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ABSORPTION, METABOLISM AND EXCRETION OF [^{14}C]MK-0524, A PROSTAGLANDIN D_2 RECEPTOR ANTAGONIST, IN HUMANS

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Abbreviations: DP1, prostaglandin D_2 receptor 1; MK-0524, [(3r)-4-(4-chlorobenzyl)-7-fluoro-5-(methylsulfonyl)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl]acetic acid; UGT, UDP-glucuronosyltransferases.

ABSTRACT

MK-0524 is a potent orally-active human DP1 (prostaglandin D_2 receptor 1) antagonist that is currently under development for the prevention of niacin-induced flushing. The metabolism and excretion of [^{14}C]MK-0524 in humans were investigated in six healthy human volunteers following a single oral dose of 40 mg (202 μCi). [^{14}C]MK-0524 was absorbed rapidly with plasma C_{max} achieved 1 to 1.5 hr post-dose. The major route of excretion of radioactivity was *via* the feces, with 68% of the administered dose recovered in feces. Urinary excretion averaged 22% of the administered dose, for a total excretion recovery of ~90%. The majority of the dose was excreted within 96 hr following dosing. Parent compound was the primary radioactive component circulating in plasma, comprising 39 to 70% of the total radioactivity in plasma for up to 12 hr. The only other radioactive component detected in plasma was M2, the acyl glucuronic acid conjugate of the parent compound. The major radioactive component in urine was M2, representing 64% of the total radioactivity in the urine. Minor metabolites included hydroxylated epimers (M1/M4) and their glucuronic acid conjugates, which occurred in the urine as urea adducts, formed presumably during storage of samples. Fecal radioactivity profiles were comprised mainly of the parent compound, originating from unabsorbed parent and/or hydrolyzed glucuronic acid conjugate of the parent compound. Therefore in humans, MK-0524 was eliminated primarily *via* metabolism to the acyl glucuronic acid conjugate, followed by excretion of the conjugate into bile and eventually into feces.

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INTRODUCTION

MK-0524, [(3R)-4-(4-chlorobenzyl)-7-fluoro-5-(methylsulfonyl)-1,2,3,4-tetrahydrocyclopenta[B]indol-3-yl]acetic acid (Figure 1), is a selective antagonist of DP1 (prostaglandin D2 receptor 1) (Sturino et al, in press) that is undergoing preclinical and clinical development for the treatment of niacin-induced flushing. Niacin (nicotinic acid), a member of the vitamin B complex, has been used as a dietary supplement in milligram quantities and as a successful plasma lipid-modifying agent when administered in gram quantities. Niacin decreases plasma concentrations of cholesterol, free fatty acids and triglycerides in humans, raises plasma HDL cholesterol (Shepherd et al, 1979) and lowers plasma VLDL and LDL cholesterol (Knopp et al, 1999). Furthermore, studies have shown that niacin can be effectively combined with statins to treat patients with low HDL cholesterol (Zhao et al, 2004, Rubenfire et al, 2004). The major side-effect with niacin therapy is the mild to severe cutaneous flushing experienced by patients that is mediated by vasodilation (Vogt et al, 2006). MK-0524, a potent DP1 antagonist has an IC_{50} value of 1.1 nM in a mouse DP1 functional assay (unpublished results) and has been shown to block niacin-induced vasodilation in the mouse by ~80% (Cheng et al, 2006). Furthermore, clinical studies have demonstrated the efficacy of MK-0524 in attenuating niacin-induced vasodilation/flushing (Cheng et al, 2006).

The objective of the present study is to investigate the absorption, metabolism and excretion of [^{14}C]MK-0524 in six male human volunteers. In preclinical species (rats and dogs), MK-0524 was eliminated primarily *via* glucuronidation, followed by excretion of the acyl glucuronic acid conjugate of the parent compound (M2) in bile (Chang et al,

2007). The in vitro metabolism of MK-0524 in non-clinical species and humans has been described elsewhere (Dean et al, 2007). The major metabolite in hepatocytes from all species was M2, whereas phase I metabolites (hydroxylated and keto derivatives) collectively comprised a minor component of the metabolic profiles (Dean et al, 2007).

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MATERIALS AND METHODS

Chemicals. MK-0524 was synthesized by Process Research at Merck Research Laboratories, Rahway, NJ. [^{14}C]MK-0524 [(3R)-4-(4-chlorobenzyl)-7-fluoro-5-(methylsulfonyl)-1,2,3,4-tetrahydrocyclopenta[B]indol-3-yl]-3a-^{14}C] acetic acid (specific activity of 5 μCi/mg) and methylsulfonyl-[^{13}C]D_3MK-0524 were synthesized by the Labeled Compound Synthesis Group at Merck Research Laboratories Rahway, NJ. The chemical purity of the tracer was 99.9%, as determined by HPLC. The dose was prepared as capsules, each containing 20 mg/101.2 μCi of [^{14}C]MK-0524.

The acyl glucuronide of MK-0524 (M2) was prepared by the Labeled Compound Synthesis Group, Drug Metabolism, Rahway, NJ. The oxidative metabolites M1, M3 and M4 were prepared by the Medicinal Chemistry Group at Merck Frosst, Canada (Nicoll-Griffith et al, in press). All other reagents were of the highest commercial quality available.

Subjects and Dose Administration. The study was conducted at Clinical Pharmacology Associates in Miami, FL. Six healthy male volunteers abstained from food and drink overnight for at least 8 hours prior to dosing. Each subject was administered a single oral dose of [^{14}C]MK-0524 as 2 capsules (total of 40 mg; 202.4 μCi/subject) with 240 mL water. After dosing, each subject received a standardized lunch and dinner at 4 and 10 hr, respectively, and a snack in the afternoon.

Sample Collection. Heparinized blood (12 mL) was collected predose and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, 36, 48, 60, 72, 96, 120, 144 and 168 hr post-dose. Blood samples were centrifuged within 30 min of collection at 3000 rpm at 4°C for 10

min. Plasma was separated and stored as two aliquots at -20°C for qualitative and quantitative analysis. Urine samples voided predose and at specific intervals (0-6, 6-12, 12-24, 24-48, 48-72, 72-96 and 96-120 hr) were stored at -20°C. All fecal samples and wipes were individually packaged during pre-dose and throughout the 7 days post-dose. Exhaled CO_2 was collected pre-dose (within 2 hr of dosing) and at 1 and 4 hr post-dose. Subjects were instructed to exhale through a one-way valve into scintillation vials containing 4 mL of 1:1 mixture of 1 M hyamine hydroxide solution and ethanol and trace amounts of 1% thymophtalein (blue indicator). The blue solution turns clear upon CO_2 collection in approximately 40 to 60 sec. Following collection, Scintisafe Gel cocktail (12 mL) was added to each sample; the samples were then stored in darkness at 4°C.

Sample Preparation. *Plasma.* Samples were stored at -20C before extraction for analysis. Previously studies were conducted to demonstrate that the acyl glucuronide metabolite (M2) underwent minimum hydrolysis (0.1%) when plasma samples were stored on ice for up to 6 hr (Schwartz et al, 2006). Human plasma was prepared for HPLC-MS/MS analysis by the addition of nine volumes of acetonitrile containing a stable isotope labeled internal standard. After mixing and centrifugation, the supernatant was acidified (using an equal volume of dilute formic acid in 40% acetonitrile) to prevent hydrolysis of an acyl glucuronide metabolite found in post dose plasma samples. The sample preparation procedure utilized 96-well plates and was partly automated using a robotic sample processor.

For metabolite profiling, an equal volume (1 mL) of plasma samples collected at 1, 2, 4, and 12 hr from each individual was pooled and mixed with 3 mL acetonitrile:water (2:1). The samples were sonicated for 10 min, vortex mixed for 10 min, and centrifuged

at 3000 rpm for 10 min. The supernatant was removed and the pellets were resuspended in 3 mL acetonitrile:water (2:1) by sonicating for 30 min and vortex mixing for 10 min. The samples were then centrifuged at 3000 rpm for 10 min and the supernatants evaporated to dryness under N_2. The dried extracts were reconstituted with water:acetonitrile:50% acetic acid (7:2:1, v/v/v).

Urine. For metabolite profiling, 0 to 120 hr pools of urine samples were prepared for each subject based on volume, wherein a fixed fraction of the total volume of urine from each interval was pooled. Aliquots (5 mL) from each of these pools were combined. Approximately 100,000 dpm (10 mL) was removed from the urine pool and dried under N_2 at 25 ^{o}C overnight. The residue was reconstituted with water: acetonitrile: 50% acetic acid (7:2:1, v/v/v), sonicated for 10 min, centrifuged at 3000 rpm for 10 min, then analyzed by LC-MS with radiometric detection. A representative metabolite profile was generated by analyzing a sample consisting of equal volumes of urine extracts from the 6 subjects.

For isolation of the urea adducts from urine, 5 to 10 ml of urine was evaporated under N2 and the residue reconstituted with water: acetonitrile: 50% acetic acid (7:2:1, v/v/v), sonicated for 10 min, centrifuged at 3000 rpm for 10 min. The extracts were analyzed by RP-HPLC and the radioactive peaks corresponding to each of the adducts were separately collected. Following evaporation of the solvent under a stream of nitrogen, the samples were reanalyzed using a new column and the pooled fractions were concentrated and subjected to HPLC and NMR analysis.

Feces. Concentrations of radioactivity and percent of radioactive dose excreted in feces were determined at Covance Laboratories, Inc. (Madison, WI). Briefly, samples

were combined by subject at 24-hour intervals and the weight of each combined sample was recorded. A weighed amount of ethanol:water (20:80, v:v) was added to the sample to form an approximate 20% fecal homogenate and the sample was homogenized using a probe-type homogenizer. Aliquots of the feces homogenate samples were transferred into combustion cones in triplicate and weighed.

For metabolite profiling, aqueous homogenates of the feces collected at 0 to 168 hr from each individual were pooled according to homogenate weights. Equal weights from the individual pools were combined across subjects. A 1.5 g aliquot containing approximately 100,000 dpm was precipitated with 5 mL acetonitrile:isopropyl alcohol (4:1), vortex mixed, and centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and evaporated to dryness under N_2. The residue was reconstituted with water:isopropyl alcohol:50% acetic acid:acetonitrile (5:3:1:1, v/v/v/v), and analyzed by LC-MS with radiometric detection. A representative metabolite profile was generated by analyzing a sample consisting of equal weights of fecal extracts from 6 subjects.

Toilet Tissue. Toilet tissue samples were combined by subject at 24-hour intervals and a weighed amount of ethanol:water (20:80, v:v) was added.

Quantitative Analysis. *Determination of MK-0524 Concentration in Plasma by LC-MS/MS.* The LC-MS system consisted of a Shimadzu LC-10AD VP HPLC pump, a Shimadzu SIL-HTC autosampler and CTU-10ACVP column heater) and a Waters Symmetry C-8 HPLC column (5.0 cm x 2.1 mm; 3 micron), connected to a 0.5 µm in-line filter were used. Additionally, a PE SCIEX API 4000 tandem mass spectrometer equipped with a Turboionspray interface, and controlled with Analyst software (PE Sciex; Thornhill, Canada) was used. Samples were analyzed using a column flow rate of

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0.3 mL/minute mobile phase (50/50 acetonitrile/water with 0.05% formic acid. The column temperature was 35°C and the injection volume was 5 μL. The MS/MS analysis was performed in the positive ionization mode using multiple reaction monitoring (MRM); the precursor-to-product ion transitions were *m/z* 436 → 125 and *m/z* 440 → 125 for the detection of parent compound and internal standard, respectively. The standard curve range was 10 to 2500 ng/mL.

Measurement of Radioactivity, Aliquots (100 to 200 μL) of plasma and urine samples were mixed with Scintisafe Gel cocktail (Fisher Scientific) and the radioactivity estimated using a Packard Liquid Scintillation Analyzer (Model 1900TR). Air-dried triplicate aliquots of feces homogenates were combusted in a Model 307 Sample Oxidizer (Packard Instrument Company) and the resulting $^{14}CO_2$ was trapped in a mixture of Perma Fluor and Carbo-Sorb. Samples were analyzed for radioactivity in Model 2900TR liquid scintillation counter (Packard Instrument Company) for at least 5 minutes or 100,000 counts. Triplicate weighed aliquots (approximately 0.5 g) of the extracts of toilet tissue were analyzed directly by LSC as above.

Metabolite Profiling. The LC-MS system consisted of a Series 200 autosampler and two pumps from Perkin Elmer (Norwalk, CT), a Packard radioactivity flow detector, and a Sciex 3000 triple quadrupole mass spectrometer controlled by Analyst software (Sciex, version 1.4, Foster City, CA). The mass spectrometer was operated in the negative ion mode. The source voltage was -3750 V, and the probe temperature 350^{o}C. A Zorbax SB-phenyl column (4.6 x 250 mm; 5 μm) was used for chromatographic separation. Column elution was achieved with a mixture of 10 mM aqueous ammonium acetate containing 0.1% acetic acid (mobile phase A) and acetonitrile:methanol (92.8:7.2, v/v)

containing 7.2 mM ammonium acetate and 0.1% acetic acid (mobile phase B). The column was eluted over a 40 minute period with a linear gradient from 25 to 65% B. The effluent from the HPLC, pumped at a rate of 1.0 mL/min, was diverted at a 24:1 ratio to the radiometric flow detector and the mass spectrometer, respectively. Scintillation cocktail (Packard Ultima Flo-M, Downers Grove, IL) was pumped at a rate of 3 mL/min into the radiometric detector. Contributions of parent compound and metabolites were calculated from the amount of radioactivity eluting in each peak relative to the total radioactivity in the HPLC radiochromatogram. Metabolites were identified by extraction of expected m/z from Q1 TIC scan and by comparison of their retention times and MS/MS spectra with those of available authentic standards.

In vitro Formation of Urea Adducts of M1 and M4. M1 and/or M4 synthetic standards (5 μM) were incubated at room temperature with predose human urine for 5 days or with 2.5 M urea for 24 hr. Acetonitrile extracts were analyzed by RP-HPLC coupled with LC-MS/MS.

NMR Analysis of Urea Adducts of M1 and M4. Proton NMR spectra of the parent and metabolites were obtained in acetonitrile-d_3/D_2O (8:1, by volume) at room temperature using a Varian Inova 600 MHz NMR spectrometer equipped with a 3-mm Nalorac probe.

Pharmacokinetics. The apparent terminal rate constant (λ) for both radioactivity and MK-0524 plasma concentrations was estimated by regression of the terminal log-linear portion of the concentration-time profile (using quantifiable concentrations only); $t_{½}$ was calculated as the quotient of ln(2) and λ. Onset of the terminal log-linear phase was determined by inspection. AUC to the last time point with a detectable plasma

concentration ($AUC_{0\text{-last}}$) was calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations. $AUC_{0\text{-}\infty}$ was estimated as the sum of $AUC_{0\text{-last}}$ and the extrapolated area given by the quotient of the last measured concentration and λ. The C_{max} and T_{max} values were obtained by inspection of the plasma concentration data.

Dose Recovery Calculations. The percentage of radioactivity dose recovered as metabolites was determined by the sum of the percentages excreted in urine, feces and exhaled air. The percentage of the radioactive dose recovered as metabolites was determined by the product of the percentages of the radioactivity dose recovered and those accounted by metabolites in metabolite profiles.

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RESULTS

Excretion of Radioactivity The excretion of radioactivity in human urine and feces following a single 40-mg oral dose of [^{14}C]MK-0524 is summarized in Table 1. The results indicated that ~22% of the administered dose was recovered in the urine within 5 days (range: 17 to 30%) in the 6 subjects who participated in this study. Fecal excretion averaged 68% of the administered dose (range: 59 to 74%) within 7 days, with a mean total recovery of radioactivity in urine and feces of ~90% (range: 87 to 91%). The majority of the dose was excreted within 96 hr following dosing. Exhaled air was collected 1 and 4 hr postdose and examined for [^{14}C]CO_2. Average radioactivity levels at these time points were not substantially different from background levels.

Plasma Concentrations The mean concentration profiles of MK-0524 and radioactivity in plasma following administration of a single oral dose of [^{14}C]MK-0524 are shown in Figure 2. Individual plasma MK-0524 and radioactivity pharmacokinetic parameters are listed in Table 2. MK-0524 accounted for approximately 29% of the radioactivity in plasma, as determined by the AUC ratio of MK-0524 to radioactivity. Following oral administration, [14C]MK-0524 was absorbed rapidly; the highest MK-0524 as well as total radioactivity concentrations in plasma (C_{max}) were achieved, on average, between 1 to 1.5 hr and ranged from 1.27 to 3.59 μM eq (mean value 2.34 μM eq) and 3.60 and 6.44 μM (mean value 5.07 μM), respectively.

Identification of Metabolites Figure 1 shows the structures of metabolites identified in human samples following oral administration of [^{14}C]MK-0524. Most of the metabolite identification was done by LC-MS analysis and/or by comparison of their retention times and MS/MS spectra with those of available authentic standards. Detailed

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LC-MS/MS information on the fragmentation of the metabolites has been described elsewhere (Dean et al, in press). Two new unknowns were detected in human urine and are discussed below.

[^{14}C]MK-0524 Metabolite Profiles in Plasma Metabolite profiles in plasma pooled across subjects at 1, 2, 4 and 12 hr are presented in Figure 3. Due to the low levels of radioactivity and the limited volume of plasma, it was not possible to obtain individual subject metabolite profiles. The major circulating components in human plasma were the parent compound, MK-0524, and the acyl glucuronide of the parent (M2). The parent compound represented 39 to 70% of the radioactivity in plasma up to 12 hr, whereas M2 comprised 28 to 56% of the radioactivity.

[^{14}C]MK-0524 Metabolite Profiles in Urine The metabolite profile of a pooled sample of urine collected at 0 to 120 hr is presented in Figure 4. MK-0524 was a minor component, comprising ~5% of the radioactivity, whereas M2 was the major component and comprised 64% of the radioactivity in human urine. Minor amounts of the acyl migrated products were also observed. Two new unknowns were observed at 18.40 min and 19.70 min in the radiochromatograms of the pooled human urine (Figure 4). These unknowns gave deprotonated molecular ion [M-H-] at *m/z* of 492, corresponding to an addition of 58 Da to the parent compound (*m/z* 434). Further fragment ions were at *m/z* 449 corresponding to the loss of a CO-NH2 group. The ^{1}H NMR spectra of the *m/z* 492 adducts revealed the loss of one proton from the methylene adjacent to the indole moiety (***i'***, see Figure 5) and a 2.5 ppm downfield shift of the remaining proton (***i***). The chemical shift of ***i*** (5.08 ppm) relative to that in the spectrum of the hydroxylated metabolite (5.15 ppm, spectrum not shown) indicated a weaker electron withdrawing group, such as an

amide nitrogen. Given the molecular weight and the prevalence of urea in urine, these data suggested a urea adduct. These urea adducts could have formed ex vivo, during the storage of urine samples. HPLC analysis of an incubation mixture of a synthetic standard of M1 (*m/z* 450) with predose urine for 5 days showed that both M1 and its epimerized product M4 were converted to their urea adducts (m/z 492) (Figure 6, panel A). Similarly, urea adducts of both M1 and M4 were formed following an overnight incubation of M4 in the presence of 2.5 M urea (Figure 6, panel B). Both incubations resulted in the formation of *m/z* 492 adducts that yielded fragment ions and ^{1}H NMR spectra identical to the adducts originally isolated from human urine. Urea adducts of the glucuronide conjugates of M1 and M4 gave an *m/z* of 668. The keto derivative, M3, was also a minor component in human urine (2% of the total radioactivity). The radioactivity in urine was thus comprised of 5% parent compound, 31% Phase I (oxidative) metabolites, and 64% Phase II (glucuronide conjugate) metabolites.

Metabolite Profiles in Feces The metabolite profile of a pooled human fecal sample collected at 0 to 168 hr is presented in Figure 7. Parent compound was the major radioactive component in feces, comprising 73% of the radioactivity. Also present in the feces were the two hydroxylated metabolites, M1 and M4, which comprised 10% of the radioactivity, and the keto derivative, M3, which comprised 17% of the total radioactivity. The radioactivity in feces was thus comprised of 73% parent compound and 27% Phase I (oxidative) metabolites.

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DISCUSSION

The objective of this study was to investigate the metabolism and excretion of an oral dose of 40 mg /202 μCi of [^{14}C]MK-0524 in 6 healthy human subjects. On average, 68% of the administered dose was recovered in feces during a 7-day period. Urinary excretion averaged 22% of the administered dose, during a 5-day period. The total mean recovery of radioactivity was ~90%. The excretion pattern was similar in all six subjects.

Absorption was rapid with peak plasma concentrations of total radioactivity and parent compound being achieved within 1.5 hr. A comparison of AUC values of the parent compound versus total radioactivity (10.1 vs. 34.6 μM.hr) indicates that MK-0524 contributed to ~30% of the circulating levels of drug-related material in plasma. Plasma radioactivity profiles at select time points (1, 2, 4 and 12 hr) revealed that the only other radioactive component, besides MK-0524, was the acyl glucuronic acid conjugate of the parent compound (M2), comprising 28 to 57% of the radioactivity. A similar plasma profile was also observed in dogs dosed orally and intravenously with [^{14}C]MK-0524, wherein the relative contributions of the parent compound and M2 were 63 to 88% and 24 to 37%, respectively (Chang et al, in press). The relative ratio of the concentrations of M2 to MK-0524 in vivo was species-specific in animal studies. Following oral and intravenous administration of MK-0524 to rats, dogs and cynomolgus monkeys, plasma AUC values of M2 were 1, 10 to 20 and 29 to 127%, respectively, of those of MK-0524. The stability of M2 in plasma was studied previously (Chang et al, in press). Briefly, when incubated with fresh plasma from rat, dog and human, M2 underwent acyl migration to form transient isomeric products and finally hydrolyzed to form the parent compound. The in vitro half-life for M2 hydrolysis in plasma ranged from 1 to 3 hr in

these species. In this study, human plasma collection methods were optimized and validated to prevent hydrolysis of M2. Studies were conducted to demonstrate that the relative extent of hydrolysis of M2 was ~0.1% when plasma samples were stored on ice for 6 hr (Schwartz et al, 2006). Glucuronidation has been considered as the most common phase II reaction and is mainly a mechanism of detoxification and elimination for a wide variety of endogenous substrates and xenobiotics in mammals. Acyl glucuronides are usually susceptible to hydrolysis by beta glucuronidases present in the gut, non-specific esterases, serum albumin and hydroxide ion (Bailey and Dickinson, 2003, Koster et al., 1985).

Following oral administration of MK-0524, about 22% of the administered radioactive dose was excreted in the urine. The major component in urine samples was M2, the acyl glucuronide, comprising ~64% of the total radioactivity, along with urea adducts of the hydroxylated epimers (M1/M4) and of their corresponding glucuronides. In addition, M3 (the keto metabolite) was a minor component in human urine. Detailed in vitro studies conducted following incubation of M1 and M4 in the presence of urea and/or control human urine, indicated that these urea adducts could have formed in vitro during the storage of study samples and appear to be chemical artifacts arising from M1/M4. Standards of M1 and M4 were found to readily epimerize when analyzed with LC-MS and formed urea adducts when allowed to stand in blank urine or 2.5 M urea. Based on these observations, a plausible mechanism for the formation of the urea adducts involves the acid-catalyzed elimination of water to form a resonance-stabilized carbocation. This carbocation can be trapped by urea to form both urea adduct epimers or water to regenerate the epimers M1/M4.

Approximately 73% of the dose was excreted into the feces following oral administration of MK-0524. The parent compound was the major component in human feces accounting for 73% of the total radioactivity excreted via this route. The presence of parent compound in feces presumably represents hydrolyzed M2, acyl glucuronic acid conjugate of the parent compound, along with contribution from the unabsorbed parent compound. Based on unpublished observations, the oral bioavailability of MK-0524 in human subjects was determined to be ~80%, which was similar to what was observed in dogs (Chang et al). Therefore, most of the parent compound present in feces is likely the result of enzymatic hydrolysis of M2, which suggests that MK-0524 was eliminated primarily by metabolism via glucuronidation and further excretion of the acyl glucuronide into bile and feces. In vitro studies also showed that MK-0524 underwent glucuronidation in incubations of human intestinal microsomes conducted in the presence of UDPGA (Dean et al). Therefore, it is likely that a portion of the oral dose of the compound may undergo first-pass glucuronidation in the gut as well during the absorption process. M2 was also the major metabolite formed in vitro in human hepatocyte incubations with MK-0524, and several recombinant human UGT enzymes (1A1, 1A3, 1A9 and 2B7) could catalyze the acyl glucuronidation of MK-0524 (Dean et al, in press).

Thus, the routes of elimination of MK-0524 in humans appear to be similar to those in preclinical species. In rats and dogs MK-0524 was cleared *via* metabolism exclusively by way of glucuronidation, followed by excretion of the acyl glucuronide (M2) into bile, which represented >95% of the total radioactivity (Chang et al, in press). Trace metabolites in rat and dog bile comprised of the hydroxylated epimers M1/M4 and the

keto derivative, M3, along with the corresponding glucuronides of these entities (~1 to 2% of radioactivity). In comparison, human feces profiles showed that M1/M4 and M3 collectively comprised ~27% of the radioactivity in a 0 to 168-hr feces profile, thus demonstrating that in humans, a significant portion of the dose was eliminated by phase I metabolism. Similarly, human urine profiles also showed that M1/M4 along with their glucuronidated derivatives and M3, accounted for a total of 30% of radioactivity. In vitro studies to identify the CYP isoforms involved in the oxidative metabolism of MK-0524 have shown that CYP3A4 was the primary isoform catalyzing the formation of M1/M4 and M3 in human liver microsomes.

In summary, following an oral dose of 40 mg/202 μCi, MK-0524 was eliminated primarily by metabolism via formation of M2, the acyl glucuronide, which was further excreted into the bile. The major circulating entity in human plasma following an oral dose of MK-0524 was the parent compound along with smaller amounts of M2, the acyl glucuronic acid conjugate of the parent compound.

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FOOTNOTES

Figure 1

[a] *Healthy volunteers received 40 mg/202.4 μCi of [^{14}C]MK-0524 in 2 drug-filled capsules. Radioactivity in plasma was determined by liquid scintillation counting; Plasma concentrations of MK-0524 were determined by an LC-MS/MS assay.*

Figure 3

[a] *Healthy volunteers received 40 mg/202.4 μCi of [^{14}C]MK-0524 in 2 drug-filled capsules. Plasma pooled from 6 subjects was subjected to protein precipitation followed by LC-MS analysis with radiometric detection.*

Figure 4

[a] *Healthy volunteers received 40 mg/202.4 μCi of [^{14}C]MK-0524 in 2 drug-filled capsules. Pooled urine extracts from 6 subjects were analyzed by LC-MS with radiometric detection.*

Figure 6

[a] *Authentic standards of M1 and M4 (m/z 450), were incubated at room temperature in the presence of pre-dose urine for 5 days (panel A) or 2.5 M urea for 24 hours (panel B), respectively. Acetonitrile extracts of the incubation mixtures were analyzed by RP-HPLC with UV detection at 310 nm. Peaks corresponding to m/z 492 were identified as urea adducts of M1 and M4.*

Figure 7

[a] *Healthy volunteers received 40 mg/202.4 μCi of [^{14}C]MK-0524 in 2 drug-filled capsules. Fecal homogenates from 6 subjects were pooled and the extracts were analyzed by LC-MS with radiometric detection.*

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FIGURE LEGENDS

Figure 1. Major In Vivo Pathways of Metabolism For [^{14}C]MK-0524 In Humans.

Figure 2. Mean Concentration-Time Profiles of MK-0524 and Total Radioactivity in Plasma Following Administration of a Single Oral Dose of 40 mg (202.4 μCi) [^{14}C]-MK-0524 to Healthy Adult Male Subjects (N=6)[a].

Figure 3. HPLC Radiochromatograms of Human Plasma Following Oral Administration of [^{14}C]MK-0524 (40 mg, 202.4 μCi)[a].

Figure 4. HPLC Radiochromatograms of Pooled Human Urine (0 to 120 hr) Following Oral Administration of [^{14}C]MK-0524 (40 mg, 202.4 μCi)[a]

Figure 5. Comparative NMR spectra of (A) MK-0524, (B) the Urea Adduct of M1/M4 Isolated from Human Urine, (C) and from Incubation of M4 with 2.5 M Urea

Figure 6. HPLC-UV Trace of Incubations of M1 and M4 with Predose Urine (A) or 2.5 M Urea (B)[a]

Figure 7. HPLC Radiochromatogram of Feces (0 to 168 hr) Following Oral Administration of [^{14}C]MK-0524 (40 mg, 202.4 μCi)[a]

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Table 1

Excretion of Radioactivity in Human Urine and Feces Following an Oral Dose of [^{14}C]MK-0524[a]

Time (hr)/ Subject	Percent Dose							
	0001	0002	0003	0004	0005	0006	Mean	SD[b]
Urine								
0-6	6.2	10.6	5.9	7.6	6.9	4.6	6.9	2.1
6-12	5.6	9.9	6.7	5.6	5.4	3.9	6.2	2.0
12-24	3.3	4.7	6.0	3.6	3.3	3.4	4.1	1.1
24-48	2.5	3.5	2.2	3.2	2.9	2.4	2.8	0.5
48-72	0.9	0.9	1.4	1.1	1.3	1.5	1.2	0.3
72-96	0.3	0.2	0.6	0.2	0.5	0.6	0.4	0.2
96-120	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1
0-120	18.9	30.0	22.9	21.3	20.4	16.7	21.7	4.6
Feces								
0-24	9.2	0.01	N.S.[d]	11.3	0.4	0.4	3.6	5.2
24-48	28.4	21.5	0.1	12.6	17.2	14.8	15.8	9.5
48-72	12.0	34.3	14.4	41.0	32.8	N.S.	22.4	15.9
72-96	13.3	1.6	35.4	3.3	15.0	54.4	20.5	20.5
96-120	3.9	0.8	11.3	0.9	2.0	N.S.	3.1	4.2
120-144	0.8	0.3	3.1	0.2	1.0	4.7	1.7	1.8
144-168	0.14	0.1	2.0	0.2	0.4	N.S.	0.5	0.8
0-168	67.8	58.6	66.3	69.5	68.7	74.3	67.5	5.2
Total[c]	86.8	88.9	89.3	91.1	89.7	91.0	89.5	1.6

[a] Six healthy volunteers received 40 mg/202.4 μCi of [^{14}C]MK-0524 in 2 drug-filled capsules. Radioactivity in fecal homogenates and urine was determined at Covance Laboratories, Inc. and expressed as percent of administered dose.

[b] SD = standard deviation.

[c] Includes radioactivity on fecal wipes.

[d] N.S.= No sample obtained.

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Table 2

Plasma Pharmacokinetic Parameters of MK-0524 and Radioactivity Following Administration of a Single Oral Dose of 40 mg [^{14}C]MK-0524 to Healthy Adult Male Subjects[a]

	MK-0524			Radioactivity			MK-0524 / Radioactivity	
Subject #	$AUC_{0-\infty}$ μM•hr	C_{max} μM	T_{max} hr	$AUC_{0-\infty}$ μM eq•hr	C_{max} μM eq	T_{max} hr	$AUC_{0-\infty}$ Ratio	C_{max} Ratio
001	10.8	1.76	1.5	31.6	3.60	1.5	0.34	0.49
002	12.6	3.59	1.0	34.2	6.05	1.0	0.37	0.59
003	10.3	1.98	1.0	33.5	4.67	1.5	0.31	0.42
004	4.32	1.27	1.5	29.5	5.17	1.5	0.15	0.25
005	12.9	2.71	1.0	48.5	6.44	1.0	0.27	0.42
006	13.6	2.73	1.0	33.3	4.50	1.5	0.41	0.61
AM	10.8	2.34	1.2	35.1	5.07	1.3	--	--
SD	3.40	0.83	0.3	6.80	1.05	0.3	--	--
AM = Arithmetic Mean; SD = Standard Deviation								

[a] Six healthy volunteers received 40 mg/202.4 μCi of [^{14}C]MK-0524 in 2 drug-filled capsules. Radioactivity in plasma was determined by liquid scintillation counting. Concentrations of MK-0524 were determined by an LC-MS/MS assay.

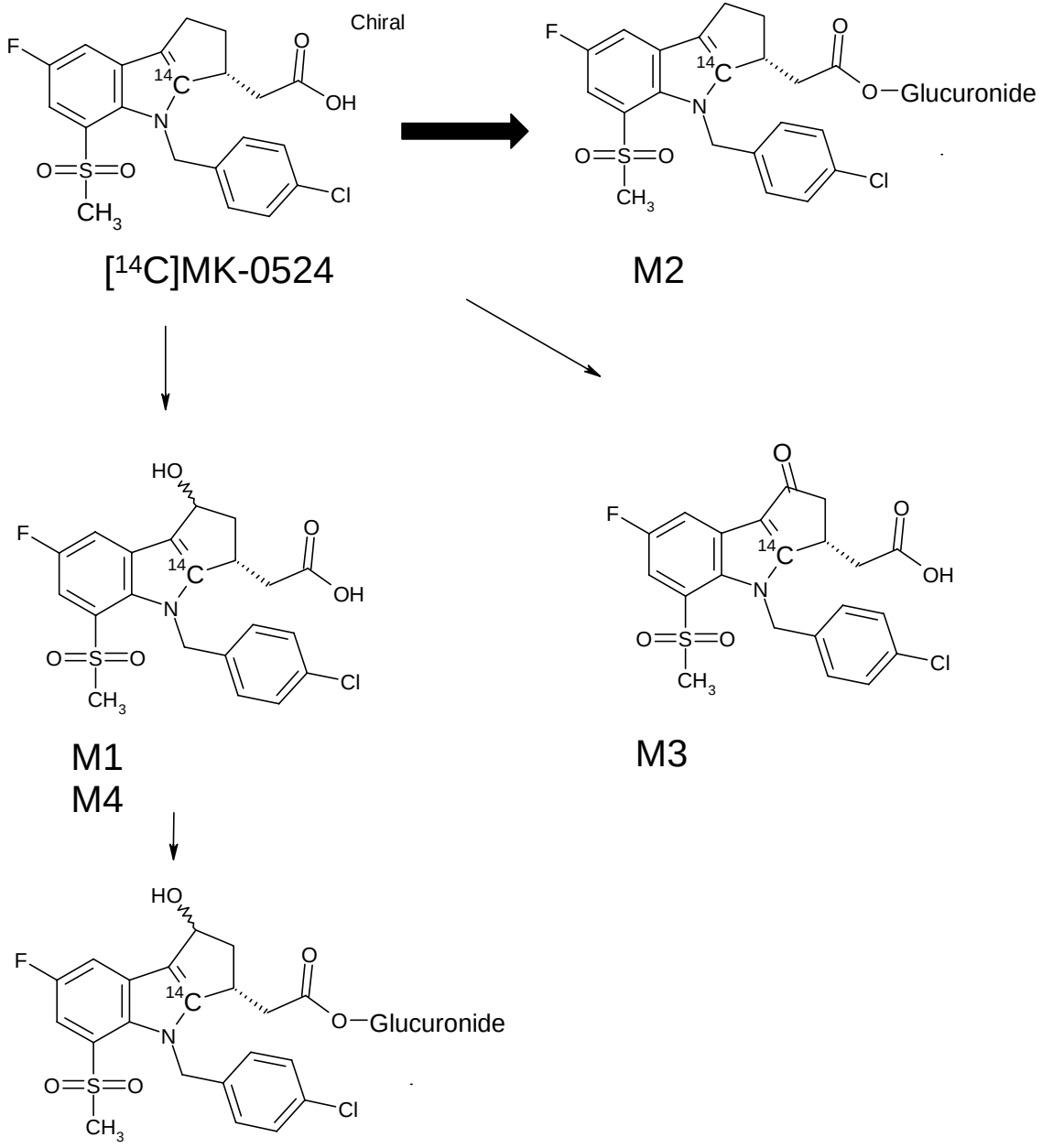


Figure 1

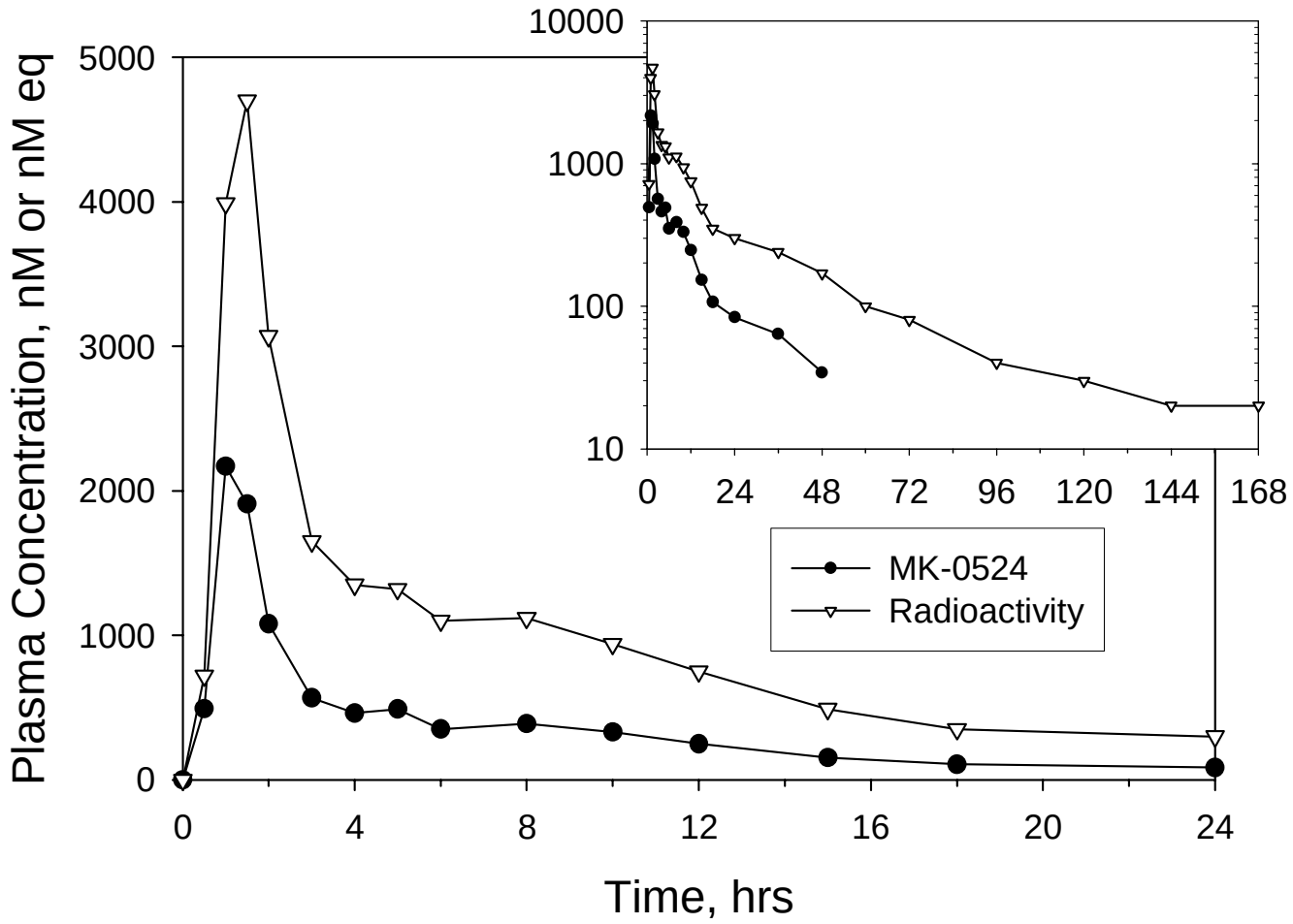


Figure 2

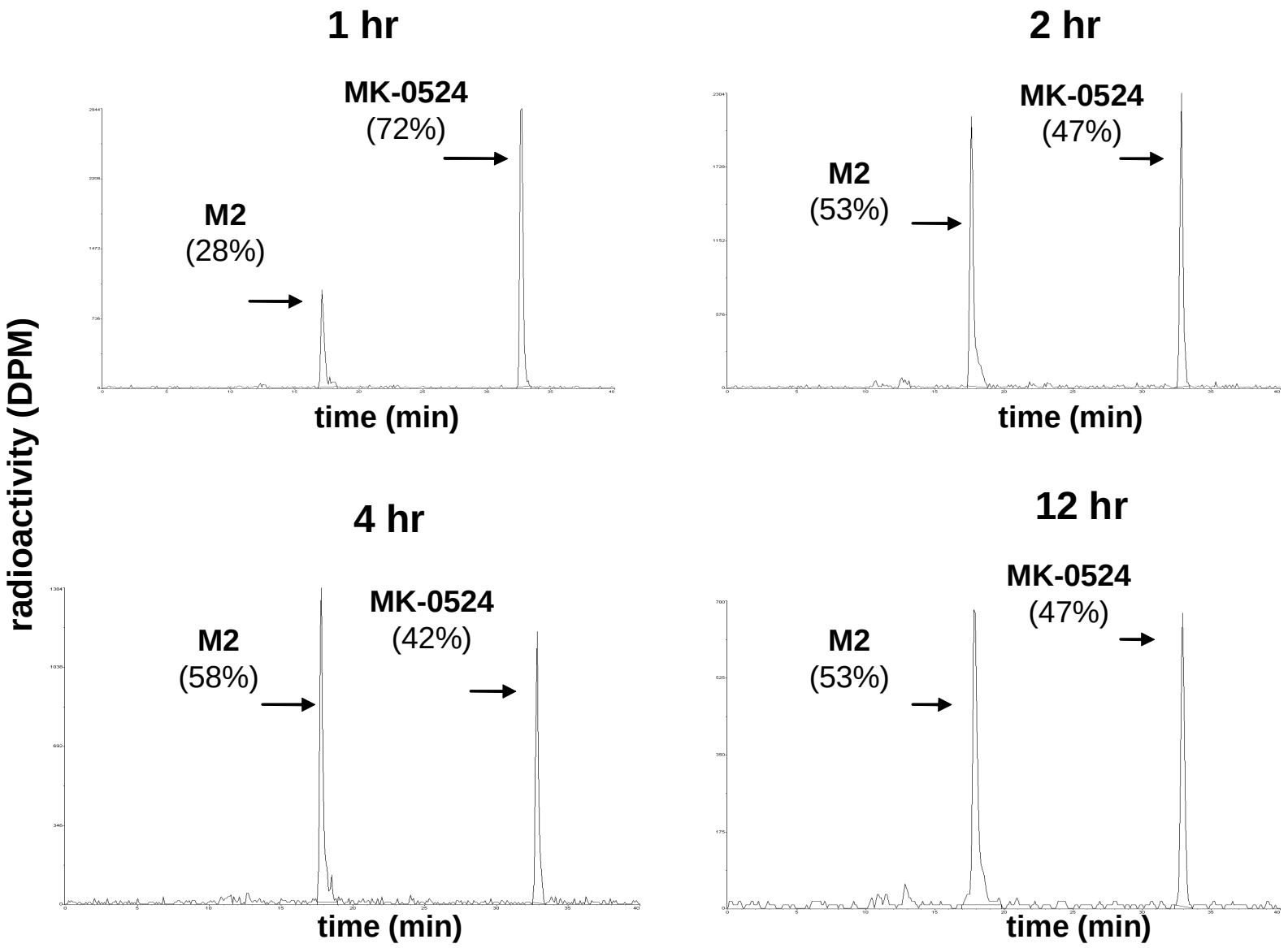
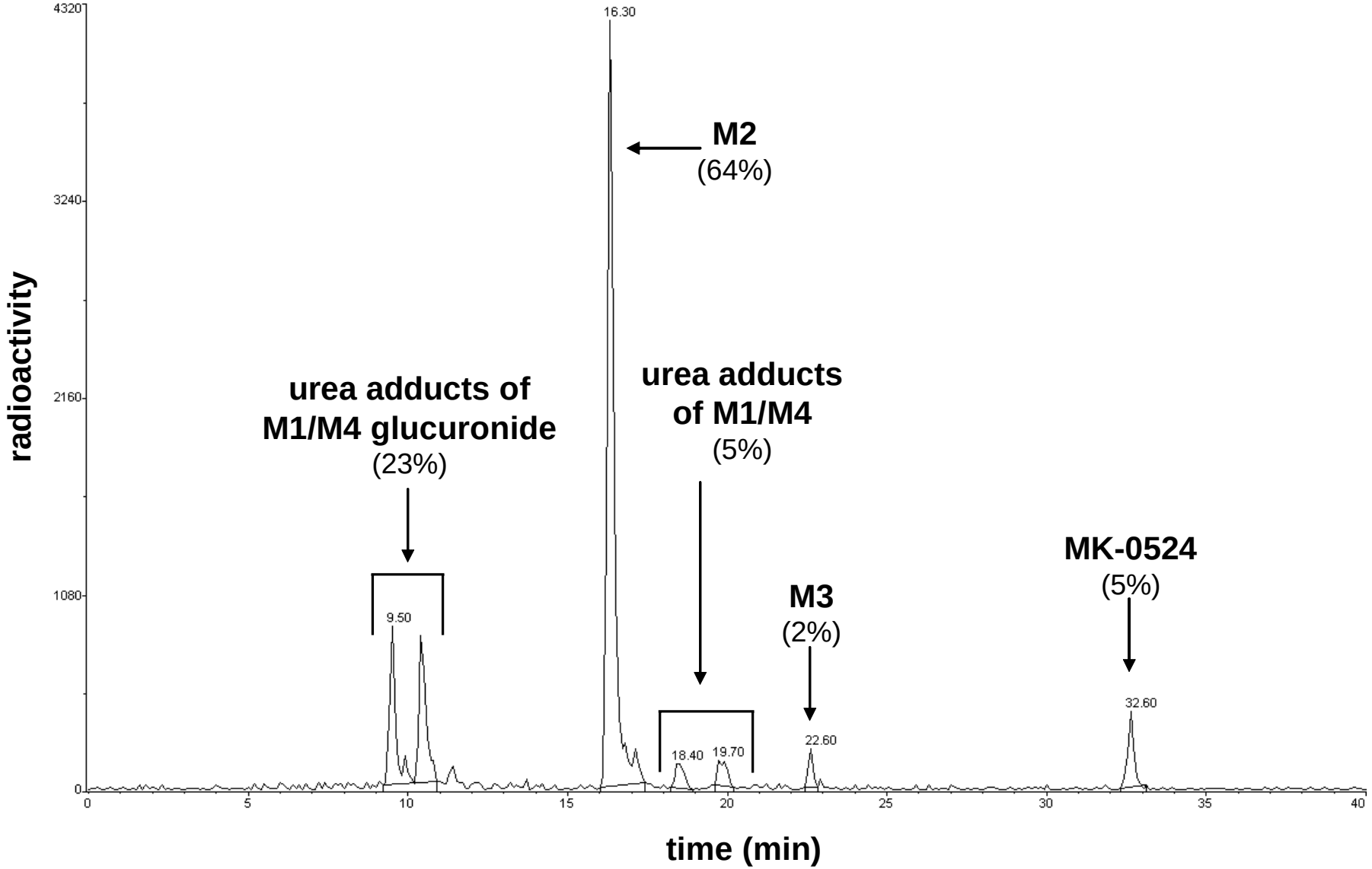


Figure 3



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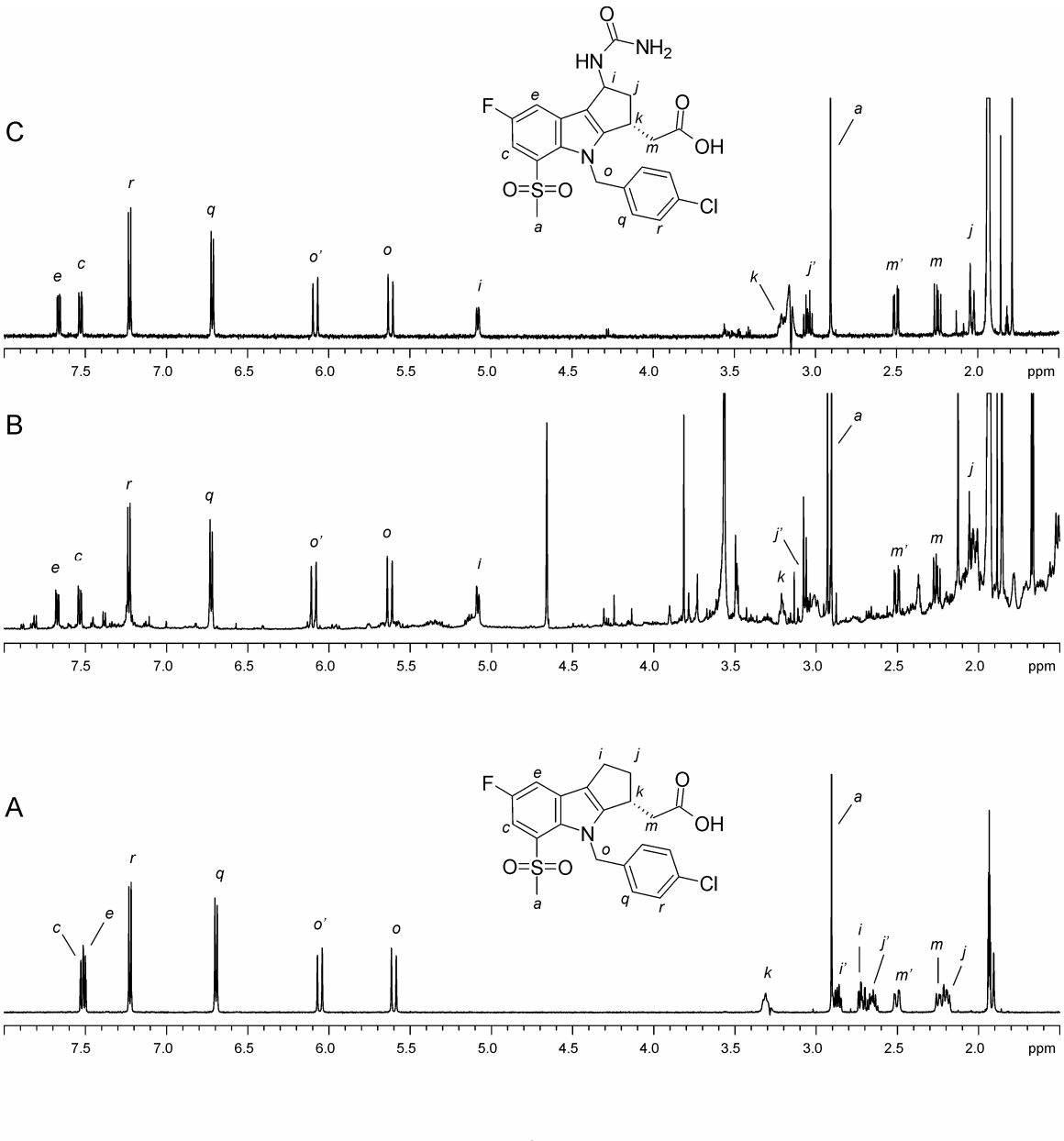

Figure 5

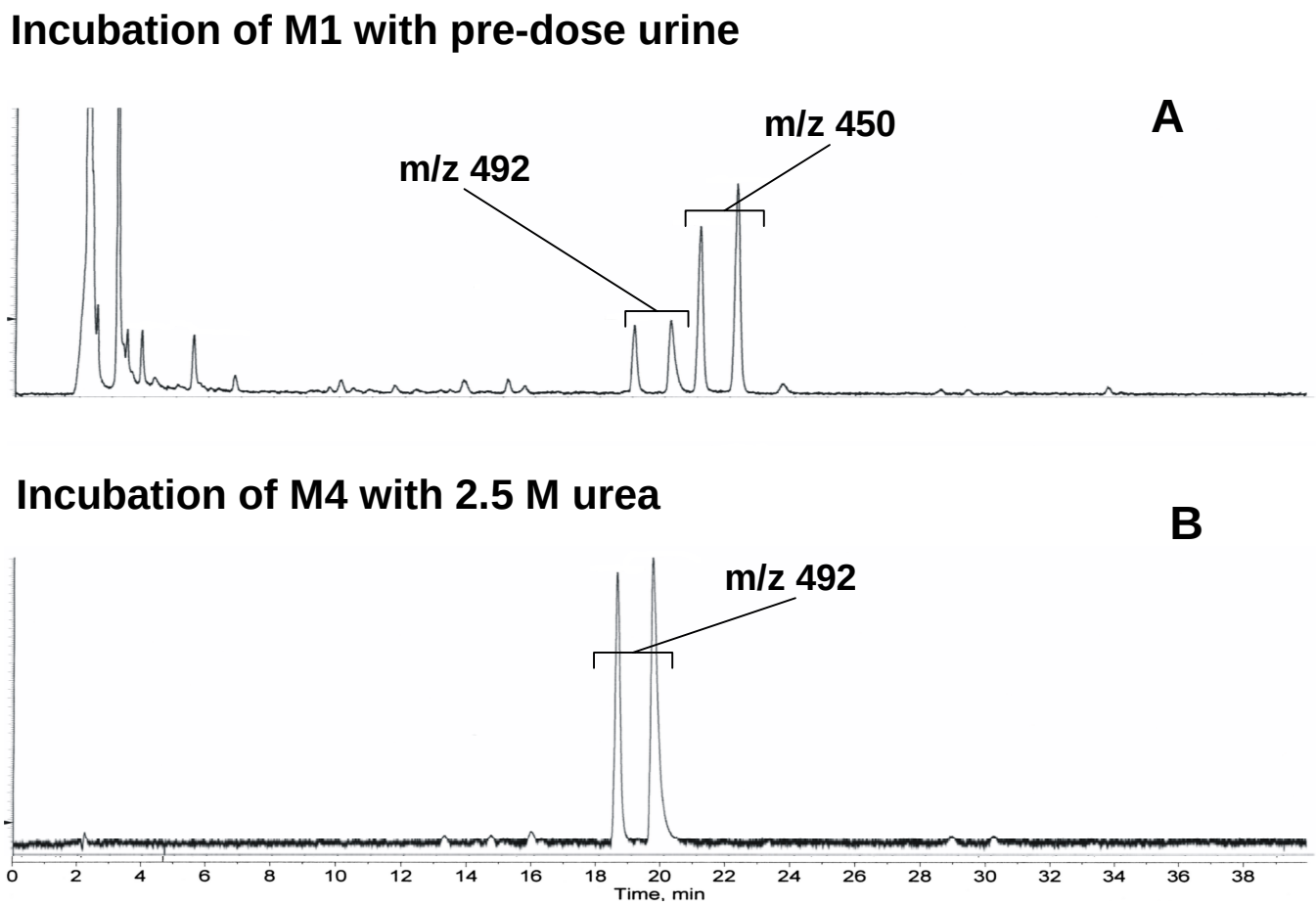

Figure 6

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Figure 7