamino)cyclopentyl]-N-(3',4'-dichlorophenyl)propanamide and its metabolites in serum The method utilizes an automated sample preparation whereby drug, metabolites and internal standard are extracted from polar serum components by adsorption chromatography onto an XAD-type resin The N-demethylated metabolites are derivatized by acetylation prior to chromatography Detection is by mass spectrometry with chemical ionization This method was utilized to determine levels of unlabeled and pentadeuterated drug and their respective metabolites in canine serum after oral co-administration No significant kinetic isotope effects were observed for either absorption or metabolism

INTRODUCTION

A new non-tricyclic anti-depressant agent, N-[*trans*-2-(dimethylamino)-cyclopentyl]-N-(3',4'-dichlorophenyl)propanamide (I), is reported to be generally more potent than imipramine with fewer side-effects [1, 2] Studies in dogs have shown that I is extensively metabolized by N-dealkylation to the

---

*Present address Mead Imaging, Miamisburg, OH 45342, U S A
**Present address Cornell University, Department of Chemistry, Baker Laboratory, Ithaca, NY 14853, U S A

desmethyl (II) and bis-desmethyl (III) metabolites [3, 4]. The parent drug and the desmethyl metabolite have previously been determined in canine serum with a packed column gas chromatographic method using electron capture detection [4] This method utilized a 3% OV-17, 2 m × 2 mm I D glass column and an isothermal temperature of 195°C Extraction of I from alkalinized serum was made with a toluene—methanol mixture followed by back-extraction into aqueous hydrochloric acid before pH adjustment and final extraction into toluene. It is the purpose of this paper to focus upon the development of the analytical procedures and synthesis of the labeled drug required to assess the bioavailability of I using stable-isotope techniques

The methodology and advantages of the use of stable isotopes in the assessment of bioavailability have been previously reviewed [5—8] A primary consideration is the power of the method to detect differences in the bioavailability of formulations [9, 10] By the simultaneous administration of a labeled drug (oral or intravenous) along with an unlabeled formulation, intra-subject variability occurring during the wash-out period of a conventional cross-over study is minimized The benefits include more accurate data, reduced animal studies since cross-over legs are eliminated and unlike radioisotopes, stable-isotope methodology can be used in humans without severe safety concerns.

A capillary gas chromatographic (GC) system with alkali flame detection (AFD) was developed for use in optimizing the extraction of I, II, III and the internal standard (IV) from serum using an automated sample extraction procedure. This method is a very sensitive technique in its own right and was found to be capable of quantitating I to subnanogram levels and II or III to nanogram levels in serum On the basis of the previously reported metabolism [3, 4], the $^2H_5$-labeled analogue of I was prepared by labelling the propanamide side-chain (Fig. 1). The AFD capillary GC procedure was then adapted for use with gas chromatography—mass spectrometry (GC—MS) to simultaneously monitor I, II, III and their stable-isotope labeled covariants in serum This technology was used to demonstrate the bioequivalence of labeled and unlabeled I in dogs
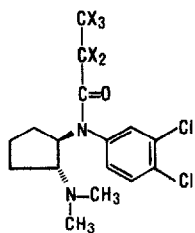

Fig 1 Structures of I (X = H) and [^2H_s]I (X = ^2H)

EXPERIMENTAL

Reagents and materials

[$^2H_6$]Propionic acid (99.1 atom %) was obtained from Merck Sharp and Dohme Isotopes Synthesis of the intermediate, *trans*-N,N-dimethyl-N'-(3,4-dichlorophenyl)-1,2-cyclopentanediamine (V) has been described previously [1]. The metabolites II and III, and I were obtained from The Upjohn

Company. Benzene, methanol, acetone, and toluene were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) All other chemicals were from standard sources. Blank dog serum was obtained from Pel-Freeze Biologicals (Rodgers, AR, U S A)

Capillary gas chromatography– alkali flame detection

The gas chromatograph used was a Hewlett-Packard Series 5880A gas chromatograph equipped with an alkali flame detector The column was 12 m X 0.2 mm fused-silica capillary column with a cross-linked methyl silicone phase, film thickness 0 33 μm (Hewlett-Packard) The injection mode was splitless with a solenoid activation (gas bypass) of 0.25 min Helium was the carrier gas (flow-rate 1 ml/min) and make-up gas (flow-rate 25 ml/min) while hydrogen (flow-rate 3 ml/min) and air (flow-rate 150 ml/min) were detector support gases Injection and detector temperatures were set at 200 and 300°C, respectively The column temperature was programmed from an initial temperature of 100°C (0.5 min) to 200°C (30°C/min), isothermal 200°C (7.2 min), 200°C to 250°C (30°C/min) and isothermal 250°C (2 3 min) after which the oven was returned to 100°C Peak areas and heights were determined by a recording integrator (Hewlett-Packard 3390A) and an in-house digital computer system

Gas chromatographic—mass spectrometric conditions

GC—MS—multiple-ion detection (GC—MS—MID) was carried out on a Finnigan 4600 gas chromatograph—mass spectrometer The GC—MS system was equipped with an on-column in-house designed injector so that on-column injection could be made into a large-bore capillary column (15 m X 0 53 mm I.D.). The bonded phase was cross-linked OV-17, film thickness 1.0 μm (Quadrex, New Haven, CT, U S A.). Chemical ionization was obtained with ammonia reagent gas (113 Pa). The injector, column (isothermal) and source temperatures were 250, 240 and 250°C, respectively The carrier gas was helium at a flow-rate of about 3 ml/min. The multiplier was set at −1700 V, pre-amp at 10^{-8}. Filament electron energy was 70 eV and trap current was 0.33 mA. MID was used to monitor the GC effluent at the following mass units m/z (a.m.u. in parentheses) internal standard IV (315), I (329), [^{2}H$_5$]I (334), III (343), [^{2}H$_5$]III (348), II (357) and [^{2}H$_5$]II (362) Peak areas were determined by manual integration methods provided by INCOS MS software The instrument was previously calibrated using perfluorotributyl amine.

Sample preparation

Serum extractions were performed by a DuPont PREP I automated sample processor (Dupont, Wilmington, DE, U.S A) Type W extraction cartridges (cross-linked styrene divinyl benzene macroreticular resin) from Clinical Systems Division of DuPont were washed by running program 14 twice using acetone and water in reservoirs 1 and 2, respectively [11]. This removed potentially interfering substances on the columns. Venous blood was allowed to clot, centrifuged, and the serum was separated The serum was frozen if further preparation was delayed by more than 8 h For each sample, 1.0 ml of serum, 0 5 ml of internal standard solution (50 ng/ml IV in water) and 1.0

ml of water were added to each cartridge Calibration standards containing I, [²H₅]I, II and III were prepared with serum to cover the expected concentration range of samples Cartridges were placed into the PREP I unit, which contained water (25 ml) and acetone (25 ml) in reservoirs 1 and 2, respectively Program No 15 was selected with a temperature of 50°C [11] After run completion (about 31 min) the extracted samples in aluminum cups were reconstituted in about 100 µl of toluene—methanol (80 20) and vortexed. The resultant mixture was transferred via a glass disposable pipet into a glass micro vial (Reacti-Vial 100 µl, Pierce, Rockford, IL, U S A). Approx 10 µl of acetic anhydride were added to each vial, the vial was capped and then heated for 30 min at 100°C The sample was mixed and allowed to cool prior to injection

In vivo testing

Animal studies were performed using purebred beagles Oral solutions were administered via gastric intubation Blood samples were drawn through an indwelling catheter consisting of a 21-gauge needle with a fluoroethylene propylene cannula, 51 mm × 1.3 mm O D , 0 86 mm I D (ABBOCATH T, Abbott Labs , North Chicago, IL, U S A) The catheter was placed into the antebrachial section of the cephalic vein of the foreleg Once secured, a Luer-Lok injection cap (Becton Dickinson, Rutherford, NJ, U S A) was placed onto the end to prevent blood flow from the catheter About 0 5 ml of 100 U.S P (United States Pharmacopeia) units of heparin per ml of saline were injected into the catheter via the cap to prevent clotting

Preparation of [²H₅] I

Benzoyl chloride (6 27 ml, 54.0 mmol) and [²H₆] propionic acid (2 01 ml, 25 3 mmol) were added to a distillation apparatus consisting of a 50-ml round-bottom flask containing a magnetic stirring bar, a one-piece 14/20 distillation head/condenser, a PTFE adaptor with thermometer, a 50-ml sidearm round-bottom flask and a nitrogen inlet system The mixture was heated strongly with an oil bath The volatile [²H₅] propionyl chloride began to distill over at about 40°C The oil bath temperature eventually reached 225°C but the internal temperature of the distillate vapor never exceeded the boiling point of the propionyl chloride (77–77 5°C) After the internal temperature fell and no more product distilled over, the bath was removed and product was transferred under nitrogen to a rubber septum capped vial Yield was 2 02 g or approx 80% To a stirred solution of 4.49 g (18 0 mmol) of V in 50 ml of dry benzene (distilled from phosphorus pentoxide) were added 2 15 ml (23.5 mmol) of [²H₅] propionyl chloride via a syringe This mixture was stirred at room temperature under nitrogen for 30 min in a three-neck 14/20 500-ml round-bottom flask equipped with a stopper, nitrogen inlet and septum At this point, 250 ml of 1 *M* sodium hydroxide were added slowly with continued stirring The reaction mixture was transferred to a 1-l separatory funnel and extracted with 250 ml of diethyl ether The ether phase was dried over sodium sulfate, filtered through Whatman No 1 filter paper into a 1-l 24/40 round-bottom flask The solvent was removed on a rotoevaporator, yielding 5 59 g of [²H₅]I as an oil

To 1 98 g (17 1 mmol) of maleic acid in 6 ml of methanol were added drop-

wise 5 59 g (16.8 mmol) of $[^2H_5]I$ dissolved in 180 ml of diethyl ether. Stirring was discontinued after addition of the ether solution was complete The resultant crystals were allowed to settle for several hours and then collected on a sintered-glass filter The product was redissolved in the frit with approx. 10 ml of warm methanol and filtered into a suction flask The recrystallization procedure was twice repeated and the purified product was collected and dried in vacuo to constant weight. Overall yield was 5 17 g (64%) of $[^2H_5]I$ maleate. Chemical purity by high-performance liquid chromatography was found to be 99 5 ± 1 9% Comparison of the intensity data from the chemical ionization (CI) spectrum of $[^2H_5]I$ for masses m/z 334, 333, 332, 331, 330 and 329 indicates that the material is approx 98 atom % deuterated in the propionyl group

Preparation of internal standard (IV)

The internal standard was prepared by a procedure analogous to that described for I previously [1] A solution of V (5 00 g, 18 3 mmol) in 20 ml of acetic anhydride was heated overnight at 90—95°C in a three-neck flask fitted with a nitrogen inlet and a reflux condenser connected to a mineral oil bubbler Water (100 ml) was added and heating was continued for 1 h to decompose the excess anhydride The solution was made basic with 50% sodium hydroxide (pH > 10 with pH paper) and was extracted with 200 ml of diethyl ether. The ether layer was dried over anhydrous magnesium sulfate, filtered through Whatman No 1 filter paper and rotoevaporated to an oil The crude oil (8.04 g) was transferred to a 500-ml flask and dissolved in 10 ml of anhydrous methanol Oxalic acid dihydrate (3 21 g) was added with stirring. After dissolution was complete, 300 ml of diethyl ether were added dropwise over a period of 1 h The oxalic acid salt was allowed to crystallize over a period of several hours after stirring was discontinued The crystals were collected on a fritted, sintered-glass filter The product was recrystallized from methanol— diethyl ether and was dried in vacuo to constant weight The yield was approx. 88% (7 32 g) The oxalate salt was used as received

RESULTS AND DISCUSSION

The automated serum extraction procedure was based on disposable columns packed with cross-linked styrene divinyl benzene macroreticular resin Serum and internal standard were added to the columns which were prewashed to remove substances which could interfere with the chromatography The internal standard (IV) was chosen because of its similarity in structure to I which gives similar extraction, solubility and chromatographic properties After centrifugation the columns were washed with about 2 ml of water to remove unwanted serum components to the waste cup This left drug, metabolites and internal standard on the column Rotational direction of centrifugation was then reversed and the compounds of interest were quantitatively eluted from the column into aluminum sample cups with acetone The samples were then derivatized by heating with acetic anhydride in toluene—methanol

(80 20). The desmethyl metabolite (II) was converted to the N-methyl amide while the primary amine metabolite (III) was converted to its mono-acetylated derivative. Confirmation of structures was made by GC—CI-MS with M+1 ions at m/z 357 (II), m/z 362 ($[^2H_5]$ II), m/z 343 (III) and m/z 348 ($[^2H_5]$ III) for the derivatized metabolites. Without derivatization, the resolution between I and II was about 0 4 It could be increased by slower linear temperature programming, however, the run time then exceeded 20 min. After acetylation the retention time of both metabolites increased.

The chromatographic system was first optimized on a gas chromatograph equipped with a cross-linked methyl fused-silica column An alkali flame detector was used to exploit the nitrogen containing molecules of interest. The AFD system utilized a rubidium-impregnated thermionic source for the specific detection of nitrogen- and phosphorus-containing molecules. The thermionic source was electrically heated and operated in a dilute hydrogen— air atmosphere Flow-rates were such that insufficient hydrogen was present to sustain a flame The minimum detectable level (MDL) for the AFD system for I was about $2 \cdot 10^{-13}$ g/s at a signal-to-noise ratio of 2. This value is similar to that reported by Sullivan [12]. Assuming the minimum quantifiable quantity (MQQ) to be ten times this value, the MQQ is 2 pg/s. Therefore, the MQQ for I in solvent can be estimated at about 2 ng/ml Taking into account a five-fold concentration step in the sample preparation yields an MQQ of 400 pg/ml. This value is similar to that obtained from the recovery study where 300— 500 pg/ml appeared to be the lower limit The sensitivity of this system depended primarily upon the control of the hydrogen gas and the temperature and condition of the rubidium bead The AFD system used featured a non-adjustable hydrogen flow controller valve stem to restrict gas flow to the detector. The rubidium bead was located inside a castle assembly and was relatively easily replaced.

Linearity of the GC—AFD system was shown by preparing toluene—methanol (80 20) solutions of varying concentrations of I with a constant concentration of IV. Samples were then chromatographed, the ratios of the responses of I and IV were plotted versus the concentrations of I Least-squares analyses of the data show a correlation coefficient and y-intercept of 1.000 and 0.0005, respectively, over the range 0 5—200 ng/ml. Recovery of drug from
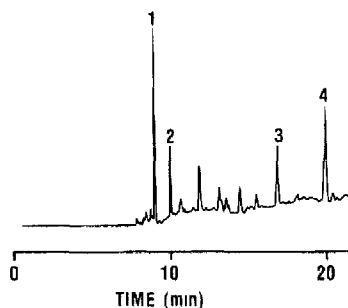

Fig 2 GC—AFD profile of sample preparation after derivatization of dog serum spiked with I (4 ng/ml) and metabolites. Conditions as described in Experimental Attenuation of 8 (0— 8 min), attenuation of 0 (8—20 min) Peaks 1 = IV, 2 = I, 3 = III (acetylated), 4 = II (acetylated)

serum was demonstrated by assaying blank serum spiked with aqueous solutions containing varying amounts of I and metabolites. Equivalent serum concentrations ranged from 0.26 to 216 ng/ml Metabolites were added to simulate actual in vivo samples and demonstrate non-interference. The results show a linear relationship over the described range between drug concentration and the response ratios Linear regression of the data yielded a correlation coefficient of 0 999 and a y-intercept of $-0\ 0015$ ($n = 11$) From the metabolite data, it appears that the detection limit is about 10 ng/ml for each metabolite Correlation coefficients of 0 999 and 0 998 were obtained for II and III, respectively, over the range 10–300 ng/ml No underivatized metabolites were detected at any level. A typical sample preparation of serum containing 4 ng/ml drug in serum is shown in Fig 2 Estimation of the precision of the method was made at two concentration levels of I in dog serum. Serum was spiked with I at the 4 ng/ml and 700 pg/ml levels. Seven 1 0-ml samples were then assayed The resulting ratios of responses of I versus IV were compared for reproducibility. The relative standard deviations (R S.D.) of seven samples were 3 9 and 7.2%, respectively. The higher R S D. for the 700 pg/ml samples reflects the greater variability as the detection limit is approached. Selectivity of the system is shown in Fig. 3 for several structurally related compounds Structures are shown in Fig 4. Generally, the retention times increased for compounds as molecular weight increased as is expected on a methylsilicone column Therefore, the elution order follows: C_{17} (VI) $> C_{16}$ (I) $> C_{15}$ (IV), n-C_{17} (VI) $>$ iso-C_{17} (VII), six-member ring (VIII) $>$ five-member ring (I). A linear dependence exists between the logarithm of the retention time versus total carbon number for C_{15} (IV) through C_{17} (VI). C_{13} (IV) does not lie on the line since it is a secondary amine with different
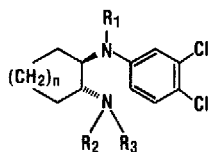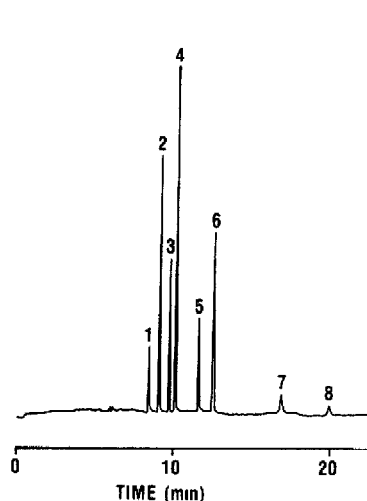
Compound	n	R$_1$	R$_2$	R$_3$
I	1	COCH$_2$CH$_3$	CH$_3$	CH$_3$
II	1	COCH$_2$CH$_3$	CH$_3$	H
III	1	COCH$_2$CH$_3$	H	H
IV	1	COCH$_3$	CH$_3$	CH$_3$
V	1	H	CH$_3$	CH$_3$
VI	1	CO(CH$_2$)$_2$CH$_3$	CH$_3$	CH$_3$
VII	1	COCH(CH$_3$)$_2$	CH$_3$	CH$_3$
VIII	2	COCH$_2$CH$_3$	CH$_3$	CH$_3$

Fig 3 GC—AFD profile of I and related compounds of about 10 µg/ml Conditions as described in Experimental Peaks 1 = V, 2 = IV, 3 = VII, 4 = I, 5 = VI, 6 = VIII, 7 = III (acetylated), 8 = II (acetylated)

Fig 4 Structures of cycloalkane 1,2-diamine compounds

TABLE I

RETENTION TIMES AND RESPONSE FACTORS OF I AND STRUCTURALLY RELATED COMPOUNDS WHEN CHROMATOGRAPHED ON THE CAPILLARY GC—AFD SYSTEM

Compound	Retention time (min)	Relative response factor (relative to I)
V	8 33	1 2
IV	9 00	1 2
III	9 52	—
VII	9 63	1 0
II	9 85	—
I	9 99	1 0
VI	11 4	0 66
VIII	12 3	0 59
Acetylated III	16 6	—
Acetylated II	19 5	—

retention mechanisms. The response factors and retention times of these compounds relative to I are given in Table I

The capillary column used in this work generated about 4300 plates per m for I with a helium flow-rate of about 1 ml/min A peak equivalent to about 50 pg/ml I (by peak area) had been observed with a retention time near to that of I However, it appeared rarely and could be checked for by running a serum blank Human serum when prepared similarly showed no substantially interfering peaks Interfering peaks produced by blood collection devices had been reported with other methods and drugs [13]. None were observed here. Use of glass pipets whenever toluene—methanol was used aided in reducing this background. The sample preparation may be stored at least five days at room temperature. Although this conventional GC—AFD method was developed as an adjunct to the GC—MS procedure, it was found to be a very sensitive method for the determination of I in conventional bioavailability studies

Gas chromatography—mass spectrometry

The primary change in the conversion of the GC—AFD methodology to GC—MS was the injection system Sensitivity, reproducibility and instrument design dictated the use of on-column injection in place of splitless injection Therefore a large-bore (530 μm) fused-silica column having the same stationary phase used for the GC—AFD system was employed. Since separation of the solutes from the serum background was not necessary with the mass spectrometer, isothermal temperature conditions were used. CI with ammonia as the reagent gas was utilized since no fragment of sufficient intensity containing the label was observed in the electron ionization (EI) spectrum (Fig 5). The parent ion of the CI spectrum at m/z 329 is easily distinguished from the m/z 334 parent ion of $[^2H_5]$I (Fig. 6). Fig. 7 shows a mass chromatogram with MID output of a serum standard containing about 10 ng/ml I and $[^2H_5]$I and 18 ng/ml IV (m/z 315) As observed with the GC—AFD system, there was slight separation as the deuterated compound eluted earlier than the unlabeled drug
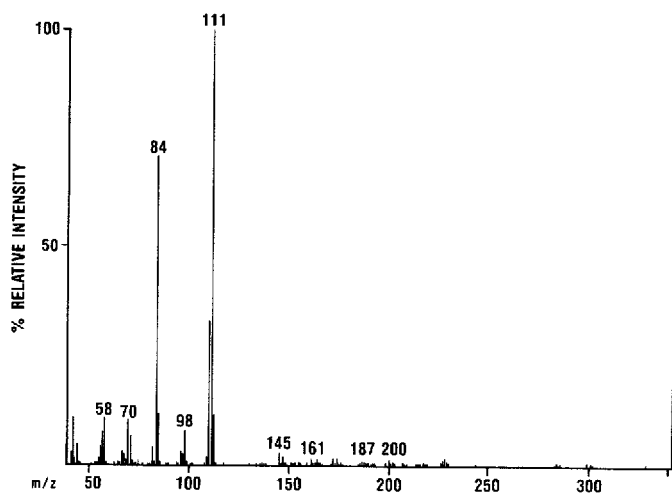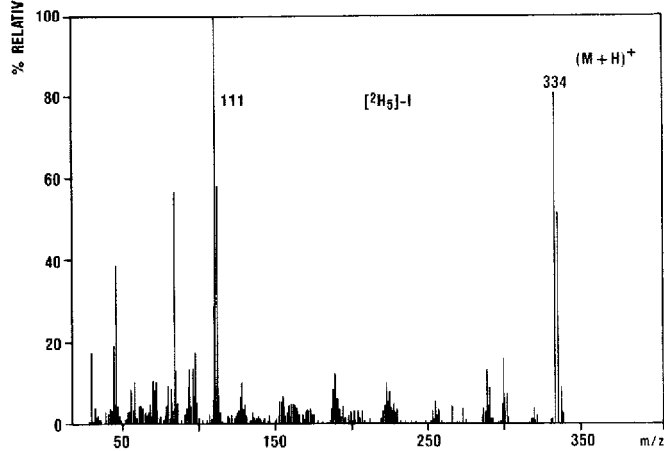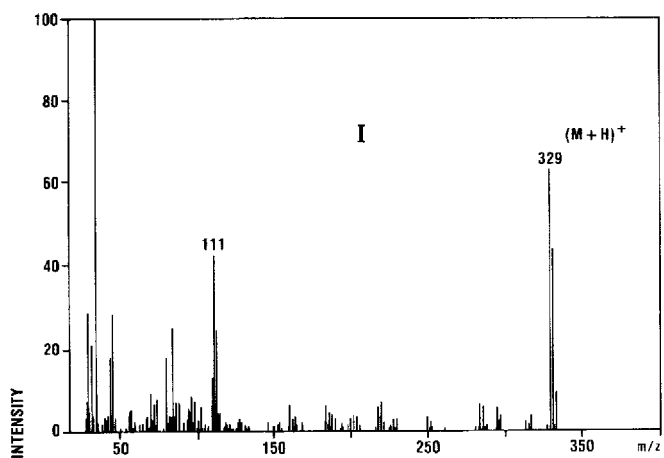
Fig 5 Electron-ionization mass spectrum of I
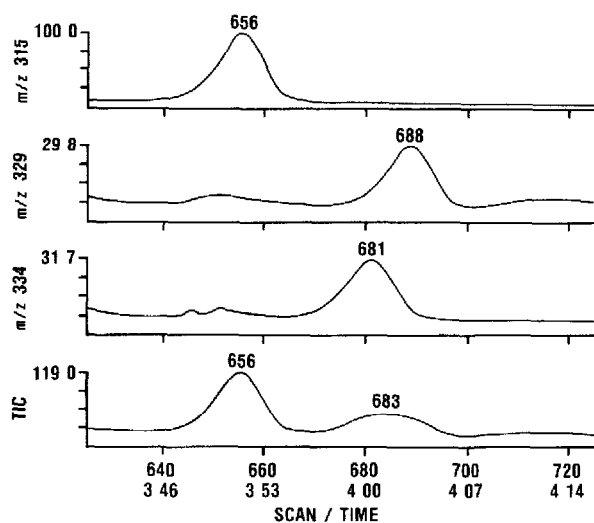

Fig 6 Chemical-ionization (ammonia) mass spectra of I and [²H₅]I

Fig 7 Multiple-ion detection output of serum standard contaning 18 ng/ml IV (m/z 315), 10 ng/ml I (m/z 329) and 10 ng/ml [$^2H_5$]I (m/z 334) TIC = Total-ion current

Recovery studies were performed usmg the automated extraction system The initial recovery study produced a bi-linear calibration curve for both I and [$^2H_5$]I Equations of lines for the lower range (0 5—10 ng/ml) and upper range (10—100 ng/ml) were $y = 0\ 0218x + 0.00297$ and $y = 0\ 0327x - 0.108$, respectively, for I Those for the respective lines of [$^2H_5$]I were $y = 0\ 0228x + 0\ 0108$ and $y = 0\ 0318x - 0.0959$ Correlation coefficients of $> 0\ 999$ were observed for all lines Subsequent calibration curves were descnbed by a single line over the entire range. This mcreased lmeanty of the system was realized by the replacement of the ion source filament and a minor system retunmg Precision of the method was assessed by determmmg the I/IV and [$^2H_5$]I/IV ratios of six samples processed from the same pool of serum for each of two levels 10 and 1 ng/ml The R S.D. values at the 10-ng level were 4.4 and 2.2% for the unlabelled and labelled ratios, respectively The R S D values at the 1-ng level were higher, 7.6 and 5 3%, reflectmg the approach to the detection limit. A blank serum sample was processed without mternal standard and was found to contam no significant mterfering substances.

Application of the method
A dog study was performed to demonstrate equivalence between I and [$^2H_5$]I m beagles. Aqueous solutions of both I and [$^2H_5$]I at 2 mg/kg (1 mg/kg each of I and [$^2H_5$]I) were given via gastric mtubation to one beagle Samples were obtaned for 24 h Visual exammation of the results (Fig 8 and Table II) mdicates a t_{max} for I at about 1 h, while II peaked at about 0 75 h and III at about 4 h The latter metabolite was detectable up to 24 h II was observed withm 15 mm after admmistration. Calibration curves for I and [$^2H_5$]I yielded regression lines descnbed by the equations $y = 0.0342x + 0.0106$ and $y = 0.0325x - 0\ 0056$ with correlation coefficients of 0 998 and 1 000, respectively Those descnbmg the regression lines for II and III were $y = 0\ 0395x - 0.0777$ and $y = 0\ 032x - 0\ 0562$ with correlation coefficients of 0 998 and 1 000, respectively
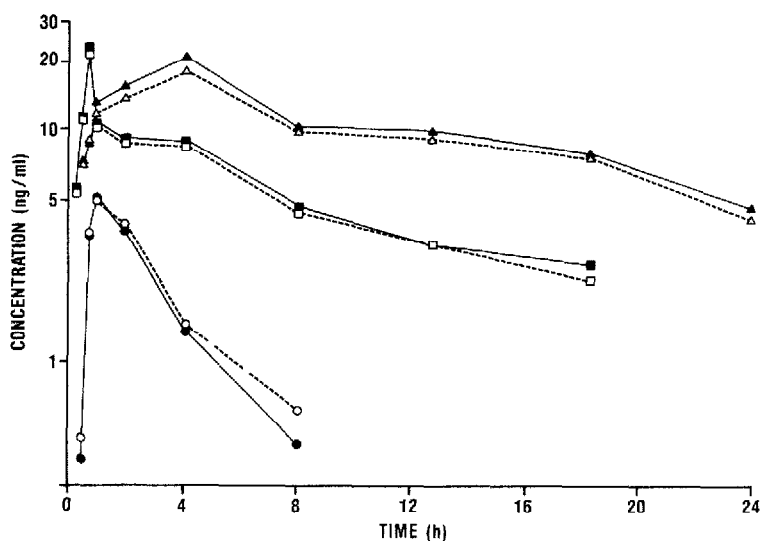
Fig 8 Profile of I (○), [²H₅]I (●), II (□), [²H₅]II (■), III (△) and [²H₅]III (▲) ın dog serum See Table II for tabulatıons

TABLE II

CONCENTRATIONS OF I, II, III AND THE ²H₅-LABELED ANALOGUES IN DOG SERUM AFTER A 1 mg/kg DOSE OF BOTH I AND [²H₅]I

Tıme (h)	Concentratıon (ng/ml)					
	I	[²H₅]I	II	[²H₅]II	III	[²H₅]III
0	N D *	N D	N D	N D	N D	N D
0 25	N D	N D	5 70	5 98	N D	N D
0 50	0 51	0 41	12 1	12 1	7 64	7 75
0 75	3 86	3 80	23 2	24 4	9 61	9 37
1 00	5 42	5 55	11 0	11 5	12 6	14 2
2 00	4 19	3 89	9 35	9 77	14 7	16 5
4 12	1 56	1,44	9 04	9 54	19 4	22 2
8 07	0 67	0 48	4 77	5 00	10 7	11 3
12 8	N D	N D	3 44	3 45	10 0	10 6
18 4	N D	N D	2 72	2 81	8 33	8 64
24 0	N D	N D	N D	N D	4 52	5 04

*N D = Not detected

Metabolısm was extensıve wıth II formıng rapıdly. Further demethylatıon of II occurred to form III. As expected a later peak serum concentratıon tıme of III occurred Thıs ıs sımılar to the metabolısm of ımıpramıne observed ın rats and humans [14] Imıpramıne, a tertıary amıne, underwent N-demethylatıon rapıdly and desmethylımıpramıne, whıch ıs also pharmacologıcally actıve, appeared ın the blood wıthın mınutes after admınıstratıon [15--17]. Further demethylatıon occurred to produce bıs-desmethyl ımıpramıne, though thıs ıs not a major pathway ın rats or humans Imıpramıne had been reported to have low oral absolute bıoavaılabılıty and sınce ıt ıs well absorbed ın the gastro-

TABLE III

RATIOS OF UNLABELLED AND LABELLED DRUG AND METABOLITE
CONCENTRATIONS

Time	I/[²H$_5$]I	II/[²H$_5$]II	III/[²H$_5$]III
0 50	—*	1 00	0 98
0 75	1 02	0 95	1 02
1 00	0 98	0 95	0 89
2 00	1 08	0 96	0 89
4 12	1 08	0 95	0 87
8 07	—	0 95	0 95
12 83	—	1 00	0 94
18 35	—	0 97	0 96
24 03	—	—	0 90
Mean ± S D	1 04 ± 0 05	0 97 ± 0 02	0 93 ± 0 05

*Serum levels too close to the detection limit to be considered

intestinal tract [14, 17—19] probably undergoes extensive first-pass metabolism Szmuszkovicz et al [1] reported similar therapeutic activity for the metabolites of I in the rat.

The ratios of the serum levels of I and [²H$_5$]I compounds were examined to determine if any significant isotope effect was present [20] These data are tabulated in Table III for I, II and III The coefficient of variation is less than or equal to 5% for the mean of the I/[²H$_5$]I ratios. The ratios remain essentially constant at all time points measured and this is considered indicative of the absence of a significant isotope effect

CONCLUSIONS

Drug I labeled with five deuterium atoms in the propanamide side-chain exhibited no significant kinetic isotope effect in beagles The capillary GC—MS method with CI was found to be a selective and sensitive assay for I, [²H$_5$]I and their respective metabolites in canine serum. This label and method are suitable for use in the determination of the bioavailability of I and its formulation(s). The capillary GC—AFD methodology developed as an adjunct to the GC—MS procedure was found to be capable of quantitating subnanogram per milliliter levels of I and should be useful for conventional determination of I in biological fluids.

REFERENCES

1 J Szmuszkovicz, P F VonVoigtlander and M P Kane, J Med Chem , 24 (1981) 1230
2 P VonVoigtlander, H Triezenberg, E Losey and D Gay, Drug Dev Res , 3 (1983) 545
3 D Lakings, personal communication
4 D Lakings, R Stryd and T L Gilbertson, J Pharm Sci , 73 (1984) 317
5 T A Baillie, Pharmacol Rev , 33 (1981) 81
6 W A Garland and M L Powell, J Chromatogr Sci , 19 (1981) 392

7   M Eichelbaum, G E von Unruh and A Somogyi, Clin Pharmacokin , 7 (1982) 490

8   J Vink, Mass Spectrom Rev , 1 (1982) 349

9   J G Wagner, Fundamentals of Clinical Pharmacokinetics, Drug Intelligence Publications, Hamilton, IL, 1975, p 301

10 H d'A Heck, S E Buttrill, Jr , N W Flynn, R L Dyer, M Anbar, T Cairns, S Dighe and B E Cabana, J Pharmacokin Biopharm , 7 (1979) 233

11 PREP I Instrument Manual and Protocols, DuPont, Wilmington, DE, 1981

12 J Sullivan, in R Grob (Editor), Modern Practice of Gas Chromatography, Wiley Inter- science, New York, 1977, p 220

13 J Shang-Qiang and M Evenson, Clin Chem , 29 (1983) 456

14 I W Taylor, C Ioannides, P Sacra, J C Turner and D V Parke, Biochem Pharmacol , 32 (1983) 641

15 M H Bickel and H J Weder, Arch Int Pharmacol Ther , 173 (1968) 433

16 A H Glassman and J M Perel, Arch Gen Psychiatry, 28 (1973) 649

17 A G Gilman, L S Goodman and A Gilman, The Pharmacological Basis of Therapeutics, Macmillan, New York, 1980, pp 422, 1712

18 L F Gram and J Christiansen, Clin Pharmacol Ther , 17 (1975) 555

19 L F Gram and K Fredericson-Overo, Clin Pharmacol Ther , 18 (1975) 305

20 R L Wolen and C M Gruber, Jr , in K S Albert (Editor), Drug Absorption and Dis- position Statistical Considerations, American Pharmaceutical Association, Washington, DC, 1980, p 69