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# DISPOSITION AND METABOLISM OF RADIOLABELLED CASOPITANT IN HUMANS

Authors:

Mario Pellegatti, Ellenia Bordini, Patrizia Fizzotti, Andy Roberts and Brendan M. Johnson

Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline R&D Verona, Italy (M.P., E.B., P.F., A.R.) and Clinical Pharmacology Modeling and Simulation, GlaxoSmithKline R&D Research Triangle Park, NC, US (B.M.J.)

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**RUNNING TITLE**

a) Disposition and metabolism of casopitant in male healthy humans

b) Mario Pellegatti, PCD DMPK GlaxoSmithkline, Via Fleming 4 37135 Verona, tel 0039 0458218403, fax 0039 0458218072, Mario.N.Pellegatti@gsk.com

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d) Abbreviations used are:

NK-1 = neurokinin-1

HPLC = high performance liquid chromatography

HPLC-MS/MS = high performance liquid chromatography tandem mass spectroscopy

Q-TOF = quadrupole/ time-of-flight

NMR = nuclear magnetic resonance

QC = quality control

AUC = area under the plasma concentration-time curve

BQL = below quantification limit

$Vd_{ss}$ = steady-state volume of distribution

$C_{max}$ = maximal plasma concentration

$t_{max}$ = time to reach $C_{max}$

CL = systemic plasma clearance

CI = confidence interval

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**ABSTRACT**

Casopitant [1-piperidinecarboxamide,4-(4-acetyl-1-piperazinyl)-N-((1R)-1-(3,5-bis(trifluoromethyl)phenyl)-ethyl)-2-(4-fluoro-2-methylphenyl)-N-methyl-(2R,4S)-; GW679769] is a novel NK-1 receptor antagonist being developed for prevention of chemotherapy-induced and post-operative nausea and vomiting. The disposition of [$^{14}$C]-casopitant was determined in a single-sequence study in six healthy male subjects after single-dose 90 mg intravenous and 150 mg oral administration. Blood, urine and feces were collected at frequent intervals after dosing. Plasma, urine and fecal samples were analyzed by High Performance Liquid Chromatography/Mass Spectrometry coupled with off-line radiodetection for metabolite profiling. Moreover, urine was also analyzed with $^{1}$H-NMR to further characterize metabolites. Plasma pharmacokinetic parameters for casopitant, a major metabolite (M13, coded as GSK525060), and total radioactivity were determined. Absorption of radioactivity after oral administration appeared to be nearly complete; elimination was principally via the feces both after oral and intravenous administration. Urinary elimination accounted only for <8% of total radioactivity. The main circulating metabolites were a hydroxylated derivative, M13 (coded as GSK525060) and, after oral administration, also M12 (coded as GSK631832), a deacetylated and oxidized metabolite. In addition, many other metabolites were identified in plasma and excreta: the principal route of metabolism included multiple oxidations, loss of the N-acetyl group, modifications or loss of the piperazine group and cleavage of the molecule. Casopitant was extensively metabolized and only negligible amounts were excreted as unchanged compound. Some phase II metabolites were also observed, particularly in urine.

## INTRODUCTION

Casopitant, also known as GW679769, is a novel, potent and selective orally available neurokinin-1 (NK-1) receptor antagonist, which has shown efficacy in the prevention of chemotherapy-induced and post-operative nausea and vomiting (CINV and PONV, respectively) (Arpornwirat et al., 2006; Aziz et al. , 2008; Chung et al., 2006; Grunberg et al. , 2008; Herrstedt et al. , 2008; Navari, 2008; Rolski et al., 2006; Singla et al., 2006; Strausz et al., 2008). The site of action of NK-1 receptor antagonists for the prevention of emesis is believed to be the nucleus tractus solitarius, where vagal afferents from the gastrointestinal tract and inputs from the area postrema and other regions of the brain important in the control of emesis converge. For the prevention of nausea and vomiting, casopitant has been administered as a single-dose or as part of an acute 3-day dosing regimen. Casopitant is administered in combination with a 5-HT3 receptor antagonist, such as ondansetron, and for the prevention of CINV, dexamethasone is also co-administered. Clinical studies have evaluated the safety and effectiveness of a single 50 mg oral dose of casopitant for PONV, and single (150 mg oral) or 3-day ( 90 mg IV or 150 mg oral on day 1, followed by 50 mg oral on days 2 and 3) regimens of casopitant for CINV.

The metabolism, excretion and pharmacokinetics of casopitant in rats and dogs were studied (unpublished results) and showed that the compound is highly metabolized with metabolites eliminated mainly in the feces. The elimination half-life was 1.8 h in rats and 4.5 h in dogs. Metabolism and brain penetration also have been studied in the ferret (Minthorn et al., 2008)

The purpose of this study was to characterize the disposition and metabolism of [$^{14}$C]-casopitant (Fig.1) after single-dose IV and oral administration to healthy male subjects.

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## METHODS

### Chemicals

[$^{14}$C]-casopitant mesylate, casopitant, [$^{13}C^{2}H_3$]-casopitant, standards of metabolites M13 (coded as GSK525060), M12 (coded as GSK631832), M31 (coded as GSK517142), M134 (coded as GSK2110277), M44 (coded as GSK1735004) and [$^{13}C^{2}H_3$]-M13 were all supplied by Chemical Development, GlaxoSmithKline R&D. The structures of metabolites are reported in Table 4. Commercially obtained chemicals and solvents were of HPLC or analytical grade. Liquid scintillation cocktails were obtained from Zinsser Analytics (Frankfurt, Germany) and Perkin Elmer Life Science and Analytical Instruments Inc (Boston, MA).

### Casopitant Formulations

Infusion solution was prepared in an infusion bag for intravenous administration diluting [$^{14}$C]-casopitant at a concentration of 15 mg/mL (0.39 µCi/mg, radiochemical purity 100%) with sterile saline to reach a final concentration of 0.36 mg/mL in 250 mL (equivalent to 90 mg and 35 µCi). For oral administration [$^{14}$C]-casopitant (0.5 µCi/mg, radiochemical purity 100%) was dissolved in sterile water and 10 mL were dispensed into individually labeled containers (equivalent to 150 mg and 75 µCi). The formulations were supplied to the study centre by Pharmaceutical Development, GlaxoSmithKline (Ware, UK).

### Study design and subjects

The clinical study was performed at Charles River Laboratories Clinical Services (Edinburgh, UK) in accordance with Good Clinical Practice and the

principles of the 1996 version of the declaration of Helsinki. The protocol was reviewed and approved, as appropriate, by the Edinburgh Independent Ethics Committee for Medical Research and the Medicine and Healthcare products Regulatory Agency (MHRA). The proposed radioactive dose was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC). Written consent was obtained from all subjects prior to any protocol-specific procedures.

Six healthy male Caucasian subjects, aged between 30 and 55 years and with a body mass index between 19-30 $kg/m^2$, participated in this study. The study design was an open label two period single-sequence study with single-dose IV administration (infusion) followed by single-dose oral administration after a washout period. Subjects were in good health as assessed by clinical examination, non-smokers, with no history of drug or alcohol abuse and were on no other medication at the time of the study, with no prescribed medication within 14 days of the study commencing.

**Study procedures**

Subjects were fasted overnight prior to casopitant administration. All subjects received a single IV infusion of [$^{14}$C]-casopitant (as mesylate salt) at target dose of 90 mg (1.3 MBq, 35 µCi) in 250 mL of sterile saline over 15 minutes. After a washout period of 43 days, the same subjects received a single oral dose of 150 mg [$^{14}$C]-casopitant (as mesylate salt) (2.8 MBq, 75µCi), dissolved in 10 mL sterile water.

Following IV administration, blood samples (7 mL for pharmacokinetic evaluation) were collected into K3-EDTA containing tubes at pre-dose, 0.25, 0.5, 1, 2, 3, 4, 6, 10, 16, 24, 36, 48 and 72 h after the start of infusion; additional

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larger samples for metabolite identification (30 mL) were collected at 0.25, 1, 3 and 6 h. Following oral administration blood samples (7 mL) were collected at pre-dose, 0.5, 1, 2, 3, 4, 6, 10, 16, 24, 36, 48 and 72 h after casopitant administration; additional samples (30 mL) were collected at 1, 3, 6 and 16 h.

Blood samples were mixed, immediately chilled on crushed ice and centrifuged for 10 minutes at 1500 x g at approximately 4°C to obtain plasma. Total radioactivity was measured on duplicate weighed aliquots of plasma; the remaining plasma was stored at -20°C prior to the assay for casopitant and M13 or profiling by HPLC. At selected time points (1, 3, 6, 16, and 24 h) blood radioactivity also was measured on duplicate aliquots of whole blood.

Urine samples were collected pre-dose and between 0-6 h, 6-12h, 12-24 h after drug administration and then at 24 h intervals until 288 h. For each collection period, after thorough mixing, the weight of the sample was recorded, and radioactivity was determined on an aliquot of 5 mL; two 30 mL aliquots were stored at -20°C for metabolite identification.

Fecal samples were collected pre-dose and at 24 h intervals until 288 h. Feces from of each 24 h interval were mixed and homogenized 1:1 with water. Total weight of homogenate was recorded and duplicate aliquots of 0.3 g used for radioactivity determination; the remaining homogenate was stored at -20°C for metabolite identification.

**Assay of total radioactivity**

Aliquots of blood (250 µL) and of fecal homogenates (ca. 0.2 g) were combusted using a Packard model 307 sample oxidizer (Perkin Elmer). The $^{14}CO_2$ generated was collected in a suitable absorbent scintillation system. Aliquots of liquid samples (e.g. urine and plasma) were subjected to liquid

scintillation counting (LSC) together with representative blank samples, whose values were subtracted to give net dpm per sample, using a Liquid Scintillation Analyzer with automatic quench correction by an external standard method (Botta et al., 1985).

**Casopitant and M13 Quantification**

Quantification of casopitant and of M13 in plasma was performed using a validated analytical method based on protein precipitation with acetonitrile, followed by HPLC-MS/MS analysis. Acetonitrile (150 µL) containing the internal standards ([$^{13}C^{2}H_3$]-casopitant, [$^{13}C^{2}H_3$]-M13, both at a concentration of 75 ng/mL) was added to plasma samples (50 µL). After vortex mixing, water (100 µL) was added and the deproteinized samples were centrifuged for 10 minutes at approximately 3000 g. The supernatant was separated using a Betasil Phenyl Hexil column (50 x 3.0 mm - 5.0 µm, Thermo, Cheshire, UK) and eluted at a flow rate of 0.7 ml/min. The isocratic mobile phase consisted of 35% 5 mM ammonium acetate (native pH) and 65% acetonitrile. The column eluate was introduced into a TurboIonSpray source of a Sciex API-3000 triple quadrupole mass spectrometer (Applied Biosystem, Concord, Ontario, Canada) operated in positive ion mode. The temperature of the probe was maintained at 350$^{o}$C with a curtain gas setting of 10 and collision gas setting of 4. Casopitant and M13 were monitored by multiple reaction monitoring of 617→167 and 633→479, respectively. [$^{13}C^{2}H_3$]-casopitant and [$^{13}C^{2}H_3$]-M13 were monitored by multiple reaction monitoring of 621→171 and 637→483, respectively.

The concentrations of casopitant and M13 present in plasma samples were determined from calibration curves constructed from analysis of samples spiked

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with known concentrations of casopitant and M13, with weighted $1/x^2$ linear regression applied in each case over the range 1.5 to 1500 ng/mL.

Quality Control samples (QC), prepared at 3 different analyte concentrations and stored with study samples, were analyzed with each batch of samples against separately prepared calibration standards. QC samples and calibration standards were prepared using independently prepared stock solutions of casopitant and M13 reference materials. For the analysis to be acceptable, no more than one-third of the QC results were to deviate from the nominal concentration by more than 15%, and at least 50% of the results from each QC concentration were to be within 15% of nominal.

**Quantification and profiling of metabolites in plasma, urine and feces**

Plasma samples from each time point were analyzed separately for each subject. Representative fecal and urine samples for each subject were obtained by pooling across sampling times by total weight ratio in order to generate sample pools containing 90% or greater of the radioactivity excreted in feces or urine.

Plasma samples (~10 mL), after thawing, were centrifuged and diluted 1:1 v/v with 10% formic acid before being extracted by solid-phase extraction (SPE). Each sample was equally split and loaded onto two Oasis® MCX (6 mL/500 mg) cartridges (Waters, Milford, MA), previously conditioned by rinsing with 5 mL methanol and then 5 mL water. Each column was washed with 3 mL of water and radioactivity eluted with 7 mL ammonium hydroxide 5% in methanol. The two extracted samples were combined, evaporated and reconstituted in 1 mL 80:20 water/methanol.

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Urine samples (40 mL) were centrifuged and diluted 4:1 v/v with 10% formic acid before being extracted by SPE. The urine mixture was applied onto Oasis® MCX (20 mL/1g) cartridges, previously conditioned with 15 mL of methanol and 10 mL of water. Cartridges were then washed with 5 mL of water and the radioactivity eluted with 9 mL of ammonium hydroxide 5% in methanol. The extracts were evaporated and reconstituted in 1 mL 80:20 water/methanol.

Fecal homogenates (ca. 4 g) were extracted by mixing with 7 mL of 50:50 methanol/acetonitrile. After centrifugation the pellet was extracted with 9 mL of methanol, centrifuged again and extracted a third time with 9 mL of methanol. Supernatants were combined and concentrated under nitrogen to 1 mL prior to HPLC analysis.

Weighed aliquots of each extract were radioassayed by LSC for the calculation of recovery after extraction and reconstitution.

Because of the large number of metabolites and the qualitative differences across the various matrices, two different HPLC methods were used as detailed below.

*HPLC method 1 (used for analysis of plasma and feces samples)*

The chromatographic instrument used consisted of an Agilent 1100 binary pump, autosampler and column oven (50$^{o}$C) (Agilent Technologies, Palo Alto, CA) using a Phenomenex (Torrance, CA) Synergi Polar RP column (250 x 4.6, 4 µm). The mobile phase consisted of 5 mM ammonium formate (pH 5) (solvent A) and 5 mM ammonium formate (pH 5) in acetonitrile (10:90 water/acetonitrile) (solvent B) at a flow rate of 1 mL/min. A gradient was used, starting at 37% B with a linear change to 57% B over 60 minutes, followed by a linear increase to 100% by 65 minutes, with these conditions being maintained for a further 5 minutes. *HPLC method 2 (used for analysis of human urine samples)* The

chromatographic method is as listed for method 1 with the exception of a different gradient, starting at 5% of B with a linear increase to 20% over 15 minutes, followed by further four linear increase: to 25% B by 40 minutes, to 45% B by 45 minutes, to 80% B by 65 minutes and then to 100% B by 65.1 minutes. These conditions were maintained for a further 5 minutes.

HPLC column recoveries were determined on selected samples by collecting the total HPLC column eluate for the appropriate run and assaying the radioactivity to assess recovery of injected radioactivity. Full recoveries of radioactivity were obtained from the HPLC eluate collected.

Radio-HPLC data were captured off line (Bruin et al., 2006): chromatographic fractions were collected using a Perkin Elmer fraction collector *mod* IntekServices FC LH200 onto 96 deep wells LUMAPLATES microtitre plates containing yttrium silicate solid scintillant (Perkin Elmer). Radioactivity determination was performed by scintillation counting (TopCount NXT counter, Perkin Elmer).

For NMR analysis of urine samples, 150 mL of urine were freeze-dried and reconstituted with 90:10 water/methanol (20 mL). After centrifugation, the sample was fractioned by preparative HPLC, using a chromatographic method as listed for method 2 but with a flow rate of 4 mL/min and column dimension, 250 x 10 mm.

**Structural identification of metabolites**

Structural characterization was performed on selected samples by HPLC-Mass Spectrometry using hybrid quadrupole/time-of-flight Q-TOF Ultima (Waters MS Technologies, Manchester, UK) tandem mass spectrometer [Morris, 1996]. Electrospray ionization, in positive or negative mode, was used.

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The HPLC flow was split (1:10) between mass spectrometer and a fraction collector model FC 204 (Gilson Inc, Middleton, WI) used for off-line radiodetection.

Metabolites were identified based on charged molecular ions, mass accuracy and their collision induced dissociation fragmentation [Oliveira, 2000]. Authentic standards, when available, were used to compare chromatographic retention times and fragmentation patterns. Supporting data from preclinical studies (unpublished) were also used in the assignment of metabolite structures. For some metabolites, confirmation of the structure has been obtained by $^{1}$H-NMR [Plumb, 1999].

NMR experiments were performed using a Bruker DRX-600 spectrometer (Rheinstetten, Germany) equipped with an inverse 5 mm TXI CryoProbe™ ($^{1}$H/$^{13}$C/$^{15}$N) operating at 600.13 MHz under the control of TopSpin. $^{1}$H NMR spectra were acquired using a standard NOESYPRESAT pulse sequence for solvent suppression with time shared double presaturation of the water and acetonitrile frequencies. In these experiments 256 transients were acquired into 64 K data points over a spectral width of 12019 Hz (20 ppm) with an inter-scan delay of 2.4 seconds giving a pulse repetition time of 5 seconds.

Fully characterized metabolites were designated by the letter M followed by a number; where a synthetic standard was available a GSK code number was assigned.

**Pharmacokinetic analysis**

Actual blood collection times and the actual dose administered were used for all pharmacokinetic calculations. Standard non-compartmental

methods were used to derive pharmacokinetic parameters using WinNonlin (Version 4.1, Pharsight, Mountain View, CA). Maximum plasma concentration ($C_{max}$) and time of $C_{max}$ ($t_{max}$) were taken directly from the pharmacokinetic concentration-time data. Area under the plasma concentration time curve (AUC) was calculated using the linear up-log down method, to the last sampling time t [$AUC_{(0-t)}$], and extrapolated to infinity [$AUC_{(0-\infty)}$] by the addition of the concentration at the last sampling time divided by the terminal elimination rate constant, $\lambda_z$. Half-life was determined as ln(2) divided by $\lambda_z$. After intravenous administration only, systemic clearance (CL) was calculated as dose divided by $AUC_{(0-\infty),}$ and the volume of distribution at steady-state ($Vd_{ss}$) was the product of CL and the mean residence time as determined by moment analysis. The ratio of oral-to-IV dose-normalized $AUC_{(0-\infty)}$ parameters were used for determination of the absolute bioavailability of the casopitant oral solution. Concentration units for radioactivity are expressed as ng-equivalents of casopitant/mL (ng-eq/mL).

## RESULTS

### Mass balance of total radioactivity

The actual dose of IV and oral casopitant administered ranged from 68-76 mg (27-30 μCi) and 141-144 mg (70-72 μCi), respectively. Subjects received a slightly lower dose of IV casopitant than was planned, most likely a result of incomplete flushing of the injection port of the IV infusion bag during preparation. This was not considered to have any significant impact on the aims of the study.

The cumulative excretion of total radioactivity following single-dose IV and oral administration of [$^{14}$C]-casopitant to healthy male subjects at a target dose of 90 mg and 150 mg, respectively, is shown in Table 1.

Following IV administration of [$^{14}$C]-casopitant in Period 1, total radioactivity was eliminated primarily in the feces, accounting for a mean of 83.8% (range 74.1% to 91.1%) of the administered dose by 288 h post-dose. An additional fecal sample was collected from all subjects (408 h and/or 432 h post-dose) and radioactivity in these samples ranged from 0.2% to 1.4% of the administered dose.

After a 43 day washout, following oral administration of [$^{14}$C]-casopitant in Period 2, total radioactivity was eliminated primarily in the feces, accounting for a mean of 71.4% (range 66.3% to 82.0%) of the administered dose by the end of the collection period. At 288 h post-dose, radioactivity was still detectable in fecal samples (0.8% to 2.8%). By comparing the amount of total radioactivity in the urine from IV and oral administration of [$^{14}$C]-casopitant, and assuming linear pharmacokinetics and no enterohepatic recirculation, the mean fraction of casopitant absorbed after oral solution administration was estimated to be 93%.

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**Pharmacokinetics**

A summary of the plasma pharmacokinetic parameters for casopitant, the major circulating metabolite M13, and total radioactivity following IV and oral administration is presented in Table 2. Median plasma concentrations of casopitant, M13 and total radioactivity (plasma and whole blood) following IV and oral administration are shown in Fig. 2. After oral administration, absorption of casopitant solution was rapid with a median $t_{max}$ of 1 h and the mean absolute bioavailability of casopitant oral solution was determined to be 0.57 (95% CI: 0.39, 0.84). Following IV and oral administration, the metabolite M13 was rapidly formed, and the terminal half-life of casopitant and M13 were similar. The AUC of M13 (based on molar concentrations) was 39-41% and 81-83% that of casopitant after IV and oral administration, respectively. The apparent terminal half-life of total radioactivity in plasma ranged from 69.4 to 172 h after IV administration and 68.6 h to 91.7 h after oral administration.

Pre-dose plasma samples collected prior to oral administration of [$^{14}$C]-casopitant (period 2) still contained levels of radioactivity slightly higher than background, indicating that a small amount of circulating radioactivity had remained following the IV dose, consistent with the long half-life of plasma radioactivity.

After both IV and oral administration of [$^{14}$C]-casopitant, mean blood: plasma total radioactivity ratios ranged from 0.64 to 0.71 over the first 24 h after IV administration and from 0.47 to 0.76 over the first 24 h after oral administration. Given the mean haematocrit of 0.4 observed in this study, the percentage of radioactivity associated with the cellular fraction of whole blood over the first 24 h post-dose was 6-15% after IV administration and 0-21% after oral administration of casopitant.

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**Metabolic profile**

*Plasma*

The mean recovery of radioactive material following solid phase extraction of human plasma samples decreased at later time points following both IV and oral administration, from ca. 99% to 84% (IV) and from ca. 90% to ca. 65% (oral). Fig. 3 shows two representative radiochromatogram profiles at 1 h after IV and oral dose. Quantification for some relevant metabolites after IV infusion and oral administration at selected time-points are summarized in Table 3.

After IV administration the percentage of radioactivity accounting for parent compound decreased from 92% at 0.25 h to 39% at 6 h. The only notable circulating metabolite was M13; there were several other circulating metabolites each accounting for less than 2% of plasma radioactivity. After oral administration, casopitant represented the major circulating component up to 16 h, decreasing on average from ca. 45% at 1 h to ca. 14% at 16 h. The major metabolites observed were M13 and M12. Other relevant metabolites were M31, M134, M2, M3, M10, M39 and M156. Some pooled plasma samples beyond 16 h also were analyzed (up to 72 h post-dose): at these later time points some oxygenated derivatives of M39 were prevalent, particularly M156. M44, a non radioactive glucuronide of a cleavage derivative, was identified and estimated by HPLC-MS/MS using a biological standard (data not reported): it resulted to be less than 5% and 10 % of parent compound in IV and oral plasma extracts, respectively. Proposed structures and supporting spectral data are shown in Table 4.

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*Urine*

Fig. 4a shows a representative radiochromatogram of urine extracts. Urinary profiles were similar after IV and oral administration. Non-radioactive metabolites, resulting from cleavage, represented most of drug related material in the urine and were identified by $^{1}$H-NMR as well as HPLC-MS/MS. The main component was the glucuronide M44, estimated by $^{1}$H-NMR to account for 65% of drug related material in the urine. Other cleavage products identified were the N-dealkylated metabolite M28 and its hydroxylated derivatives M21, M138 and M139. Parent casopitant was present only in negligible amounts (<0.1% of the dose). Other metabolites were identified but each accounted for less than 0.2% of the dose. Proposed structures and supporting spectral data are shown in Table 4.

*Feces*

Radio-HPLC analysis of fecal extracts after both IV and oral administration of [$^{14}$C]-casopitant showed many radiocomponents. Metabolic profiles were similar after oral and intravenous administrations. A representative radiochromatogram of fecal extracts is shown in Fig. 4b.

As higher amounts of radioactivity were administered by the oral route compared to the IV route, the summary below is restricted to the fecal metabolite profile after oral administration where enhanced radiochemical signals facilitated metabolite identification and quantification.

More than 40 metabolites were identified in feces, but the majority did not represent more than 1% of the dose. The principal radiolabeled compounds in feces were derived from oxidation and opening of the piperazine ring, together with multiple oxidations of the parent structure. Among these, M123 and M137

accounted for an average of 5% of the fecal radioactivity each (ca. 3.5% of the dose). M123 was derived from loss of the N-acetyl group and extensive metabolism of the piperazine ring to most likely an acid moiety; M137 lost the N-acetyl group and had both the piperazine and the fluorobenzylmethyl group oxidized. N, N-deethylated oxidized derivative M141 accounted for ca. 4% of the fecal radioactivity (ca. 3% of the dose).

Several other Phase I metabolites (e.g., M50, M63, M73, M77, M80, M140, M146 and M157) each represented 2-3% of radioactivity in the feces, corresponding to less than 2% of the dose. Proposed structures and supporting spectral data are shown in Table 4.

Parent was detected only in negligible quantities.

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## DISCUSSION

Single-dose [$^{14}$C]-casopitant was administered in a single-sequence study to six healthy male subjects by both the IV and oral routes. Casopitant was rapidly and almost completely (estimated to be 93%) absorbed following oral solution administration, and the absolute bioavailability was determined to be 57%, suggestive of pre-systemic metabolism. Following IV and oral administration, total radioactivity was eliminated primarily in feces, with urinary excretion of total radioactivity totaling less than 8% of the dose after the 9 day collection period. Total radioactivity was eliminated slowly and still detectable in fecal samples at the end of the continuous collection period (12 days).

The slightly lower total recovery after oral administration is most likely a result of small amounts of radioactive species that are slowly eliminated and confounded by the technical difficulties in the accurate determination of small amounts of radioactivity in feces samples at later time points. This is not an uncommon finding with highly metabolized compounds [Roffey, 2007].

Systemic exposure to total radioactivity in plasma ($AUC_{(0-t)}$ and $AUC_{(0-\infty)}$, respectively) were approximately 5.63 and 14.5-fold (IV) and 13.1- and 27.6-fold (oral) greater than corresponding estimates for the parent compound casopitant, demonstrating more extensive metabolism following oral administration. These data also are consistent with pre-hepatic and pre-systemic metabolism of casopitant. In addition, mean half-life estimates were appreciably longer for total radioactivity compared with parent casopitant. This observation, together with greater systemic exposure observed for total radioactivity, cannot be accounted for by casopitant and the major metabolite M13 alone, and reflects the formation of other radiolabeled metabolites or

breakdown products which persist in the circulation longer than the parent compound.

Systemic plasma exposure to major metabolite M13 (based on molar AUC ratios) were between 39-41% (IV) and 81-83% (oral) of the corresponding estimates for parent casopitant. M13 and other metabolites (associated with total radioactivity) after both oral and IV administration appear rapidly, and the similar half-life estimates for casopitant and M13 also suggest this metabolite is formation-rate limited, whereas some other metabolites (associated with total radioactivity) may be elimination rate-limited.

Mean estimates of casopitant systemic plasma clearance were low compared with both normal hepatic and renal plasma flow rates in man, suggesting that clearance of casopitant from plasma would not be limited by perfusion rates, and the large estimates of volume of distribution at steady-state were appreciably greater than total body water and indicate that casopitant distributes extensively [Davies, 1993].

The identification of many metabolites in complex matrices such as plasma, urine and feces posed a significant analytical challenge; HPLC-MS/MS in combination with off-line radioactivity monitoring, and in some instances $^{1}$H-NMR, were employed for metabolite identification. For metabolite quantification, HPLC separation followed by off-line radioactivity measurement was employed to increase sensitivity.

Based on the structure of metabolites a putative simplified metabolic scheme is shown in Fig. 5. After oral administration, the principal route of metabolism involved multiple oxidations of the parent molecule, loss of the N-acetyl group, oxidation with progressive opening and modification of both the piperazine and piperidine rings. N-demethylation was also observed. A relevant

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route of metabolism observed in urine was the cleavage of the molecule with formation of some radiolabeled phase I metabolites (M28 and its hydroxylated derivatives) and of non-radiolabeled phase II metabolites, mainly M44.

The principal circulating metabolites after single oral administration were M13 and M12. Their affinity to NK-1 receptors was determined in vitro, and was similar to casopitant (data not presented). However, as these metabolites have reduced brain penetration when compared to casopitant in animals [Minthorn et al., 2008], and lower clinical plasma exposure than casopitant (Tables 2 and 3), their contribution to the overall pharmacological activity of casopitant is expected to be small.

During the clinical development of casopitant, validated assays for M13 and M12 were established and demonstrated that these metabolites were present in the plasma of animal species used for toxicological investigations at concentrations similar to or higher than those determined in humans, therefore, their contribution to the overall toxicity of casopitant was established during standard toxicological investigations.

Both casopitant and M13 are primarily metabolized by CYP3A4 and show some potential for inhibition of CYP3A4 in vitro (unpublished results). A series of clinical drug-drug interaction studies have been conducted [Adams et al., 2009; Johnson et al., 2009], and further results will be the subject of future publications.

In conclusion, casopitant is well absorbed after oral administration, and undergoes first-pass metabolism resulting in an absolute bioavailability of approximately 60%. After both oral and IV administration, casopitant is extensively metabolized; metabolites are excreted primarily in feces. Two major circulating metabolites were characterized, M13 and M12, and while many other

metabolites were identified, none represented more than 5% of the exposure to drug related material.

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## LEGENDS FOR FIGURES

Figure 1:

Structure of [$^{14}$C]-casopitant

Figure 2:

Median concentrations of casopitant, M13 and total radioactivity in plasma and whole blood (total radioactivity only) following 90 mg single-dose IV (a) and 150 mg oral (b) administration of [$^{14}$C]-casopitant in 6 subjects.

Figure 3:

Representative radiochromatograms of human plasma at 1 hour after IV infusion (a) and after oral administration (b) of [$^{14}$C]-casopitant at the target dose of 90 and 150 mg, respectively

Figure 4:

Representative radiochromatograms of human urine (a) and feces (b) after oral administration of [$^{14}$C]-casopitant at the target dose of 150 mg

Figure 5:

Putative simplified metabolic scheme for casopitant in humans

# TABLES

## TABLE 1

*Mean (SD) cumulative total radioactivity excreted through urine and feces (% of dose) after single-dose IV and oral administration of [$^{14}$C]-casopitant at target doses of 90 and 150 mg respectively to six healthy volunteers*

| Elapsed time | IV | | | | Oral | | | |
|---|---|---|---|---|---|---|---|---|
| from dose (h) | N | Urine | Feces | Total | N | Urine | Feces | Total |
| 0-24 | 6 | 3.0 (0.2) | 0.2 (0.2) | 3.1 (0.2) | 6 | 3.3 (0.9) | 0.1 (0.1) | 3.3 (0.9) |
| 0-48 | 6 | 4.6 (0.2) | 11.9 (12.1) | 14.5 (12.0) | 6 | 4.4 (0.9) | 12.6 (10.2) | 17.0 (10.3) |
| 0-72 | 6 | 5.5 (0.2) | 32.7 (11.9) | 38.1 (12.0) | 6 | 5.3 (1.0) | 25.2 (12.0) | 30.5 (12.4) |
| 0-96 | 6 | 6.1 (0.2) | 43.7 (13.3) | 49.8 (13.5) | 6 | 5.8 (1.0) | 43.2 (5.1) | 49.0 (5.0) |
| 0-120 | 6 | 6.5 (0.2) | 56.9 (5.8) | 63.4 (5.9) | 6 | 6.1 (1.0) | 49.8 (4.2) | 56.0 (4.5) |
| 0-144 | 6 | 6.8 (0.2) | 62.7 (7.5) | 69.5 (7.7) | 6 | 6.4 (1.0) | 57.6 (4.5) | 64.1 (4.1) |
| 0-168 | 6 | 7.1 (0.2) | 67.3 (9.9) | 74.4 (10.1) | 6 | 6.6 (1.0) | 61.6 (6.0) | 68.2 (5.6) |
| 0-192 | 6 | 7.3 (0.2) | 74.7 (5.4) | 82.0 (5.4) | 6 | 6.8 (1.0) | 63.9 (4.9) | 70.7 (4.4) |
| 0-216 | 6 | 7.5 (0.2) | 77.1 (5.5) | 84.6 (5.5) | 6 | 7.0 (1.0) | 66.3 (5.7) | 73.2 (5.3) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0-240 | 6 | - | 81.0 (7.1) | 88.6 (7.0) | 6 | - | 68.5 (5.3) | 75.4 (4.7) |
| 0-264 | 6 | - | 82.9 (6.9) | 90.4 (6.9) | 6 | - | 69.4 (5.7) | 76.4 (5.2) |
| 0-288 | 5 | - | 83.8 (7.3) | 91.3 (7.3) | 5 | - | 71.4 (6.2) | 78.3 (5.7) |

**TABLE 2**

*Geometric Mean (95% CI) casopitant, M13, and total radioactivity plasma pharmacokinetic parameters following single-dose IV (target dose: 90 mg) and oral (target dose: 150 mg) administration of [$^{14}$C]-casopitant to humans*

| Parameter | IV | | | Oral | | |
|---|---|---|---|---|---|---|
| | Casopitant | M13 | Total Radioactivity | Casopitant | M13 | Total Radioactivity |
| N | 6 | 6 | 6 | 6 | 6 | 6 |
| $AUC_{(0-\infty)}$ (ng.h/mL) [a] | 6980 (5490, 8890) | 2940 (2110, 4100) | 101000 (69700, 148000) | 6620 (3640, 12100) | 5620 (3610, 8760) | 183000 (161000, 208000) |
| $AUC_{(0-t)}$ (ng.h/mL) [a] | 6730 (5330, 8500) | 2720 (1990, 3710) | 37900 (32100, 44800) | 6240 (3570, 10900) | 5160 (3690, 7230) | 81700 (72000, 92600) |
| $C_{max}$ (ng/mL) [a] | 2080 (1700, 2560) | 110 (97.1, 124) | 228 (1810, 2870) | 761 (509, 1140) | 297 (250, 352) | 2160 (1970, 2360) |
| $t_{max}$ (h) [b] | 0.25 (0.25, 0.25) | 3.50 (0.50, 4.00) | 0.25 (0.25, 0.25) | 1.00 (0.50, 2.00) | 2.02 (1.00, 4.00) | 2.50 (1.00, 4.00) |
| $t_{1/2}$ (h) | 15.6 (11.2, 21.8) | 16.9 (11.9, 24.1) | 107 (74.9, 153) | 17.0 (11.5, 25.1) | 17.4 (11.0, 27.5) | 78.6 (68.0, 91.0) |
| CL (L/h) | 12.9 (10.1, 16.4) | - | 0.887 (0.610, 1.29) | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Vdss (L) | 194 (156, 240) | - | 136 (114, 162) | - | - | - |

[a] Concentration units for total radioactivity are ng-eq/mL.

[b] Median (range)

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**TABLE 3**

*Mean (n=6) percentage of radioactivity of casopitant and its relevant metabolites in human plasma after a single-dose IV and oral administration at target doses of 90 and 150 mg of [$^{14}$C]-casopitant, respectively*

| Metabolite I.D. | Mean % of plasma radioactivity [a] | | | |
|---|---|---|---|---|
| | IV administration | | Oral administration | |
| | 0.25 h | 6 h | 1 h | 16 h |
| Casopitant | 92.2 | 38.8 | 44.9 | 14.1 |
| M13 | BQL | 13.4 | 13.4 | 12.2 |
| M12 | BQL | BQL | 8.3 | 1.9 |
| M2 | ND | ND | 1.5 | BQL |
| M3 [b] | ND | ND | 3.1 | BQL |
| M10 [b] | ND | ND | 2.6 | 0.9 |
| M31+M134 | ND | ND | 1.2 | BQL |
| M76+M111 | 1.8 | BQL | ND | ND |
| M156 [b] | ND | ND | 1.0 | 7.4 |

*a* Observed metabolite radioactivity was determined by 96-well fraction collection with scintillation counting for 5 minutes after a chromatographic separation was performed by HPLC. Percentages obtained from the radio-chromatogram have been adjusted for the extraction recovery. Mean (n=6) percentage of radioactivity per time point does not equal 100% since only distinct radioactive peaks were assigned values and a few minor metabolites were not reported.

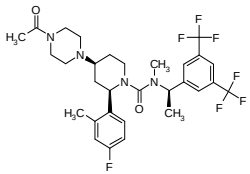
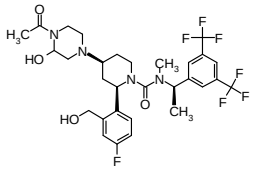
*b* The quantification was affected by at least one minor co-eluting metabolite

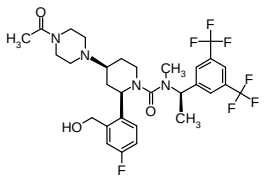
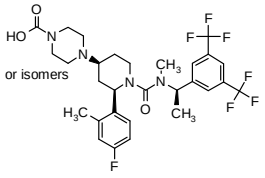
BQL = below quantification limit, set to 25 cpm as peak area

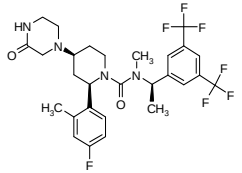
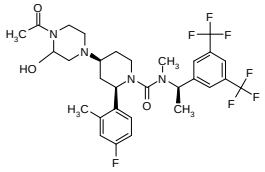
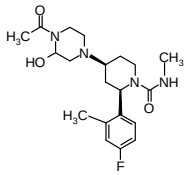
ND = not detected

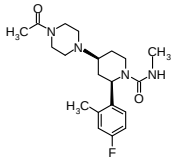
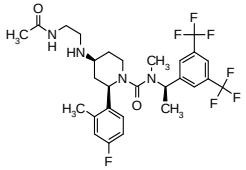
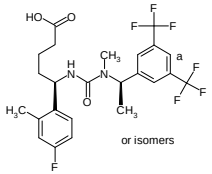
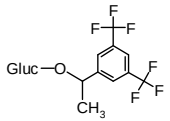
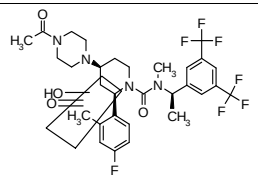
## TABLE 4

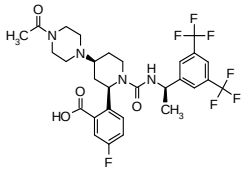
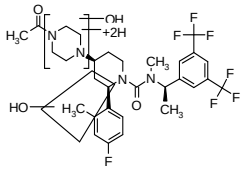
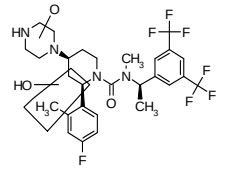
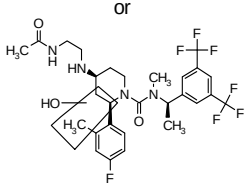
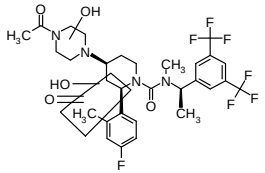
*Relevant metabolites of [$^{14}$C]-casopitant in human plasma, urine and feces after a single-dose IV (90 mg, 35 $\mu$Ci ) and oral (150 mg, 75 $\mu$Ci ) administration of [$^{14}$C]-casopitant*

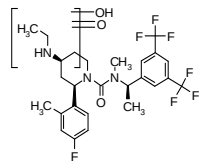
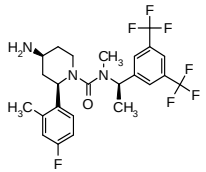
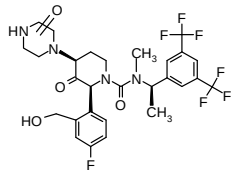
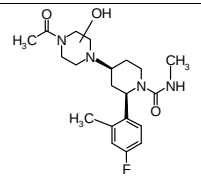
| **Matrix** | **Proposed Structure** | **Positive [M+H]+ or Negative [M+H]-ion MS Products Ions (m/z)** | **$^{1}$H-NMR (600Mhz, 1:1 ACN:$D_2O$) (where available)** |
|---|---|---|---|
| PL, FE |  Casopitant | 617 $[M+H]^+$<br>489, 481, 346, 327, 298, 277, 241, 210, 167 | 7.89 (brs, 1H), 7.59 (brs, 2H), 7.21 (dd, 1H), 6.88 (dd, 1H) 6.76 (dt, 1H), 5.32 (q, 1H, *NCHCH$_3$*), 4.23 (m, 1H), 3.49 (m, 1H), 2.86 (m, 1H), 2.69 (s, 3H, *NCH$_3$*), 2.34 (s, 3H), 1.46 (d, 3H, *CHCH$_3$*), several piperidine and piperazine signals v. broad. |
| PL |  M2 | 649 $[M+H]^+$<br>631, 479, 291, 245, 241, 208, 183, 173, 165 | 7.90 (brs, 1H), 7.60 (brs, 2H), 7.25 (m, 1H), 7.07 (dd, 1H) 6.83 (m, 1H), 5.78 and 5.38 (m, 1H, rotamers, |

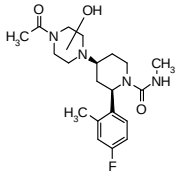
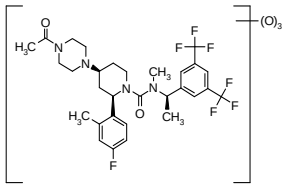
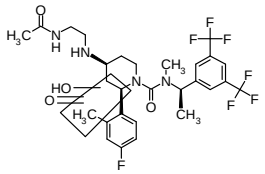
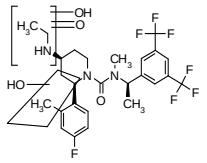
| | | | |
|---|---|---|---|
| | | | *CHOH*) 5.35 (q, 1H), 4.75 and 4.65 (d, 1H each, *$CH_2OH$*), 3.44 (m, 1H), 2.81 (m, 1H), 2.65 (s, 3H), 1.46 (d, 3H), several signals broad and/or obscured. |
| PL, FE |  M3 | 633 $[M+H]^+$<br>615, 505, 481, 369, 362, 241, 210, 173, 167 | 7.90 (brs, 1H), 7.60 (brs, 2H), 7.25 (dd, 1H), 7.07 (dd, 1H) 6.84 (dt, 1H), 5.35 (q, 1H), 4.74 and 4.64 (d, 1H each, *$CH_2OH$*), 2.65 (s, 3H), 1.46 (d, 3H), several signals broad and/or obscured. |
| PL, FE |  M10 | 619 $[M+H]^+$<br>489, 327, 298, 241, 175 | - |

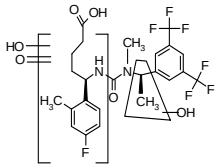
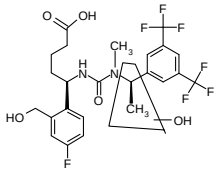
| | | | |
|---|---|---|---|
| PL |  GSK631832 (M12) | 589 $[M+H]^+$<br>489, 453, 249, 241, 182, 139 | - |
| PL |  GW525060 (M13) | 633 [M+H]+<br>615, 497, 489, 479, 461, 275, 183, 165 | 7.88 (brs, 1H), 7.59 (brs, 2H), 7.16 (m, 1H), 6.84 (dd, 1H) 6.72 (m, 1H), 5.81 and obscured (m, 1H, rotamers, *CHOH*) 5.35, several signals broad and/or obscured. |
| UR |  M21 | 393 $[M+H]^+$<br>MS/MS of $[M+H]^+$ - $H_2O$: 344, 275, 239, 208, 165 | 7.20 (dd, 1H), 6.92-6.98 (m, 2H) , 5.74 and 5.39 (m, 1H, rotamers, *CHOH*), 2.49 (s, 3H), 2.34 (s, 3H) several signals broad and/or obscured. |

| | | | |
|---|---|---|---|
| UR |  M28 | 377 $[M+H]^+$<br>241, 210, 167 | 7.21 (dd, 1H), 6.92-6.97 (m, 2H) , 2.49 (s, 3H), 2.34 (s, 3H) several signals broad and/or obscured. |
| PL, FE |  GSK517142 (M31) | 591 $[M+H]^+$<br>489, 455, 327, 320, 251, 241, 184, 175, 141 | - |
| PL, FE |  M39 | 523 $[M+H]^+$<br>315, 241, 209, 208, 191, 149 | 7.89 (brs 1H), 7.75 (brs, 2H), 7.20 (dd, 1H), 6.89-6.83 (m, 2H) , 2.62 (s, 3H), several signals broad and/or obscured. |
| PL, UR |  GSK1735004 (M44) | 433 $[M-H]^-$<br>415, 241, 213, 175 | 7.91 (brs, 2H), 8.03 (brs, 1H), 5.18 (q, 1H) 4.09 (d, 1H, anomeric)1.45 (d, 3H) |
| FE |  M50 | 647 $[M+H]^+$<br>332, 272, 241, 210, 204, 167 | - |

| | | | |
|---|---|---|---|
| FE |  M63 | 633 $[M+H]^+$<br>467, 350, 332, 290, 222, 210, 204, 167 | - |
| FE |  M73 | 651 $[M+H]^+$<br>633, 505, 499, 380, 369, 327, 298, 241, 228, 185 | - |
| PL, FE |  or  M77 | 607 $[M+H]^+$<br>589, 455, 336, 241, 190, 184, 173, 141 | - |
| FE |  M80 | 663 $[M+H]^+$<br>645, 330, 305, 241, 208, 204 | - |

| | | | |
|---|---|---|---|
| FE |  M123 | 564 $[M+H]^+$<br>489, 428, 327, 315, 298, 272, 241, 224, 175, 157, 114 | - |
| PL, FE |  GSK2110277 (M134) | 506 $[M+H]^+$<br>370, 315, 298, 241, 175 | - |
| FE |  M137 | 619 $[M+H]^+$<br>304, 286, 276, 272, 241, 204, 182 | 7.91 (brs, 1H), 7.61 (brs, 2H), 7.26 (dd, 1H), 7.07 (dd, 1H) 6.84 (dt, 1H), 5.35 (q, 1H), 4.74 and 4.66 (d, 1H each, *$CH_2OH$*), 2.62 (s, 3H), several signals broad and/or obscured. |
| UR |  M138 | 393 $[M+H]^+$<br>MS/MS of $[M+H]^+$ - $H_2O$ ion: 344, 275, 239, 208, 165 | -- |

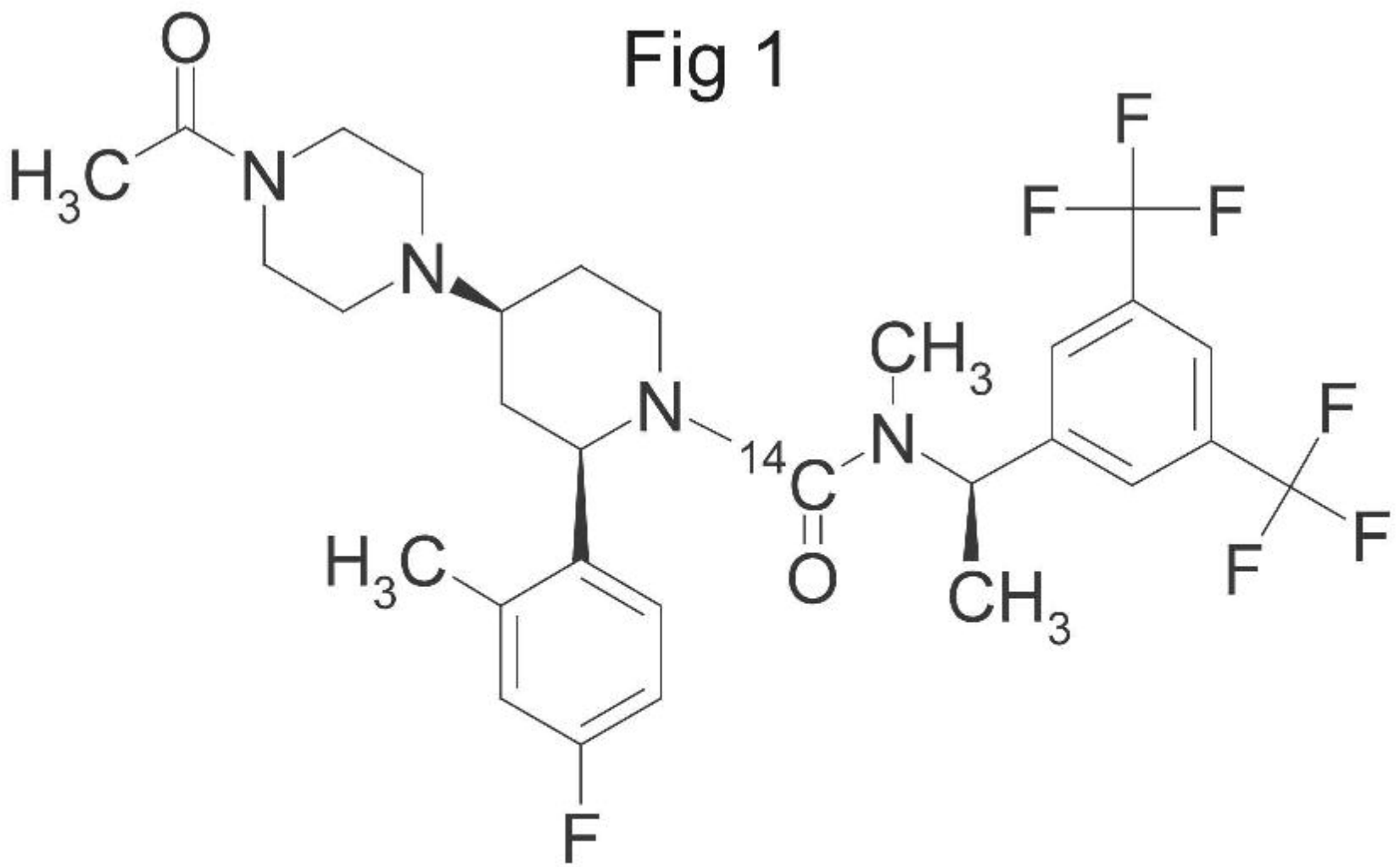
| | | | |
|---|---|---|---|
| UR |  M139 | 393 $[M+H]^+$<br>MS/MS of $[M+H]^+$ - $H_2O$ ion: 275, 239, 208, 165 | - |
| FE |  M140 | 665 $[M+H]^+$<br>No MS/MS Data | - |
| FE |  M141 | 621 $[M+H]^+$<br>350, 306, 290, 241, 184, 141 | - |
| FE |  M146 | 580 $[M+H]^+$<br>562, 241, 173, 157, 114 | - |

| | | | |
|---|---|---|---|
| PL |  M156 | 567 $[M-H]^-$<br>314, 296, 253, 208 | - |
| PL, FE |  <u>M157</u> | 553 $[M-H]^-$<br>314, 282, 270, 213, 194, 163 | 7.90 (brs, 1H), 7.61 (brs, 2H), 7.24 (dd, 1H), 7.07 (dd, 1H) 6.84 (dt, 1H), 4.74 and 4.65 (d, 1H each, *$CH_2OH$*), 2.65 (s, 3H), several signals broad and/or obscured. |

PL = plasma, UR = urine and FE = feces

The underlined metabolites in the table have been confirmed with either NMR or comparison with standard

Fig 1




$^{14}$C-casopitant

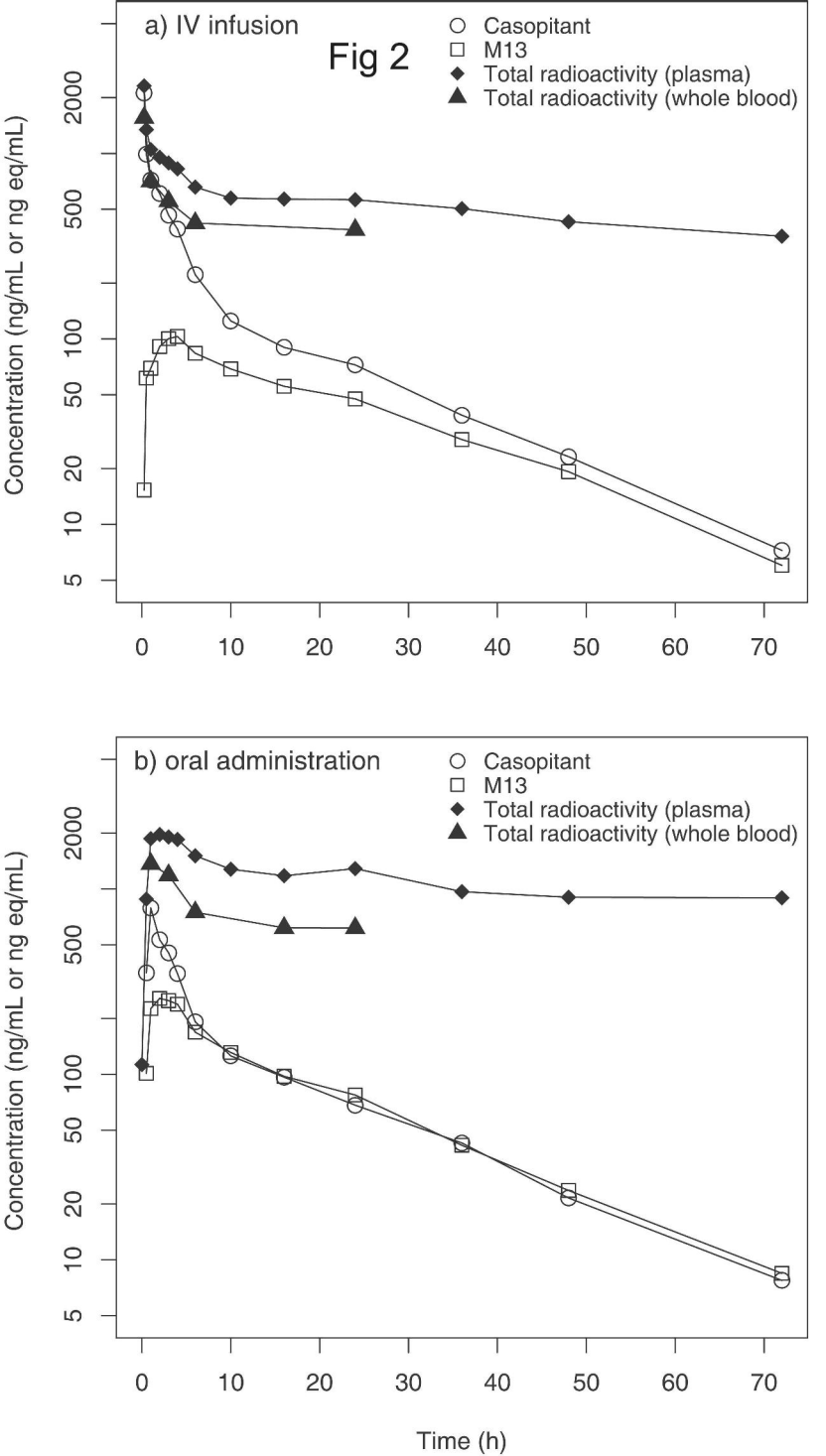
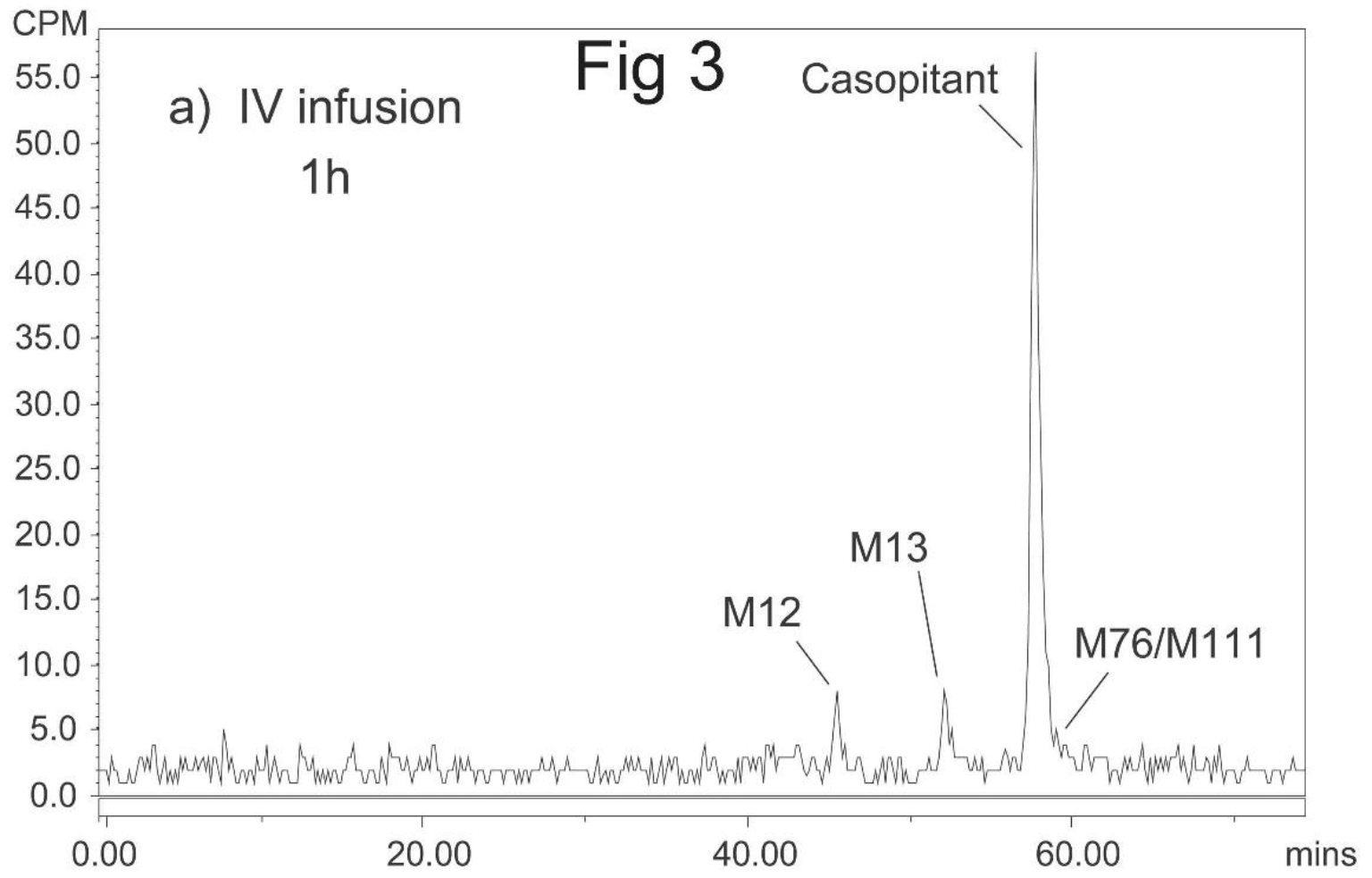

a) IV infusion
Fig 2
Casopitant
M13
Total radioactivity (plasma)
Total radioactivity (whole blood)
Concentration (ng/mL or ng eq/mL)
5
10
20
50
100
500
2000
0
10
20
30
40
50
60
70
b) oral administration
Casopitant
M13
Total radioactivity (plasma)
Total radioactivity (whole blood)
Concentration (ng/mL or ng eq/mL)
5
10
20
50
100
500
2000
0
10
20
30
40
50
60
70
Time (h)

Fig 3


CPM
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
a) IV infusion
1h
Casopitant
M13
M12
M76/M111
0.00
20.00
40.00
60.00
mins

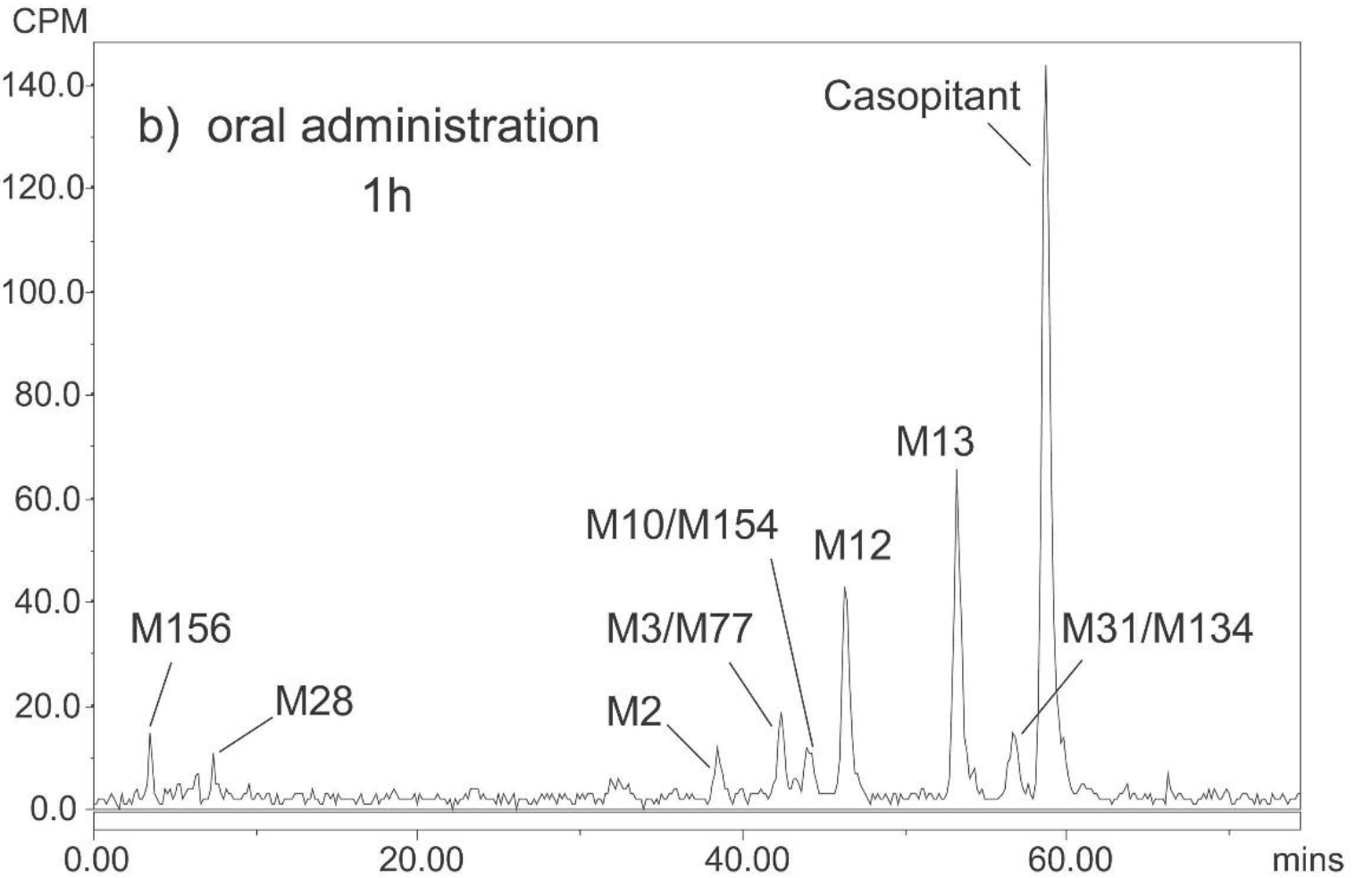
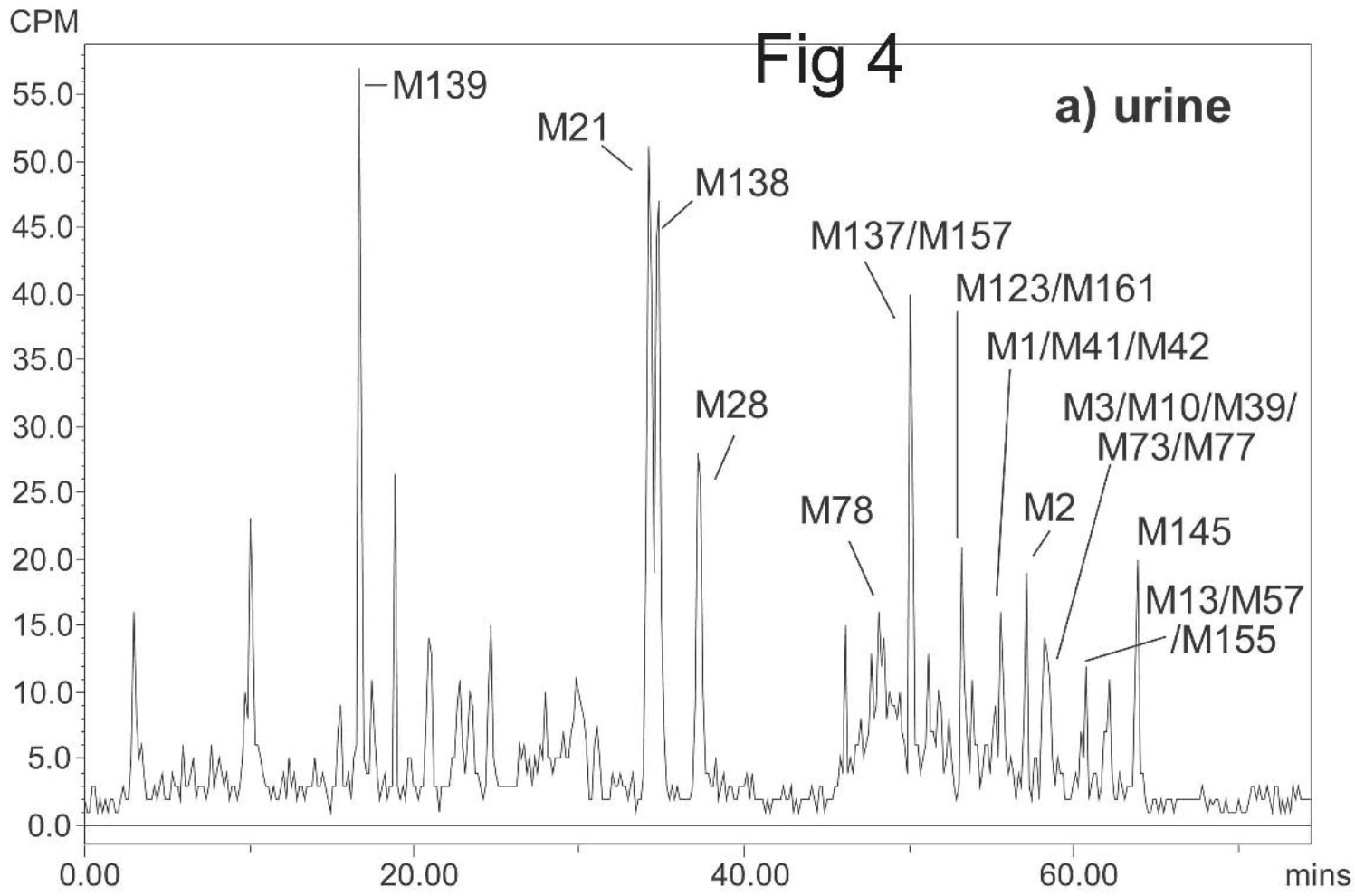
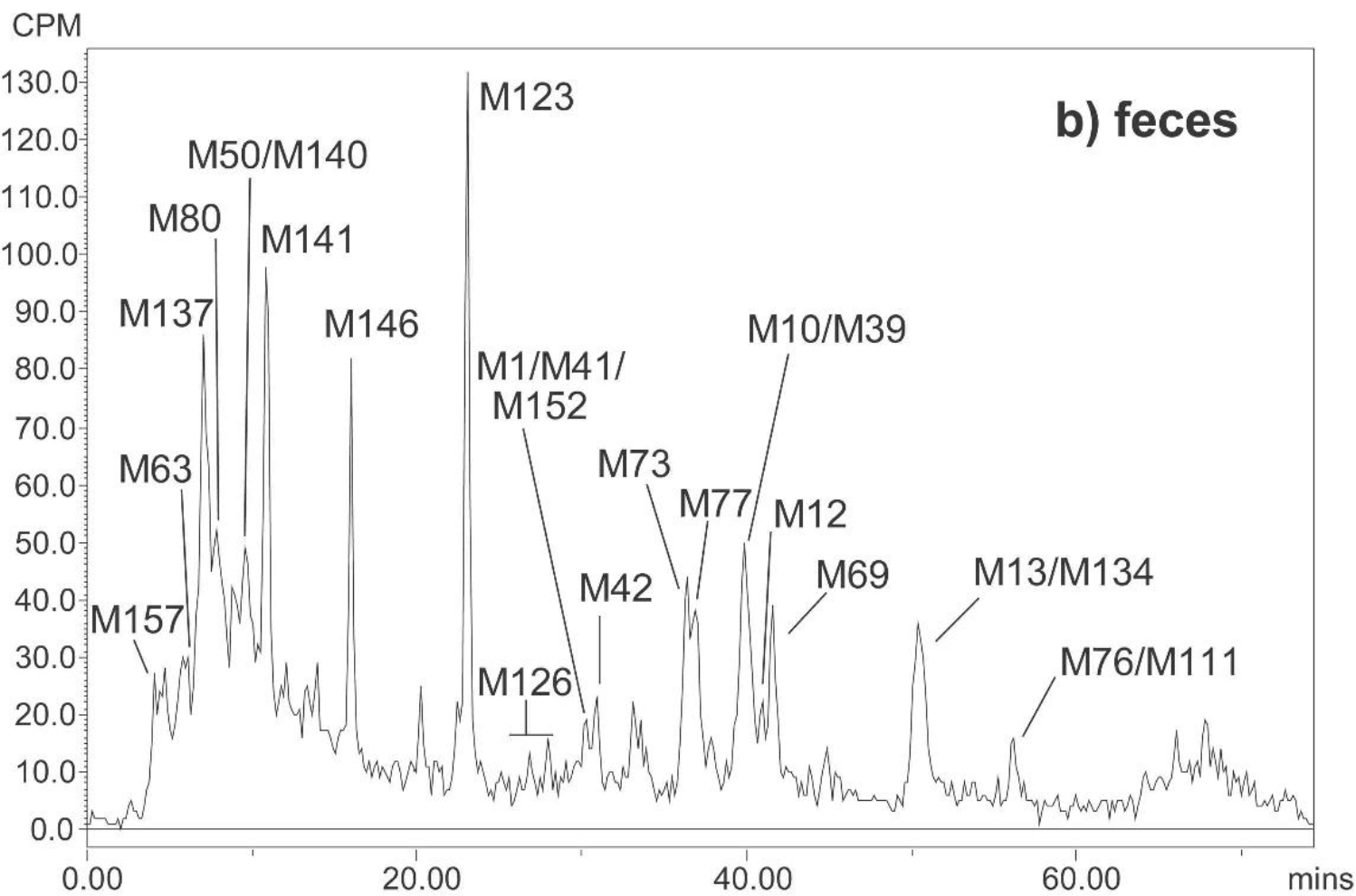
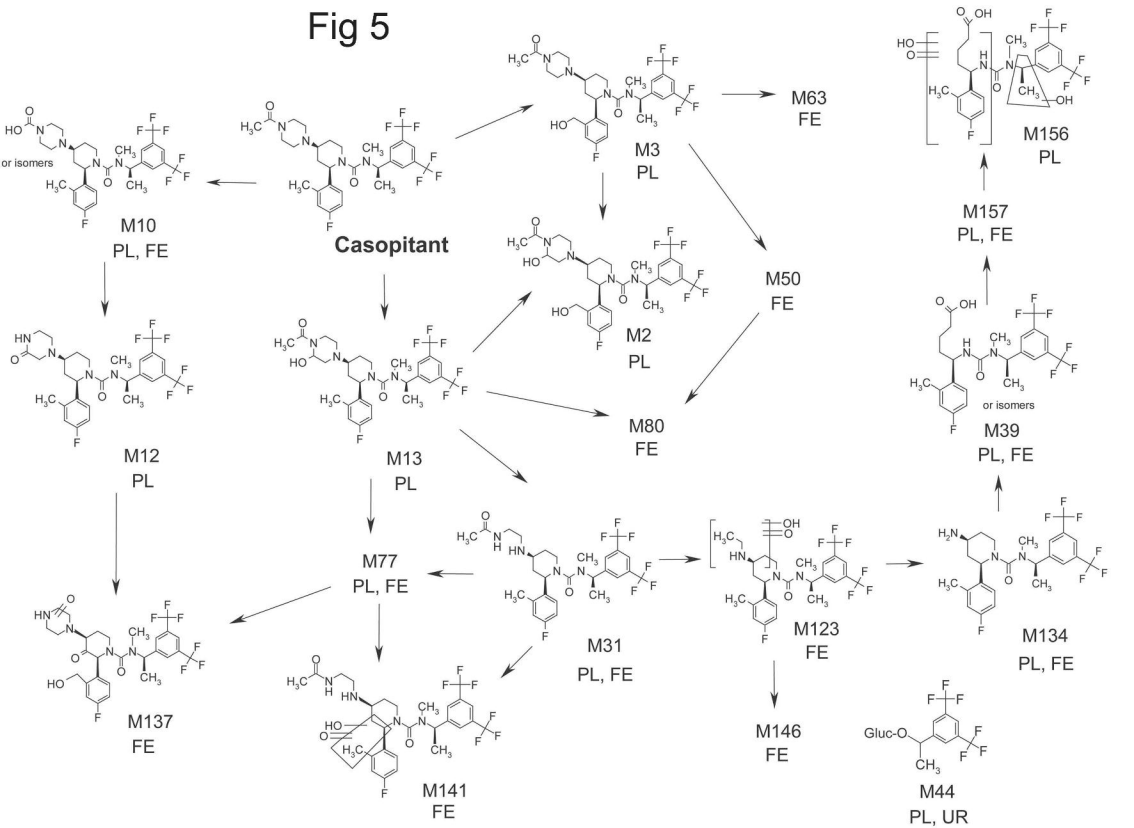

CPM
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
b) oral administration
1h
Casopitant
M13
M10/M154
M12
M156
M3/M77
M31/M134
M28
M2
0.00
20.00
40.00
60.00
mins

Fig 4


CPM
a) urine
M139
M21
M138
M137/M157
M123/M161
M1/M41/M42
M28
M3/M10/M39/
M73/M77
M78
M2
M145
M13/M57
/M155
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
55.0
0.00
20.00
40.00
60.00
mins


CPM
b) feces
M123
M50/M140
M80
M141
M137
M146
M10/M39
M1/M41/
M152
M63
M73
M77
M12
M69
M13/M134
M42
M157
M126
M76/M111
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
0.00
20.00
40.00
60.00
mins

# Fig 5




Key: PL = plasma, FE = feces, UR = urine

Major drug-related component in urine