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PHARMACOKINETICS, DISPOSITION, AND METABOLISM OF BICIFADINE IN HUMANS

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Running Title: Metabolism of Bicifadine in Humans

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BIcifadine, DOV 220,075 [(±)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane HCl]; DOV 255,828, 5-(4-methylphenyl)-3-azabicyclo[3.1.0]hexan-2-one; amu, atomic mass unit; COX, cyclooxygenase; DA, dopamine; equiv, equivalents; HPLC, high performance liquid chromatography; IC_{50}, concentration that produces 50%

inhibition; LC/MS, liquid chromatography/mass spectrometry; MAO, monoamine oxidase; *m/z*, mass to charge ratio; NE, norepinephrine; R_t, HPLC column retention time; 5-HT, serotonin; C_{max}, maximum plasma concentration; T_{max}, time to reach maximum plasma concentration; AUC_{0-t}, area under the plasma concentration versus time curve from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration versus time curve from time zero to infinity; λ_z, terminal phase rate constant; t½, eliminate phase half-life; V_z,/F, terminal phase apparent oral volume of distribution; CL/F, apparent oral clearance.

ABSTRACT

Bicifadine [DOV 220,075; (±)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane HCl)] is a non-narcotic analgesic that has proven to be effective for the treatment of acute pain in clinical studies. The pharmacokinetics, disposition, and metabolism of bicifadine were determined in 8 healthy adult male subjects following a single oral dose of 200 mg [^{14}C]bicifadine in solution. The maximum concentration of total drug equivalents and bicifadine in plasma was at approximately 1 h; the elimination half-life was 2.6 and 1.6 h for radioactivity and bicifadine, respectively. Unchanged bicifadine represented 15% of the area under the concentration time curve for total drug equivalents; the rest was due mainly to the lactam (M12), the acid (M3), and the lactam acid (M9). Total recovery of the dose was 92% with most of the radioactivity recovered in the urine in the first 24 h; fecal excretion accounted for only 3.5% of the dose. Approximately 64% of the dose was metabolized to M9 and its acyl glucuronide; another 23% was recovered as M3 and its acyl glucuronide. Neither bicifadine nor M12 were detected in urine or feces. There were no reported serious or severe adverse events during the study.

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Chronic peripheral neuropathic pain is difficult to treat and side effects may limit the usefulness of a drug. While opiates have been used for neuropathic pain management, tolerance develops to their analgesic efficacy, and they cause drowsiness and constipation. Empirical observations have demonstrated the efficacy of tricyclic antidepressants in treating peripheral neuropathy (Sindrup et al., 2005). Research has shown that this effect is produced by inhibition of the neuronal reuptake of NE and 5-HT, as well as blocking the postsynaptic α-adrenergic, H_1-histaminergic, and muscarinic cholinergic receptors. Duloxetine is a selective 5-HT and NE reuptake inhibitor that has been approved for treatment of diabetic neuropathy. Bupropion is a weak blocker of NE and DA, but not 5-HT, reuptake (Ascher et al., 1995) but was effective in reducing neuropathic pain in patients with human immunodeficiency virus (Semenchuck et al., 2001). The involvement of DA in analgesic mechanisms suggests that a broadened analgesic range may result from the ability to enhance dopaminergic neurotransmission, in addition to activating serotonergic and noradrenergic pathways. Thus, agents which simultaneously inhibit DA, NE, and 5-HT transporters may possess a unique analgesic profile,

Bicifadine [(±)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane HCl; DOV 220,075, Figure 1] is being developed for the treatment neuropathic pain. It inhibits NE (IC_{50} = 55 nM) and 5-HT (117 nM) reuptake, with lesser potency in blocking DA reuptake (910 nM), as determined using recombinant human transporter systems (Basile et al., 2007). *In vivo* microdialysis studies in the brains of rats indicate that bicifadine increases the extracellular levels of all three

neurotransmitters following oral administration of analgesic doses (Basile et al., 2007). Bicifadine was an effective antinociceptive agent in both the early (acute) and late (tonic) phases of paw-licking in the formalin test using rats and mice, unlike duloxetine or COX inhibitors, which are active only on the late stage (Iyengar et al, 2004). It is not an inhibitor of either COX-1 or COX-2 (Basile et al., 2007) and does not induce dependence in either rodents or primates after as much as 48 days of administration (unpublished observations). Clinically, it has been shown to be effective in the treatment of acute dental (Stern et al., 2005) and bunionectomy pain (Riff et al., 2006); studies in humans with diabetic neuropathy are ongoing.

A human metabolism study utilizing [^{14}C]bicifadine was conducted to support its clinical trials and registration. In addition to assaying the plasma concentrations of total drug equivalents and unchanged bicifadine, the mass balance of drug-derived radioactivity in the urine and feces was determined. Metabolites in the plasma, urine, and feces were identified and compared with those formed *in vitro* (Erickson et al., 2007) and in the species used for toxicology studies (Musick et al., 2007).

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MATERIALS AND METHODS

Radiolabeled Bicifadine and Dosage Form. [^{14}C]Bicifadine was synthesized by Vitrax Company (Placentia, CA) and supplied as a bulk powder with a specific activity of 0.52 μCi/mg. The radiochemical purity was 99.9% as determined by HPLC. The compound was formulated by Covance Clinical Research on the morning of dosing. [^{14}C]Bicifadine powder was dissolved in sterile water for irrigation to produce a solution with a concentration of 91.9 mg/g. The appropriate volume was divided into three 00 capsules, which were then double-encapsulated in 000 capsules. The capsules were administered within 20 min of preparation. The mean dose and amount of radioactivity administered was 201 mg (range 201-202) and 105 μCi (range 104-105 μCi), respectively.

Subjects and Dose Administration. The study was reviewed and approved by the Covance Clinical Research Unit Institutional Review Board. Informed consent was obtained from each volunteer prior to study initiation. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

Eight healthy male subjects (7 Caucasian and 1 African-American) participated in the study. They had a mean age of 26.4 years (range 19 to 43 years), a mean weight of 79.4 kg (range 61.3 to 93.2 kg), a mean body mass index of 25.5 kg/m^2 (range 20.2 to 28.6 kg/m^2), and met all of the eligibility criteria. Subjects were in good health based on their medical history, a physical examination, a 12-lead electrocardiogram, and clinical laboratory test results.

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The mean hematocrit was 44 ± 1%. After an overnight fast, the subjects ingested the [^{14}C]bicifadine in 3 capsules with 240 ml of water. They were fed a standard lunch approximately 4 h after dosing. The subjects were confined to the study site until at least 168 h postdose, at least 90% of the administered radioactivity was recovered, or until their excreta contained non-detectable amounts of radioactivity for at least 48 h. Samples were collected for 168 h for 5 subjects and 192 h for the other 3 subjects.

Sample Collection. Blood samples (10 ml) were collected into chilled, heparinized tubes at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 192 h postdose. Additional blood samples were collected at 0.5, 2, 4, and 6 h postdose for plasma metabolite profiling and identification. Before centrifugation, a 2-ml sample was saved for analysis of radioactivity in whole blood. The rest was centrifuged within 45 min of collection in a refrigerated centrifuge and the plasma was divided into two tubes. One sample was used for the determination of the plasma radioactivity concentration; the other was for analysis of concentrations of bicifadine and the lactam metabolite, M12 (DOV 255,828). Plasma and blood samples were stored at -70ºC.

Urine was collected predose and at intervals of 0-4 h, 4 to 8 h, 8 to 24 h, and at 24-h intervals thereafter up to 192 h postdose. Samples were refrigerated until the end of the block collection periods when the total volume of each urine block was recorded. Samples were stored at -70ºC.

All bowel movements and fecal wipes were collected before administration of [^{14}C]bicifadine and at 24-h intervals up to 192 h postdose. The fecal samples

were refrigerated until assayed for total radioactivity; the wipes were not assayed. After homogenization and sampling, the remainder of the sample was stored at approximately -20ºC until extracted for metabolite profiling.

Radioactivity Assay. All samples were assayed on a daily basis for total radioactivity by liquid scintillation counting using a Packard Model 2900TR liquid scintillation counter (Packard Instrument Co., Meriden, CT). Plasma and urine samples (0.2 ml) were added to Ultima Gold XR scintillation cocktail and counted. Blood and fecal homogenates (0.2 g) were oxidized in a Packard Model 307 Sample Oxidizer; the resulting $^{14}CO_2$ was trapped in a mixture of Perma Fluor and Carbo-Sorb (Perkin Elmer, Inc., Waltham, MA). Samples with less than 2 times the background dpm were recorded as zero.

Bioanalytical Assay. The analysis of plasma samples for bicifadine and M12 (DOV 255,828) were performed using a validated liquid chromatography with tandem mass spectrometery assay as detailed in the accompanying report (Musick et al., 2007). The only changes were that 0.5 ml of plasma was assayed and a Symbiosis Pharma System (Spark Holland, Inc., Plainsboro, NJ) was used. The latter consisted of a Symbiosis Pharma LC system, Reliance Autosampler, and an Applied Biosystems API 4000™ tandem quadrupole MS with a heated nebulizer probe.

Pharmacokinetic Analysis. Blood and plasma concentrations of radioactivity, bicifadine, and M12 above their respective lower limit of quantitation were used for pharmacokinetic analyses using noncompartmental methods (Gibaldi and Perrier, 1982). WinNonlin, version 4.1 (Pharsight Corp, Mountain View, CA) was

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used for the calculations. The values of C_{max} and T_{max} were the observed values for each subject. AUC_{0-t} was determined using the linear trapezoidal rule whenever the concentration data were increasing and the logarithmic trapezoidal rule any time that the concentration data were decreasing. The apparent λ_z was calculated as the negative slope of the log-linear terminal portion of the blood concentration-time curve using linear regression. A minimum of 3 observations was used to calculate λ_z. The t½ was calculated as ln $2/\lambda_z$. AUC_{0-t} was extrapolated to infinity ($AUC_{0-\infty}$) as $AUC_{0-\infty} = AUC_{0-t} + C_{last}/\lambda_z$, where C_{last} was the last quantifiable concentration. CL/F of bicifadine was calculated as Dose/$AUC_{0-\infty}$. V_z/F was calculated as Dose/(λ_z x $AUC_{0-\infty}$). Variation around the mean is expressed as the standard deviation.

Metabolite Profiling and Identification. Plasma samples (approximately 0.6-1.3 ml) collected from each subject at 0.5, 2, 4, and 6 h postdose were combined to provide a single pooled plasma sample at each time point. Each pool was extracted twice with 3 volumes of acetonitrile and centrifuged. Extraction recoveries ranged from 82.6 to 131%. Each supernatant was evaporated to dryness under a stream of nitrogen and reconstituted in water:acetonitrile (v:v, 1:1). The supernatant was dried again and reconstituted in water. Urine samples collected from 0 to 24 h postdose were pooled such that approximately 0.2% of each urine sample was included to prepare a single 0-24 h pooled urine sample for each subject. A percentage of each individual's pooled urine was combined for a single overall pooled urine sample for metabolite profiling. After clarification by centrifugation through a centrifugal filtration device, recovery of

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radioactivity was 102%. Approximately 3% of each fecal homogenate was included in the pools for each individual such that they represented greater than 80% of the amount excreted into feces. Due to differences in the rate of fecal excretion, pooled samples from individuals were profiled separately. Samples were extracted twice with 3 volumes of acetonitrile and centrifuged. The extracts were combined and analyzed by LSC; extraction efficiencies were 74 to 83%. Each supernatant was evaporated to dryness under a stream of nitrogen and reconstituted in water:acetonitrile (v:v, 1:1). For all samples, extraction and recovery efficiencies were determined by liquid scintillation counting and corrections were made to subsequent data.

HPLC and Radiometric Detection. The HPLC system used for profiling of metabolites consisted of an HP 1100 series pump, autoinjector, column heater, and ultraviolet detector set to 254 nm (Hewlett Packard, Palo Alto, CA). The column was a Zorbax SB-phenyl column (250 x 4.6 mm, 5 μm particle size; Agilent Corp., Foster City, CA) with a Phenomenex propylphenyl guard column (4 mm x 3 mm; Phenomenex, Inc., Torrance, CA). The initial mobile phase was 95% 0.05% formic acid in reverse osmosis water/5% acetonitrile. The percentage of acetonitrile increased linearly to 25% over 45 min and then increased linearly to 95% over the next 5 min, where it was held for 6 min. The column was re-equilibrated to the initial conditions over the next 11 min. The flow rate was 1.0 ml/min. Column fractions were collected every 10 sec for 62 min using an ISCO Foxy 200 fraction collector (Cole-Parmer Instrument Co., Vernon Hills, IL). The fractions were analyzed using a TopCount Microplate

Scintillation and Luminescence counter (Packard Instrument Co.). To determine the percentage of the total dose in each radioactive metabolite from the pooled urine and fecal extracts, the percentage of radioactivity that eluted in each peak was multiplied by the percentage of administered radioactivity excreted in the pooled sample and corrected for extraction and reconstitution recoveries.

Full scan LC/MS analyses were conducted using an Applied Biosystems 4000 Q Trap with a turbospray source (Foster City, CA). The mass spectrometer software was Analyst 1.4.1 from Applied Biosystems. The HPLC system was a Shimadzu model SIL-HTc autoinjector and system controller, and Shimadzu Model LC-10AD VP pumps (Columbia, MD). A Radiomatic Series 500 with Flo-One software, version 3.65, (Packard Instruments) was used for radiochemical detection. The HPLC column, solvents and gradient were the same as used for metabolite profiling. After passing through the column switcher, the column effluent was split with approximately 25% diverted to the mass spectrometer and 75% to the radiometric detector. To minimize contamination of the mass spectrometer source, the first 4.5 min of each run was diverted to waste. Samples were scanned in the positive ion full-scan mode from 80 to 700 amu with a Q1 scan time of 0.8 sec for 52 min. The ion spray voltage was 5000 V, the source temperature was 500ºC, and the exit potential was 10 V. Nitrogen was used as the curtain, nebulizer (GS1), and turbo (GS2) gas. Product ion analyses used the same instrumentation and conditions as LC/MS with the following exceptions: enhanced product ion was used for the scan function; the ionization mode was positive turbo spray; nitrogen was used as the collision gas;

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and the collision energy, mass range, and scan time were variable. The contribution of the ^{14}C-tracer to the mass of the metabolites was low enough (ca. 0.15%) so that it did not contribute to the *m/z* ratios as determined by mass spectrometry. For consistency across studies, the metabolite numbering system is the same as that used in the *in vitro* and nonclinical metabolism studies (Erickson et al., 2007; Musick et al., 2007).

The high resolution mass spectrum of the ion at the nominal *m/z* 135 in M9 was obtained using a Thermo LTZ/Orbitrap Fourier transform mass spectrometer (Thermo Fisher Scientific, Inc.,Waltham, MA). A solution of M9 was infused using an electrospray ionization source. The product ion spectrum was obtained at a resolution of 60,000 and using a lock mass of 218.08117 amu (protonated molecular ion of M9).

RESULTS

Pharmacokinetics of Radioactivity, Bicifadine, and M12. The mean concentrations of radioactivity in blood and plasma concentrations of radioactivity, bicifadine, and M12 (DOV 255,828) following a single oral dose of [^{4}C]bicifadine in solution are presented in Figure 2. C_{max} of radioactivity in plasma was 5154 ng equiv·hr/g at 1.19 h (Table 1). Its t½ was approximately 2.6 h; the last measurable concentration of plasma radioactivity was at 12.0 h postdose. C_{max} and $AUC_{0\text{-}t}$ values of radioactivity in blood were 53-54% of the values in plasma, indicating that bicifadine and its metabolites did not concentrate in red blood cells. The subjects' mean hematocrit of 44% is in

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agreement with these results.

The C_{max} of bicifadine, 1780 ng/ml, was observed at 1.06 h postdose (Table 1). Its AUC_{0-t} was 3373 ng·hr/ml, approximately 15% of the total drug equivalents in plasma. Both the t½ and the time to the last measurable concentration of plasma bicifadine were slightly shorter than for plasma radioactivity. CL/F was 59.1 l/h and V_z/F was 125 l; based on the weight of the subjects, CL/F was 0.74 ± 0.28 l/h/kg and V_z/F was 1.58 ± 0.32 l/kg. The lactam metabolite, M12, had a C_{max} that was slightly lower than that of bicifadine and a T_{max} that occurred later. While exposure to M12 was approximately 50% higher compared to that of bicifadine, their t½ values were similar.

Excretion of Radioactivity. Almost the entire orally-ingested radioactivity was excreted into urine (Figure 3). By 24 h postdose, 87.3 ± 5.3% of the dose was recovered in the urine; another 1.5% was excreted over the next 72 h. Only 3.52% of the dose was excreted into the feces over the collection period. Overall recovery of radioactivity was 92.3 ± 5.2% (range = 88.1-104%). Two subjects have recoveries that were slightly below 90% (88.1% and 89.4%); neither had detectable amounts of radioactivity in their feces and urine from 96 h to 192 h postdose.

Metabolite Profiling. *Plasma.* The pooled plasma contained 4 main radioactive peaks (Figure 4). The highest concentration of bicifadine occurred at 0.5 h, and then declined to nondetectable levels by 6 h postdose (Table 2). The main metabolites identified were the acid M3, the lactam acid M9, and the lactam M12. With the exception of M3 at 0.5 h, all of the metabolites had concentrations that

were higher than bicifadine. The highest concentration of the metabolites was at 2 or 4 h, and declined by 6 h. These data are consistent with the results using the validated assay that indicated that most of the plasma radioactivity was due to metabolites rather than bicifadine. A number of smaller radioactive peaks were detected in the chromatograms with concentrations that were too low to obtain reliable mass spectra. All of them had concentrations that were ≤ 31 ng equiv/g plasma with the exception of a peak at R_t 51.5 min, which had a concentration of 63.5 ng equiv/g plasma at 0.5 h.

Urine. The major urinary metabolite was the lactam acid M9; it constituted 55.6% of the administered radioactivity (Table 3, Figure 5). A series of smaller peaks that corresponded to the acyl glucuronide of M9 and its rearrangement products eluted with R_t's ranging from 18.8 to 23.2 min. These peaks represented 7.3% of the dose and, together with M9, accounted for 62.9% of the dose. The other carboxylic acid-containing metabolite, M3, and its acyl glucuronide(s), M14A-F, were also detected in urine. Combined, M3 and its conjugate accounted for another 22.4% of the dose. A smaller unidentified peak, M32, eluted at R_t 31.7 min, but it represented only 0.57% of the dose. Unchanged bicifadine and the lactam M12 were not detectable in the pooled urine.

Feces. M3 and M9 represented most of the radioactivity in feces (0.85% and 1.34% of the dose, respectively; Table 3, Figure 5). Two other unidentified metabolites, with R_t's of 46.0 and 50.6 min, accounted for <0.4% of the dose.

Metabolite Structure Identification. The proposed metabolic scheme for bicifadine in humans is displayed in Figure 6.

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Bicifadine, M9, and M12. The product ion mass spectra of bicifadine, M9, and M12 in the pooled plasma collected 4 h after the oral dose of [^{14}C]bicifadine were essentially identical to the mass spectra of the corresponding standards (Table 4). The HPLC retention times of the plasma peaks were also the same as the standards.

The high resolution mass spectrum of the ion at *m/z* 135 in the product ion mass spectrum of M9 was also determined. The fragment had an observed amu of 135.04405; the calculated amu for $C_8H_7O_2^+$ is 135.04406. The difference between the calculated and observed amu was 0.07 ppm. This empirical formula corresponds to an ion with the structure HO_2C-phenyl-CH_2^+. Two other possible formulas with a nominal amu of 135 were $C_9H_{11}O_2^+$ and $C_8H_9NO^+$ but their calculated molecular weights were 269 and 176 ppm higher, respectively, than the observed. A similar fragmentation mechanism was observed for bicifadine (*m/z* 105), M3 (*m/z* 135) (Figure 7), and M2 (*m/z* 121) (Musick et al., 2007).

M3 (bicifadine carboxylic acid). The full scan LC/MS mass spectrum of M3 had a $[M+H]^+$ at *m/z* 204, 30 amu (+2 oxygen - 2 hydrogen) higher than bicifadine (Figure 7). The presence of ions at *m/z* 135 and 163 in the product ion mass spectrum of M3 indicated that the methyl group had been oxidized to a carboxylic acid.

M14A-F (M3 acyl glucuronides). A series of peaks eluted between 5.24 and 8.42 min in the extract of the 0-24 h pooled urine sample. The $[M+H]^+$ of all of the peaks was at 380 amu, 176 amu (glucuronic acid) higher than M3. The ion at *m/z* 204 was due to the loss of the glucuronic acid moiety. It could not be determined

which of the 6 peaks was the initial 1-O-β-acyl glucuronide.

M31B-F (M9 acyl glucuronides). The metabolites M31B-F (R_t = 18.8 to 23.2 min) all had a $[M+H]^+$ at 394 amu, 176 amu (glucuronic acid) higher than that of M9 (Figure 7). They all had product ions at *m/z* 218 (-glucuronic acid), 200 (*m/z* 218 – water), and 171, which were also detected in the spectrum of M9.

M32, M33, and M34. The urine and feces contained 3 minor metabolites for which mass spectra could be obtained but possible structures were not identified. M32 had a $[M+H]^+$ at 366, 192 amu higher than that of bicifadine. The ions at *m/z* 190 and 105 indicated the loss of glucuronic acid and that the methyl group had not been oxidized, respectively. M33 was detected in fecal samples and had a $[M+H]^+$ of 232, 58 amu higher than that of bicifadine. Its enhanced product ion spectrum had similarities to M3. M34 (R_t = 49.7 min) had a $[M+H]^+$ at *m/z* 174 and enhanced product ion MS that was virtually identical to the bicifadine standard, but its R_t of 49.7 min indicated that it had a different structure. Two other metabolites, constituting <0.4% of the dose, with R_t's of approximately 30 and 50 min were detected in fecal extracts but reliable mass spectra could not be obtained.

Safety. There were 28 adverse events that were either possibly or probably related to the study drug; they were all mild or moderate in severity. There were no clinically significant changes or abnormalities in the clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead electrocardiograms in the study. The compound was overall well tolerated.

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DISCUSSION

When [^{14}C]bicifadine was administered as an oral solution, the C_{max} of both bicifadine and total drug equivalents was approximately 1 h. The elimination half-life of unchanged parent drug was 1.6 h. The material used in clinical trials is a sustained release formulation with a significantly longer half life (unpublished observations).

The oral clearance of bicifadine in this study was 59 l/h. The plasma flow of the human liver is approximately 48 l/h (Davies and Morris, 1993), slightly less than the oral clearance of bicifadine. Extrahepatic clearance of the compound is likely since the main route of metabolism is formation of the lactam metabolite by MAO-B (Erickson et al., 2007), which is widely distributed in the body (Cesura and Pletscher, 1992).

Exposure to unchanged bicifadine, based on values of AUC_{0-t}, accounted for approximately 15% of the total drug-derived radioactivity. The rest of the plasma radioactivity was due predominantly to M12 and the two carboxylic acids, M3 and M9. At 4 h postdose, these two acids were the predominant peaks in plasma. The concentration of M12 had declined by 4 h, while the concentration of bicifadine was minor. No acyl glucuronide conjugate of M3 was detected in human plasma, as it was in mouse (Musick et al., submitted).

Bicifadine was well absorbed with approximately 89% of the dose recovered in the urine. The small amount in the feces was composed only of metabolites. Neither unchanged bicifadine nor M12 were detected in urine or feces.

M9 and its glucuronide accounted for 64.3% of the radioactive dose in the excreta over the 192 h study period, while M3 and its glucuronide represented another 23.3% of the dose. It was not determined which of the M14 peaks was the initial 1-*O*-acyl-β-glucuronide, which are known to rearrange at neutral to slightly basic pH (Compernolle et al., 1987; Hasegawa et al., 1982; Janssen et al., 1982). Monkeys and humans produced the glucuronide conjugate of M9 (M31B-F), but rats and mice did not (Musick et al., 2007).

In conclusion, bicifadine was well absorbed by humans when administered as an oral solution. The T_{max} of radioactivity and bicifadine was approximately 1 h. Most of the drug-derived radioactivity in plasma was due to metabolites, especially the lactam M12, the acid M3, and the lactam acid M9. Most of the radioactivity was recovered in urine in the first 24 h. M9 and its acyl glucuronide conjugate accounted for almost two-thirds of the dose; M3 and its glucuronide represented another 23% of the dose. No human-specific metabolites were identified and the compound was well tolerated by the subjects with no reported serious or severe adverse events.

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Footnote

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Figure Legends

Fig. 1. Structure of bicifadine.

The asterisk indicates the location of the [^{14}C]radiolabel.

Fig. 2. Concentration of radioactivity (plasma and blood), bicifadine, and the lactam metabolite M12 following a single oral dose of 200 mg [^{14}C]bicifadine in solution to healthy adult male subjects (N = 8).

Fig. 3. Cumulative recovery of radioactivity in healthy adult male subjects following a single oral dose of 200 mg [^{14}C]bicifadine to healthy adult male subjects (N = 8).

Fig. 4. HPLC chromatogram of plasma radioactivity from male subjects at 0.5 h (top panel) and 4 h (bottom panel) following a single oral dose of 200 mg [^{14}C]bicifadine.

Both chromatograms are from pooled plasma at the 2 time points.

Fig. 5. HPLC chromatogram of radioactivity in pooled urine (0-24 h) (top panel) and feces (Subject 6, 24-120 h) (bottom panel) from male subjects following a single oral dose of 200 mg [^{14}C]bicifadine.

The urine was a pooled sample from all 8 subjects. Feces from individual subjects were profiled due to differing times for excretion of radioactivity.

Fig. 6. Proposed metabolic scheme of bicifadine in adult male subjects.

Fig. 7. Enhanced product ion mass spectrum of M3 (left panel) and M31B (right panel), an acyl glucuronide conjugate of M9, in pooled plasma collected 4 h after administration of [^{14}C]bicifadine.

The bottom panels are the proposed fragmentation patterns of the two metabolites.

Table 1

Mean Pharmacokinetics of Radioactivity, Bicifadine and M12 in Male Subjects (N = 8) Administered a Single Oral Dose of [^{14}C]Bicifadine in Solution

Parameter (units)	Plasma ^{14}C	Blood ^{14}C	Bicifadine	M12
C_{max} (ng/ml)[a]	5154 ± 683	2716 ± 392	1780 ± 695	1205 ± 362
T_{max} (h)	1.19 ± 0.26	1.25 ± 0.38	1.06 ± 0.42	1.50 ± 0.46
AUC_{0-t} (ng·h/ml)[a]	21,413 ± 3311	11,577 ± 1822	3373 ± 1851	4641 ± 1803
$AUC_{0-\infty}$ (ng·h/ml)	NC	NC	3427 ± 1934	4697 ± 1819
t½ (h)	2.57 ± 0.28	2.62 ± 0.17	1.63 ± 0.56	1.83 ± 0.42
tf (h)	12.0 ± 0.0	12.0 ± 0.0	10.0 ± 2.1	13.5 ± 4.2
CL/F (l/h)	NC	NC	59.1 ± 23.7	NC
V_z/F (l)	NC	NC	125 ± 31.5	NC

a Units for C_{max} and AUC_{0-t} of radioactivity are ng equiv/g and ng equiv·hr/g.

NC Not calculated

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Table 2

Concentration of Bicifadine and Metabolites in Plasma of Male Subjects Following a Single Oral Dose of 200 mg [^{14}C]Bicifadine

	Hour Postdose			
Metabolite	0.5 h[a]	2 h	4 h	6 h
Bicifadine	289	148	37.7	-
M3	38.8	464	614	342
M9	313	922	914	496
M12	508	1210	537	186

a Metabolite concentrations are ng equiv/g plasma and were determined from metabolite profiling of pooled plasma from the 8 subjects.

- Indicates that the metabolite was not detected at that time period.

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Table 3

Percent of Radioactive Dose as Bicifadine Metabolites in Pooled Urine and in Feces of Subjects Administered a Single 200 mg Oral Dose of [^{14}C]Bicifadine

Proposed Identification	Retention Time (min)	Percent of Dose in Urine[a]	Mean Percent of Dose in Feces[b]
M3	12.8	5.11	0.85
M14A-F	4.7-8.0	17.3	-
M9	34.2	55.6	1.34
M31B-F	18.8-23.3	7.33	-
M32	31.7	0.57	-
M33	46.0	-	0.11
M34	49.7	1.75	-
Unknown	50.6	-	0.23

a Percent of dose in urine determined from radiochemical profile of pooled urine from all 8 subjects.
b Percent of dose in feces determined from radiochemical profile of pooled feces for each individual subject using samples that accounted for >80% of fecal radioactivity.
- Indicates that the metabolite was not detected at that time period.

TABLE 4

Major Product Ions of Metabolites of Bicifadine

Metabolite	$[M+H]^+$	Major Fragment Ions (*m/z*)
Bicifadine standard	174	157 (-NH_3), 142, 133 (base peak, -C_2H_3N), 132, 129, 118, 105 (*m/z* 133-C_2H_4), 91 (tropylium ion), 82
M9 standard	218	201 (-NH_3), 200 (-H_2O), 190 (-CO), 182 (-2H_2O), 171, 164, 146, 135 (base peak), 129, 107, 96, 91, 83
M12 standard	188	171 (-NH_3), 170 (-H_2O), 146, 145, 143, 134, 128, 117, 105 (base peak), 96, 79
Bicifadine	174	157 (-NH_3), 142, 133 (base peak, -C_2H_3N), 132, 129, 118, 105 (*m/z* 133-C_2H_4), 91 (tropylium ion), 82
M3	204	187 (-NH_3), 186 (-H_2O), 169, 163 (base peak, -C_2H_3N), 143 (*m/z* 189-CO_2), 135 (*m/z* 163-C_2H_4), 128, 117, 115, 107, 91
M9	218	201 (-NH_3), 200 (-H_2O), 190 (-CO), 182 (-2H_2O), 171, 164, 146, 135 (base peak), 129, 107, 96, 91, 83
M12	188	171 (-NH_3), 170 (-H_2O), 146, 145, 143, 134, 128, 117, 105 (base peak), 96, 79
M14A	380	363 (-NH_3), 362 (-H_2O), 344 (*m/z* 362-H_2O), 339 (-C_2H_3N), 282, 270, 204 (-glucuronic acid), 186 (base peak, *m/z* 204 -H_2O), 169, 163, 157, 143, 128, 119
M14B	380	363 (-NH_3), 362 (-H_2O), 344 (*m/z* 362-H_2O), 339 (-C_2H_3N), 282, 270, 204 (-glucuronic acid), 186 (base peak, *m/z* 204 -H_2O), 175, 169, 163, 157, 143, 128, 119
M14C	380	363 (-NH_3), 362 (-H_2O), 344 (*m/z* 362-H_2O), 339 (-C_2H_3N), 282, 270, 204 (-glucuronic acid), 187 (base peak-NH_3), 186 (*m/z* 204-H_2O), 175, 169, 163, 157, 146, 143, 128, 119
M14D	380	363 (-NH_3), 362 (-H_2O), 339 (-C_2H_3N), 204 (-glucuronic acid), 186 (base peak, *m/z* 204-H_2O), 169 (*m/z* 169-H_2O), 157, 143, 119
M14E	380	363 (-NH_3), 362 (-H_2O), 339 (-C_2H_3N), 321 (*m/z* 339-H_2O), 204 (base peak, -glucuronic acid), 186 (*m/z* 204-H_2O), 175, 169, 157, 143, 119, 113, 85
M14F	380	363 (-NH_3), 362 (-H_2O), 345 (*m/z* 363-H_2O), 339 (-C_2H_3N), 321 (*m/z* 339-H_2O), 204 (-glucuronic acid), 186 (base peak, *m/z*-H_2O), 175, 169, 157, 143, 128, 119, 113, 85
M31B	394	376 (-H_2O), 358 (*m/z* 376-H_2O), 330 (*m/z* 376-H_2CO), 218 (base peak, -glucuronic acid), 200 (*m/z* 218-H_2O), 171, 113, 85
M31C	394	376 (-H_2O), 358 (*m/z* 376-H_2O), 340 (*m/z* 358-H_2O), 330 (*m/z* 376-H_2CO), 218 (-glucuronic acid), 200 (base peak, *m/z* 218-H_2O), 171, 141, 119, 115, 113, 85
M31D	394	376 (-H_2O), 358 (*m/z* 376-H_2O), 340 (*m/z* 358-H_2O), 314, 296, 284, 218 (-glucuronic acid), 200 (base peak, *m/z* 218-H_2O), 171, 141, 115

		m/z 218-H_2O), 171, 141, 115
M31E	394	376 (-H_2O), 358 (*m/z* 376-H_2O), 340 (*m/z* 358-H_2O), 314, 296, 284, 218 (-glucuronic acid), 200 (base peak, *m/z* 218-H_2O), 171, 141, 135, 115
M31F	394	376 (-H_2O), 358 (*m/z* 376-H_2O), 340 (*m/z* 358-H_2O), 330 (*m/z* 376-H_2CO), 218 (-glucuronic acid), 200 (base peak, *m/z* 218-H_2O), 171, 156, 146, 141, 135, 115, 113, 85
M32	366	349 (-NH_3), 348 (-H_2O), 190 (base peak, -glucuronic acid), 172 (*m/z* 190-H_2O), 157, 148, 145, 142, 131, 117, 105, 85
M33	232	214 (-H_2O), 204, 187, 175, 169, 157, 143, 135, 128 (base peak), 115, 107, 91
M34	174	157, 142, 133 (base peak), 132, 129, 118, 105, 91, 82, 79

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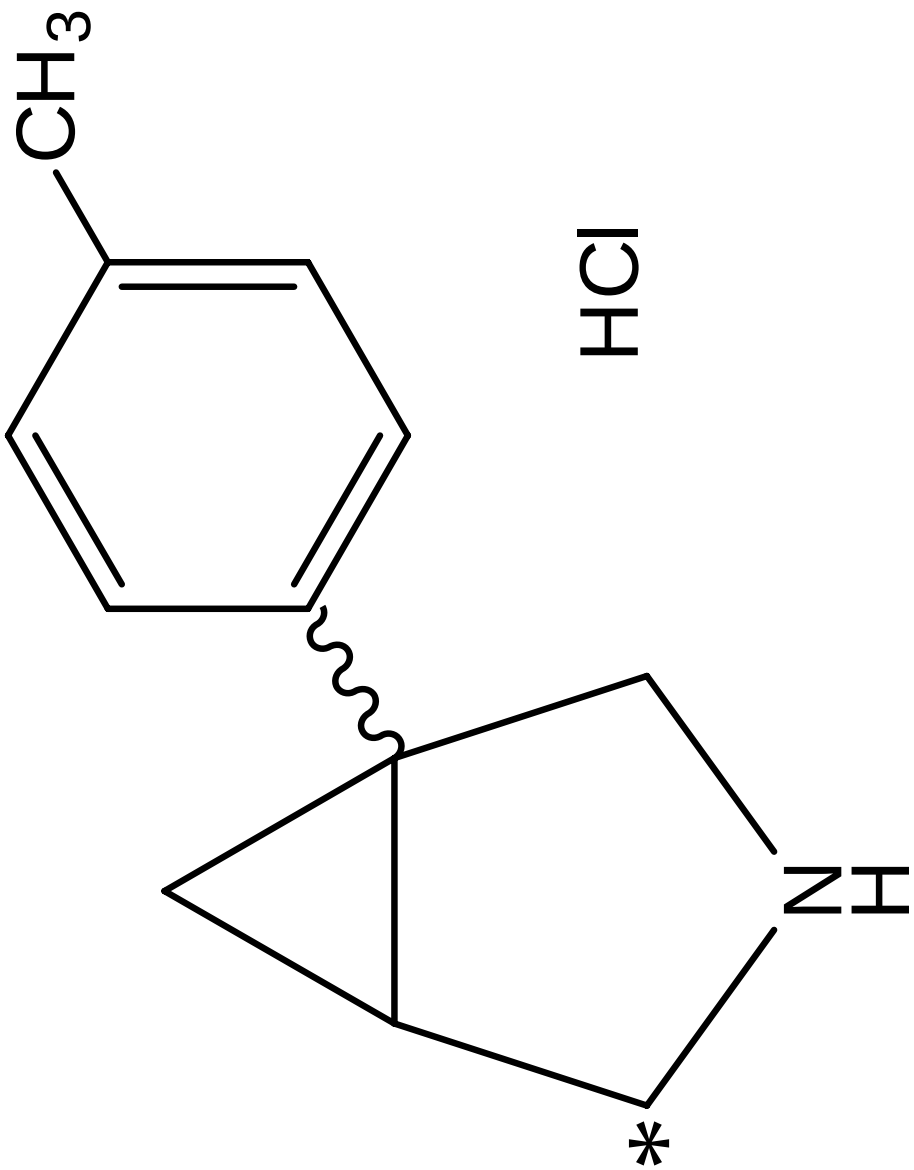


Figure 1

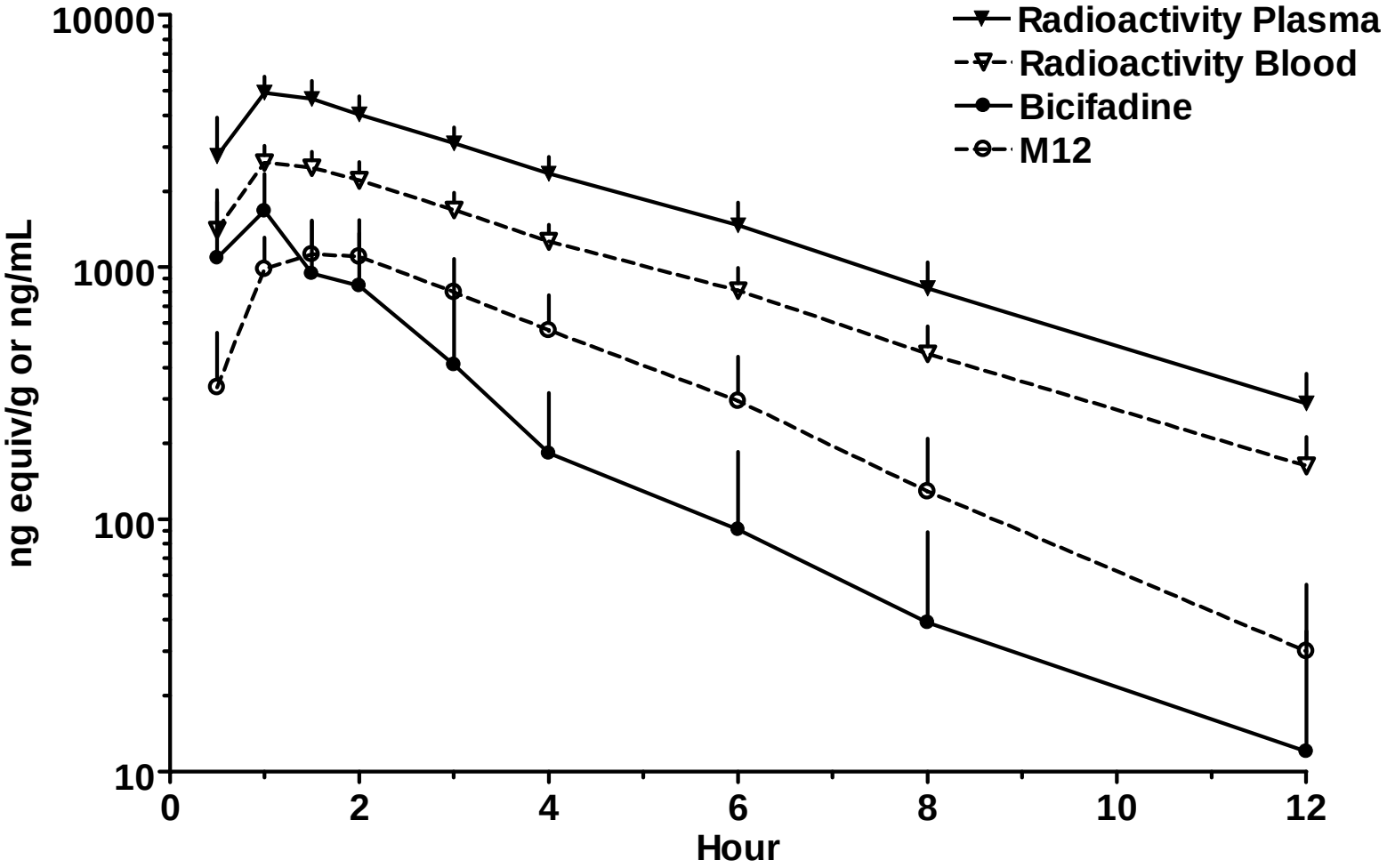
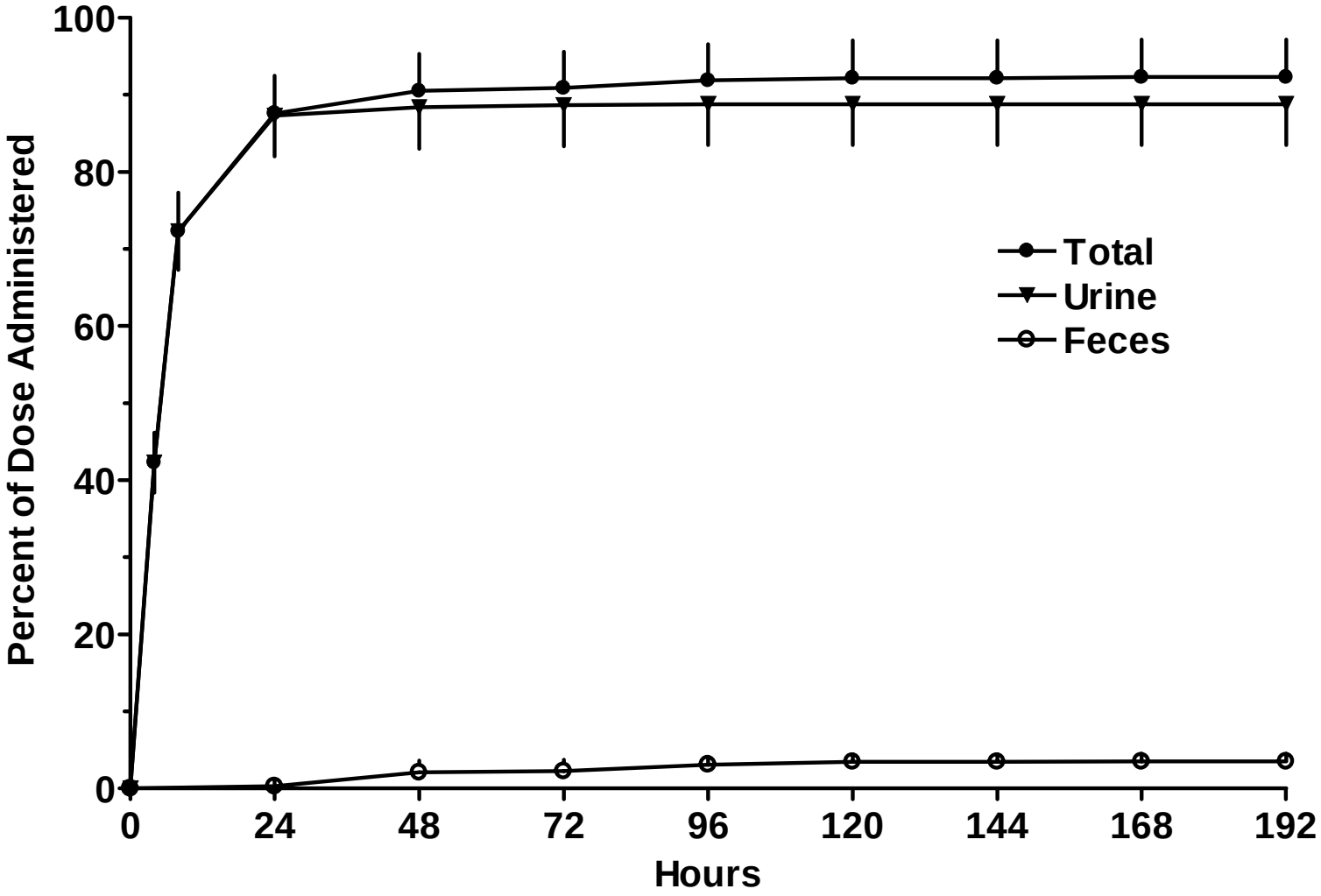


Figure 2



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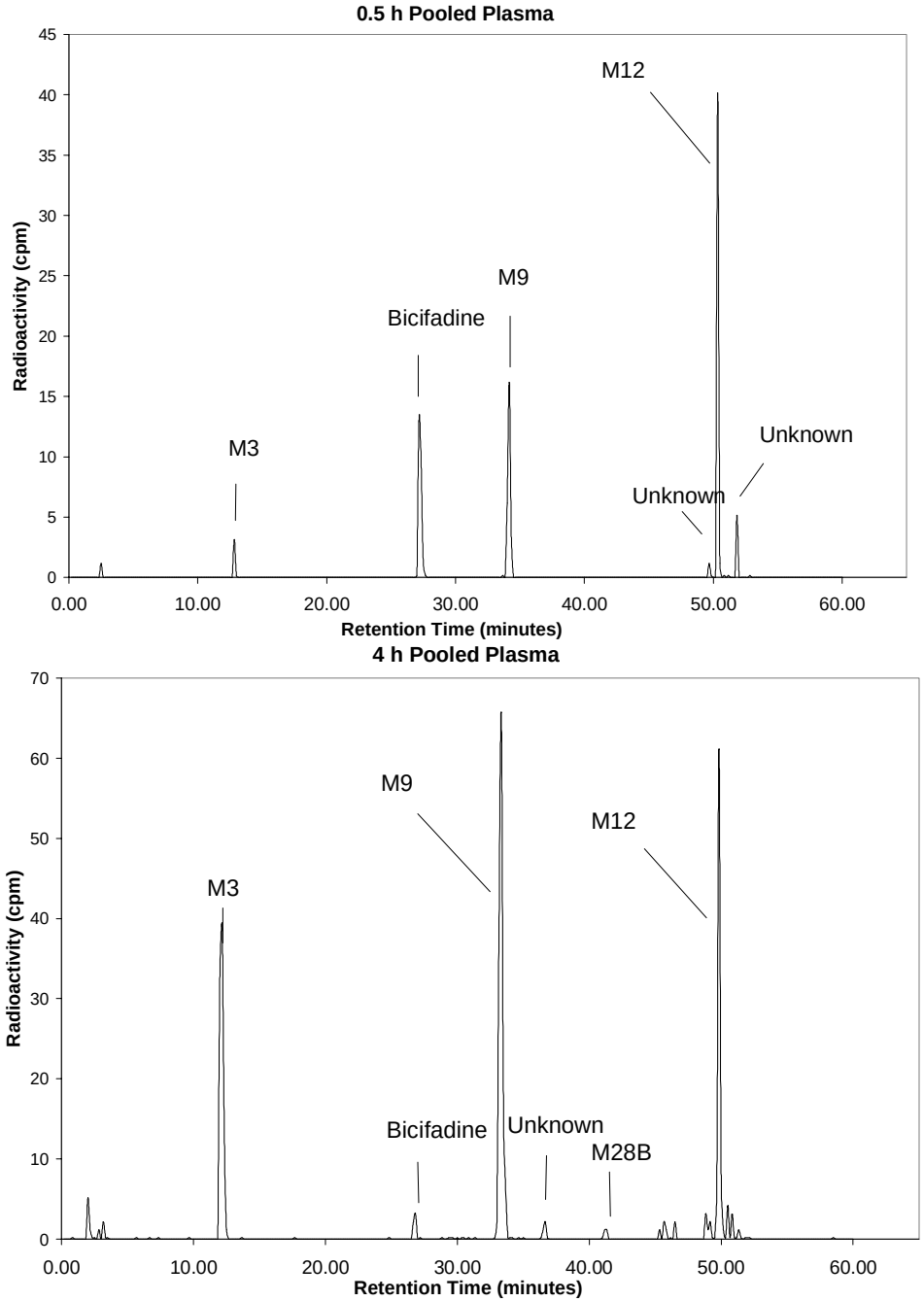
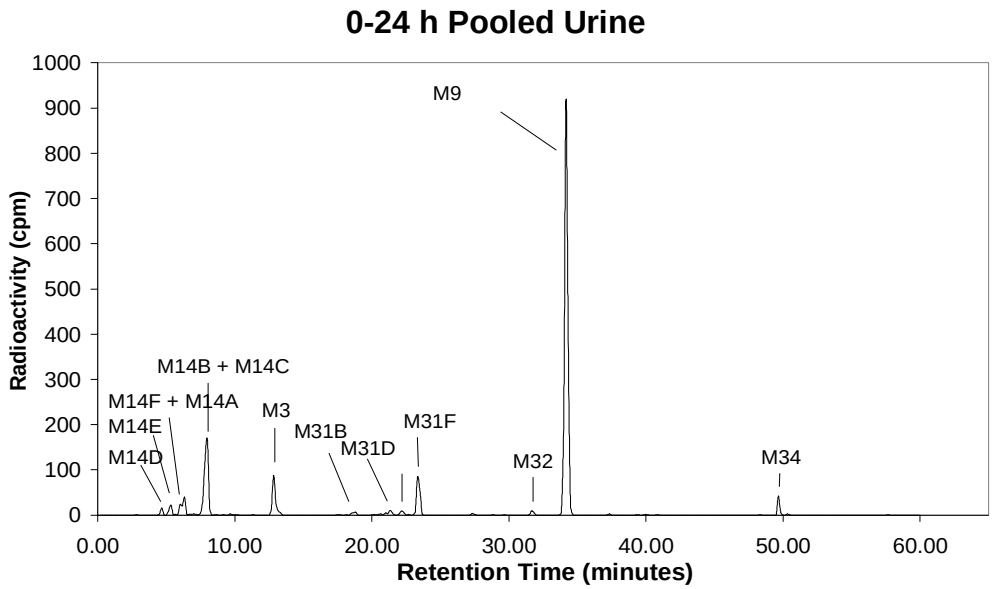


Figure 4



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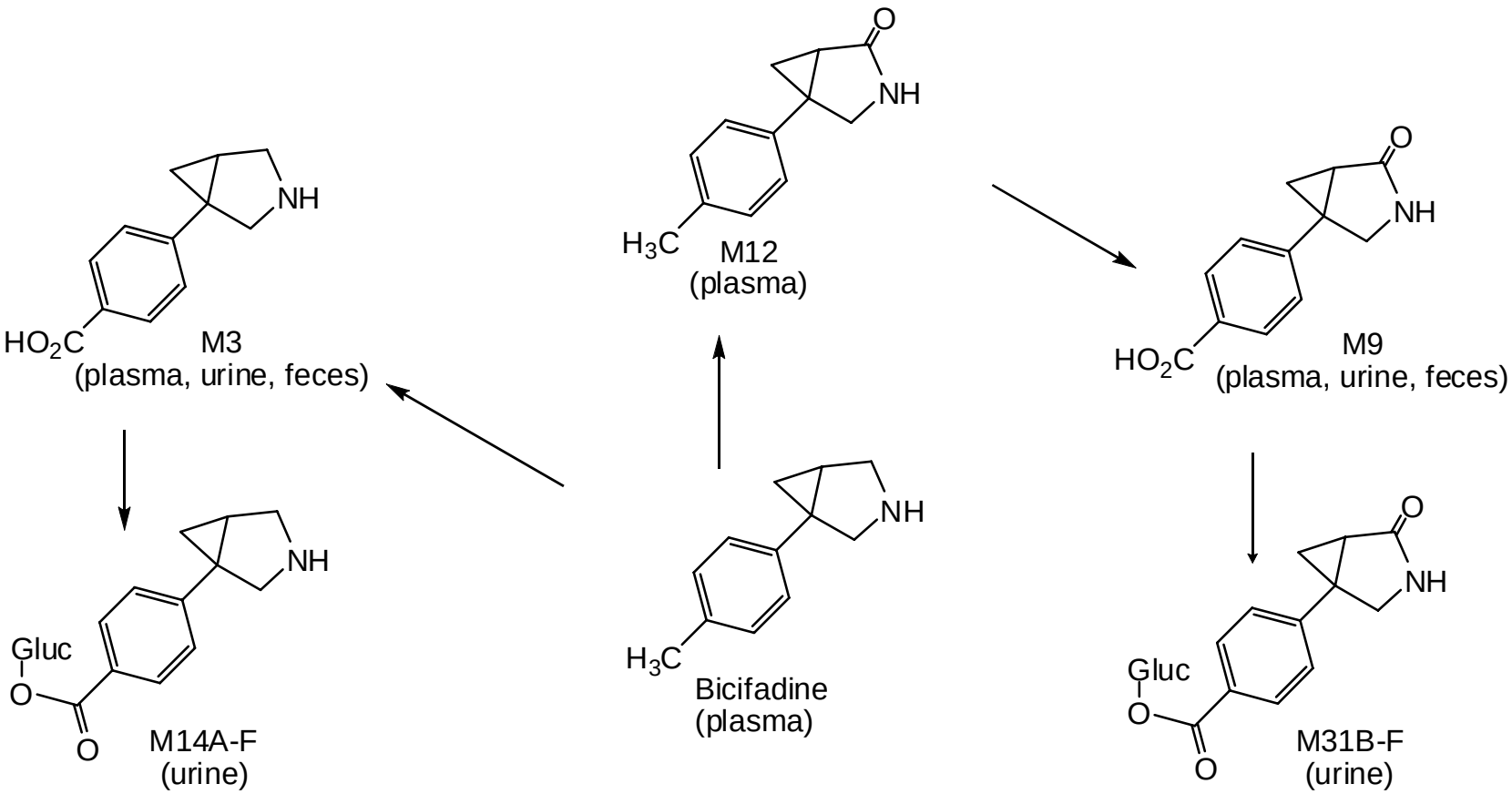

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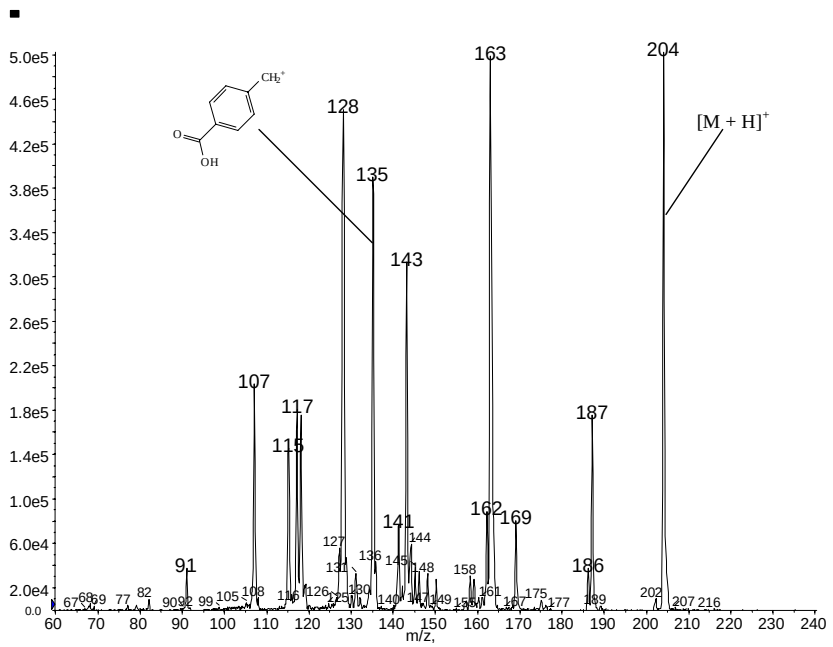
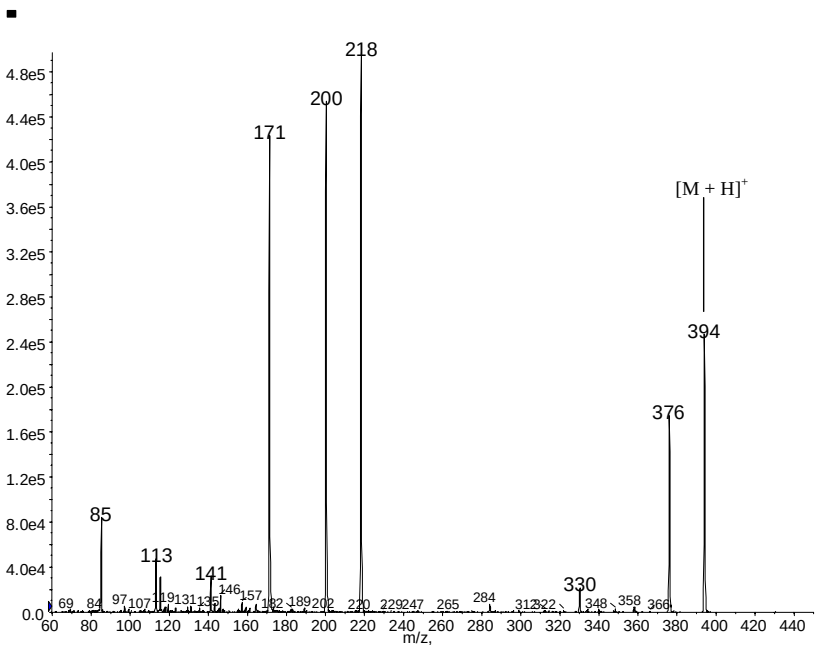
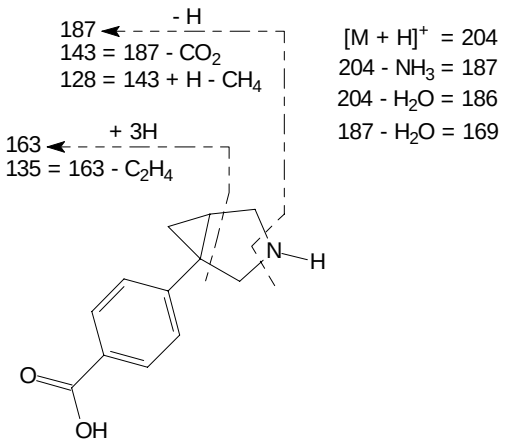
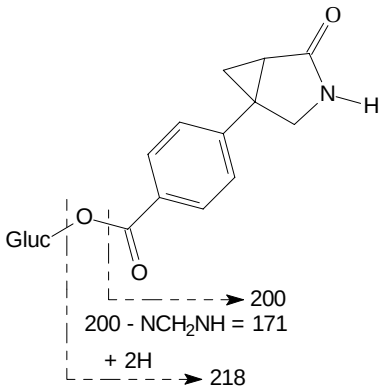






[M + H]+ = 204
204 - NH3 = 187
204 - H2O = 186
187 - H2O = 169



[M + H]+ = 394
394 - H2O = 376
376 - H2O = 358
376 - HCO2H = 330

Figure 7