ISOLATION AND IDENTIFICATION OF MAJOR URINARY METABOLITES OF RIFABUTIN IN RATS AND HUMANS

ILYA UTKIN, TATIANA KOUDBIAKOVA, TONI THOMPSON, CHARLES COTTRELL, EUGENIA IATSIMIRSKAIA, JOHN BARRY,
PAUL VOUROS, AND NICHOLAS GERBER

Department of Pharmacology, College of Medicine (I.U., T.K., E.I., N.G.) and Campus Chemical Instrument Center (C.C.),
The Ohio State University; and Department of Chemistry (T.T., J.B., P.V.), Northeastern University

(Received December 4, 1996; accepted April 24, 1997)

ABSTRACT:
The antitubercular drug rifabutin is extensively metabolized in humans and laboratory animals. About 40% of the dose is excreted in urine as unchanged drug, and lipophilic (extractable with 1-chlorobutane) and polar metabolites. Polar metabolites accounted for 59.1 ± 2.5% and 88.8 ± 4.4% of radioactivity in urine collected over 96 hr after intravenous administration of 25 and 1 mg/kg of [14C]rifabutin to Sprague-Dawley rats, respectively. After 48 hr, all urinary radioactivity consisted of polar metabolites. The most abundant polar metabolite, identified by electrospray ionization-MS, collision-induced dissociation-MS, and comparison of HPLC retention times with the synthetic standard, was N-isobutyl-4-hydroxy-piperidine. Lipophilic metabolites accounted for <20% of urinary radioactivity. Major lipophilic metabolites, 25-O-deacetyl-rifabutin, 27-O-demethyl-rifabutin, 31-hydroxy-rifabutin, 32-hydroxy-rifabutin, and 20-hydroxy-rifabutin were isolated from both human and rat urine by HPLC and identified by electrospray ionization-MS, collision-induced dissociation-MS, and NMR spectrometry. In addition, two metabolites formed by the oxidation of the N-isobutylpiperidyl group of rifabutin were found in the urine of rats, but not humans.

Rifabutin, 4-deoxy-3,4-[2-spiro-(N-isobutyl-4-piperidyl)]-1H-imidazo-(2,5-dihydro)-rifamycin S, is a semisynthetic derivative of rifamycin S (1), with a broad spectrum of antituberculosis activity in vitro (2–4). The drug has been used for the prevention of Mycobacterium avium complex in patients with AIDS (5–7) and for the treatment of tuberculosis (8–10).

Rifabutin is extensively metabolized in humans, monkeys, rabbits, and rats, and eliminated by both urinary and fecal excretion (11). The route of administration of [14C]rifabutin (po or iv) did not affect significantly the amount of total radioactivity or unchanged drug excreted in the urine or the pattern of urinary metabolites in female Sprague-Dawley rats (12). In humans, rifabutin is metabolized to >20 products (13). Several metabolites, isolated from human urine, were identified by MS and NMR as 25-O-deacetyl-rifabutin, 30-hydroxy-rifabutin, 31-hydroxy-rifabutin, 32-hydroxy-rifabutin, 32-hydroxy-25-O-deacetyl-rifabutin, and 25-O-deacetyl-rifabutin-N-oxide (13, 14). Battaglia et al. (11) reported the excretion of 31-hydroxy-rifabutin and 25-O-deacetyl-rifabutin in rat urine after administration of rifabutin, and noted that the HPLC profiles of urinary metabolites were similar in humans and rats. After a single po dose of [14C]rifabutin, the 0–24 hr urine of rats and humans contained 48 and 60%, respectively, of unidentified polar metabolites. These metabolites accounted for >93% of radioactivity in the 24- to 48-hr urine (11). Previous work from this laboratory (15) also showed that after iv administration of [14C]rifabutin to rats, the major portion of urinary radioactivity consisted of polar metabolites. However, these metabolites have not been characterized.

Recently, we reported briefly on the major lipophilic metabolites of rifabutin isolated from rat urine by HPLC (15). The current study describes in detail the isolation and identification of the major urinary polar metabolite of rifabutin in the rat and compares the major lipophilic metabolites of rifabutin in the urine of rats and humans.

Materials and Methods

Chemicals. Rifabutin, 25-O-deacetyl-rifabutin, and two analogs of rifabutin bearing the spiro-piperidyl group N-substituted with an isopropyl or heptyl chain were provided by Pharmacia (Dublin, OH). Rifampicin, rifamycin SV, and rifamycin B were purchased from Sigma Chemical Co. (St. Louis, MO). Starting material for the synthesis of [14C]rifabutin, 3-amino-4-deoxy-4-imino-rifamycin S, was a gift from Dr. Bruno Sardi (Pharmacia, Milan, Italy). Radiolabeled rifabutin (59.3 mCi/mmol) was synthesized at New England Nuclear (Boston, MA) as described by Fontana et al. (16) and Marsili et al. (1). The purity of rifabutin and [14C]rifabutin was >98% and >97%, respectively. N-isobutyl-4-hydroxy-piperidine was obtained by reduction of N-isobutyl-4-piperidone with sodium borohydride. N-isobutyl-4-piperidone was produced by acidic hydrolysis of rifabutin at 60°C for 3 hr as described elsewhere (14). Acetonitrile (HPLC grade), methanol, 1-chlorobutane, α-phosphoric acid, and liquid scintillation cocktail ScintiSafe Plus 50% were purchased from Fisher Scientific (Fair Lawn, NJ). Dowex 1, Dowex-50W, MRFA and apomyoglobin were purchased from Sigma. Charcoal was from MCB Manufacturing Chemists (Cincinnati, OH). Other chemicals were received from Sigma or J. T. Baker, Inc. (Phillipsburg, NJ).

Fourier transform-NMR spectra at 500 and 600 MHz were obtained at The Ohio State University Campus Chemical Instrument Center by Dr. C. E. Cottrell, using equipment funded by the National Institutes of Health Grants 1-S10-RR01458-01A1 and RR08299, and by the National Science Foundation Grant BIR-9921639. Mass spectra were obtained at the laboratory of Paul Vouros (Northeastern University) supported by the National Institutes of Health Grant IROICA 69390-01.

1 Abbreviations used are: po, oral; iv, intravenous; MRFA, L-methionyl-arginyl-phenylalanyl-alanine; TEA, triethylamine; RT, retention time; ESI-MS, electrospray ionization-mass spectrometry; CID-MS, collision-induced dissociation-mass spectrometry; COSY, correlated spectroscopy; HMQC, heteronuclear correlation through multiple quantum coherence.

Send reprint requests to: Dr. Ilya Utkin, Department of Pharmacology, The Ohio State University, 333 West 10th Avenue, 5084 Graves Hall, Columbus, OH 43210.
HPLC Analysis. All samples were analyzed on a Hewlett-Packard HPLC system (models 1050 or 1090 M, Palo Alto, CA) equipped with a UV diode-array detector. Radioactivity was measured on-line with a Radiomatic A-500 series Flow Scintillation Analyzer (Packard, Meriden, CT) with a solid scintillation flow cell. Separation was achieved using an analytical Hypersil C18 column (250 × 4.6 mm, 5 µm; Phenomenex, Torrance, CA) at a mobile phase flow rate of 1.5 ml/min. The mobile phases A, B, C, and D contained acetonitrile and/or one of the following aqueous solutions: 3% TEA and 0.3% trifluoroacetic acid titrated with phosphoric acid to pH 2.0 (A), 0.1% TEA and phosphoric acid (pH 6.8 –7.0) (B), 0.01% TEA and phosphoric acid (pH 7.0) (C), and 1.5% TEA and phosphoric acid (pH 6.0) (D).

Animals. Male Sprague-Dawley rats (Charles River Laboratories, North Wilmington, MA) weighing between 223 and 463 g, acclimated in a 12-hr light/dark cycle constant-temperature (24°C), and 1.5% TEA and phosphoric acid (pH 6.0). 10 min), and washed twice with 600 ml of 0.1 N KOH. The metabolites were separated by centrifugation (1200 g) by shaking for 10 min, and then separated by centrifugation (1200 g) by shaking for 10 min). The supernatant was filtered through a 0.2-µm filter, and injected (200 µl) directly into the HPLC. Rifabutin and metabolites were separated as described previously (15) using a linear gradient of 27–42% acetonitrile in aqueous solution A over 45 min (fig. 1A). Separation of individual polar metabolites designated as PM1 and PM2 from the peak labeled P in fig. 1 was achieved by using 100% aqueous solution B as a mobile phase (fig. 2). Total radioactivity in urine was measured by liquid scintillation counting (100 µl) using scintillation cocktail ScintiSafe Plus 50% (5 ml).

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine (200 µl) was injected directly into the HPLC and metabolites separated using a linear gradient of 27–42% acetonitrile in aqueous solution A over 45 min. P, peak of polar metabolites; R, rifabutin; U1 (25-O-deacetyl-rifabutin); U2 (31-hydroxy-rifabutin + 32-hydroxy-rifabutin); U3 (27-O-demethyl-rifabutin); U4 (20-hydroxy-rifabutin); U5 (a mixture of two metabolites of rifabutin resulting from hydroxylation in the N-isobutyl-piperidyl group).

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.

Isolation of Lipophilic Metabolites. The 1-chlorobutane extract of rat urine was dried, the residue dissolved in mobile phase of rifabutin and lipophilic metabolites was removed and dried under nitrogen.
A dose of 25 mg/kg was injected. Two metabolites were derived from each of peaks U2 (U2A and U2B) and U5 (U5A and U5B). The other three peaks (U1, U3, and U4) contained only one major metabolite each.

**Human Urinary Metabolites.** After fasting for 8 hr, 10 healthy male volunteers, aged 18–45 years, were each given a single oral dose of 300 mg of rifabutin (two 150 mg capsules of Mycobutin). Urine was collected 0–12 hr before the dose and for 0–48 hr after the dose and stored at −20°C. The protocol was approved by The Ohio State University Human Subjects Biomedical Sciences Review Committee.

For metabolic profiles, samples (0.4 ml) of predose and 0–48 hr urine from each subject were mixed with an equal volume of 2 mol of potassium phosphate buffer (pH 7.0) and extracted with 2 ml of 1-chlorobutane. The organic phase was dried under nitrogen at 40°C. The residues were redissolved in 75 μl of mobile phase, 40 μl was injected into the HPLC, and the metabolites were separated as described for the rat.

To isolate metabolites, 1.8 liters of urine pooled from all subjects was supplemented with 1.8 ml of isomyl alcohol and extracted 3 times with 200 ml of 1-chlorobutane by shaking in a separatory flask. The combined organic phase was dried under nitrogen at 40°C and metabolites isolated by HPLC as described for rat lipophilic metabolites.

**MS.** ESI mass spectra were obtained using a TSQ 700 mass spectrometer (Finnigan MAT, San Jose, CA). Samples were dissolved in a 50% (v/v) solution of methanol in deionized water. The molecular mass of compounds was determined by scanning the first quadrupole in the profile mode from m/z 10 to m/z 1000. For the analysis of lipophilic metabolites, the ESI mass spectrometer was tuned and calibrated using MRFA and apomyoglobin tune standard. To analyze PM2, the first quadrupole was tuned with triethanolamine as well as sodium [M+Na]+ and potassium [M+K]+ adducts. The fragmentation of rifabutin ESI-MS of the major polar metabolite PM2 showed [M+H]+ at m/z 158. The CID spectrum of the m/z 158 ion is consistent with an assignment of a structure for the PM2 metabolite corresponding to hydroxylation of the piperidine ring, possibly forming N-isobutyl-4-hydroxy-piperidine (fig. 3). The HPLC RT of PM2 matched that of synthetic N-isobutyl-4-hydroxy-piperidine that confirmed this structural assignment.

**Lipophilic Metabolites.** Radiochemical and spectrophotometric detection (275 nm) of rifabutin and lipophilic metabolites yielded similar HPLC profiles, suggesting that rifabutin and lipophilic metabolites had similar extinction coefficients. ESI-MS of rifabutin and the metabolites U1, U2A, U2B, U3, U4, U5A, and U5B produced a protonated molecular ion [M+H]+ as well as sodium [M+Na]+ and potassium [M+K]+ adducts. The fragmentation of rifabutin
O-deacetyl-rifabutin. Thus, metabolite U1 was identified based on the mass spectra of this metabolite matched those of the authentic standard of O-deacetyl-rifabutin, indicating the loss of the acetyl group. The HPLC RT, ESI and CID spectra of U1 and U3 were obtained using argon with a collision energy of 25 eV. For the other metabolites, collision energy was set at 51 eV.

Diagnostic daughter ions in the CID spectrum of U3 matched those of rifabutin (fig. 5). Furthermore, the signal of proton H37 observed in the 1H NMR spectrum of rifabutin (s at 3.06 ppm) was missing in the spectrum of U3. On this basis, U3 was identified as 27-O-demethyl-rifabutin.

Metabolites U2A and U2B. The daughter ions of U2A and U2B at m/z 112 and 423 corresponding to the unchanged fragments G and F (table 1, fig. 4), respectively, suggest no modification on that part of the molecule for either metabolite. For U2A, the diagnostic fragments at m/z 561 (545 + 16), 589 (573 + 16), and 648 (632 + 16) pointed to carbons C31, C20, C19, C18, C17, C16, and C30 as possible oxidation sites (table 1, fig. 4). In an HMBC experiment, the disappearance of the C31 signal at 17.4 ppm and correlated H31 signal at 0.85 ppm were accompanied by the appearance of a carbon signal at 63 ppm correlated with a proton signal at 3.54–3.60 ppm (tables 2 and 3). Daughter ions of U2B at m/z 545, 573, and 648 (632 + 16) implicated atoms C32, C32, and C31 as possible oxidation sites (table 1, fig. 4). The signal corresponding to H32 of parent drug (d at 1.04 ppm) disappeared, and a proton signal (3.80 and 3.98 ppm) appeared that correlated with H22 at 1.78 ppm in 1H-1H COSY and with C32 at 61.9 ppm in 1H-13C HMBC experiments (tables 2 and 3). The downfield shifts and splitting of the H31 and H32 signals (table 2) in 1H NMR spectra of U2A and U2B, respectively, suggest hydroxylation of the corresponding methyl groups. Hydroxylation caused the methylene protons to become nonequivalent (appearing as an AB of ABX pattern), because they are adjacent to a chiral center. Consequently, splitting of the proton signals occurred. Downfield shifts of the corresponding C31 and C32 signals (table 3) confirm that hydroxylation occurred in the aforementioned positions. Thus, the metabolites U2A and U2B are 31-hydroxy-rifabutin and 32-hydroxy-rifabutin, respectively.

Metabolite U4. Diagnostic fragments observed in the CID spectrum of U4 at m/z 423 and m/z 561 (545 + 16) indicated that an oxidation site was located in the ansa chain of rifabutin between carbons C15 and C31 (table 1, fig. 4). In the 1H NMR spectrum of U4, the H20 signal disappeared; the signals of protons H19 and H31 became a doublet and a singlet, respectively; and there were no changes in the chemical shifts and multiplicities of H17 and H18 (table 2). In an 1H-13C COSY experiment, signals of coupling interactions between protons H30 and H41, H30 and H19, and H20 and H31 disappeared (fig. 6). Also, no signal was observed for C50 in an HMBC experiment, confirming that there is no attached proton, and C31 shifted downfield.

Table 1: Diagnostic daughter ions in CID spectra of rifabutin and urinary metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>[M + H]^+</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifabutin</td>
<td>847</td>
<td>815 (100)</td>
<td>755 (17)</td>
<td>632 (0.8)</td>
<td>573 (0.4)</td>
<td>545 (0.2)</td>
<td>423 (1.5)</td>
<td>112 (13)</td>
</tr>
<tr>
<td>U1</td>
<td>805</td>
<td>773 (100)</td>
<td>755 (1.4)</td>
<td>632 (0.06)</td>
<td>573 (0.02)</td>
<td>545 (0.01)</td>
<td>423 (0.6)</td>
<td>112 (2.7)</td>
</tr>
<tr>
<td>U2A</td>
<td>863</td>
<td>831 (100)</td>
<td>771 (16)</td>
<td>648 (1.2)</td>
<td>589 (0.2)</td>
<td>561 (0.06)</td>
<td>423 (1.0)</td>
<td>112 (6.4)</td>
</tr>
<tr>
<td>U2B</td>
<td>863</td>
<td>831 (40)</td>
<td>771 (7.9)</td>
<td>648 (0.3)</td>
<td>573 (2.2)</td>
<td>545 (0.9)</td>
<td>423 (13)</td>
<td>112 (93)</td>
</tr>
<tr>
<td>U3</td>
<td>833</td>
<td>815 (100)</td>
<td>755 (19)</td>
<td>632 (4.4)</td>
<td>573 (2.4)</td>
<td>545 (6.5)</td>
<td>423 (7.2)</td>
<td>112 (19)</td>
</tr>
<tr>
<td>U4</td>
<td>863</td>
<td>831 (23)</td>
<td>ND*</td>
<td>ND*</td>
<td>545 (0.2)</td>
<td>423 (0.7)</td>
<td>112 (8.6)</td>
<td></td>
</tr>
<tr>
<td>U5A</td>
<td>863</td>
<td>831 (100)</td>
<td>771 (15)</td>
<td>648 (1.4)</td>
<td>589 (0.4)</td>
<td>561 (0.6)</td>
<td>439 (0.4)</td>
<td>128 (17)</td>
</tr>
<tr>
<td>U5B</td>
<td>863</td>
<td>831 (100)</td>
<td>771 (21)</td>
<td>648 (1.8)</td>
<td>589 (0.7)</td>
<td>NA^b</td>
<td>439 (1.3)</td>
<td>128 (4.7)</td>
</tr>
</tbody>
</table>

CID spectra of U1 and U3 were obtained using argon with a collision energy of −81 and −61 eV, respectively. For the other metabolites, collision energy was set at −51 eV.

a ND, not detected.
b NA, no data available.
to 30 ppm (table 3). Thus, it is concluded that hydroxylation occurred at carbon C20, and metabolite U4 is assigned the structure of 20-hydroxy-rifabutin.

Metabolites U5A and U5B. There was a 16 mass units increase in the mass of all characteristic fragments observed in the CID spectra of U5A and U5B, compared with the parent drug (table 1), thus indicating that these metabolites contained an additional oxygen atom in the N-isobutyl-piperidyl moiety (fig. 4). The lack of authentic standards and difficulties in interpretation of the CID and NMR spectra did not permit a more complete structural characterization of these metabolites.

Human Urinary Metabolites. Figure 7A shows a typical HPLC profile of lipophilic metabolites of rifabutin extracted with 1-chlorobutane from 0- to 48-hr human urine. Individual peaks were collected and rechromatographed in a gradient of 30–50% acetonitrile in aqueous solution D (see Materials and Methods) for 1 hr. This demonstrated that HU1, HU3, and HU4 consisted of one major metabolite each, whereas HU2 was composed of two compounds designated as HU2A and HU2B. Chemical identity of the metabolites HU1, HU2A, HU2B, HU3, and HU4 with the corresponding rat metabolites U1, U2A, U2B, U3, and U4 was established by comparison of HPLC RTs, NMR, and/or mass spectra. Thus, the major lipophilic metabolites were identified as 25-O-deacetyl-rifabutin, 31-hydroxy-rifabutin, 32-hydroxy-rifabutin, 27-O-demethyl-rifabutin, and 20-hydroxy-rifabutin.

ESI-MS of metabolite HU0 showed a [M + H]+ at m/z 821 suggesting the loss of the acetyl group and the presence of an additional oxygen atom. The loss of the acetyl group was confirmed by the absence of the H36 signal in the 1H NMR spectrum of HU0. The unchanged fragment F at m/z of 423 in the CID spectrum of HU0 indicated that oxidation occurred in the ansa chain. However, the amount of HU0 was insufficient for complete characterization by NMR and MS.

Discussion

Our results showed that radiolabeled polar metabolites PM1 and PM2 (figs. 1 and 2) accounted for most of the radioactivity excreted in rat urine after iv administration of [14C]rifabutin. The retention of radiolabel indicated that these polar metabolites were derived from the N-isobutyl-piperidyl group of rifabutin (fig. 4) and, possibly, its lipophilic metabolites. PM2 was identified as N-isobutyl-4-hydroxy-piperidine. Similarity of physiochemical properties of PM1 and PM2 (chemical stability, binding to a strong cation exchanger and activated charcoal, and the absence of chromophoric groups) suggested that the more polar metabolite PM1 is a hydroxy derivative of PM2. The
metabolite PM1 could not be identified conclusively because of the lack of essential synthetic standards.

The proportion of different urinary metabolites of rifabutin in the rat was dose-dependent. The fraction of the dose excreted in urine as polar metabolites was lower at a higher (25 mg/kg) dose than at a lower (1 mg/kg) dose apparently due to saturation of enzymatic and/or transport systems involved in the formation and excretion of these metabolites. This is in agreement with our previous findings (15) that first-pass metabolism of rifabutin was more significant at the lower dose (bioavailability after po administration of 25 mg/kg and 1 mg/kg of rifabutin was >90% and 44%, respectively).

The proportion of polar metabolites was higher in urine collected at later times; unchanged drug and lipophilic metabolites were not detected in 48- to 96-hr urine. Large amounts of polar metabolites in urine and an increase in their percentage at later times were observed previously after po administration of [14C]rifabutin to humans and rats (11) and after iv administration to rats (12). The predominant urinary excretion of radioactivity as polar metabolites indicates that the cleavage of the N-isobutyl-piperidyl group plays a major role in renal elimination of rifabutin after iv and po administration of the drug.

The mechanism of PM2 formation in vivo is unclear. We hypothesize that it is a two-step process that includes oxidative N-dealkylation of rifabutin by cytochrome P450 to form N-isobutyl-4-piperidone and subsequent reduction of N-isobutyl-4-piperidone to N-isobutyl-4-hydroxy-piperidine by alcohol dehydrogenase or carbonyl reductase.

It has been shown that polar metabolite(s) of rifabutin are present in human urine (11) and also in incubations of the drug with human liver and enterocyte microsomes (17). However, the identity of these metabolites remains to be established conclusively.

In previous work (11), two rat urinary metabolites were identified as 25-O-deacetyl-rifabutin and 31-hydroxy-rifabutin by comparison of their HPLC RTs with those of authentic standards. In addition to these metabolites, we found in rat urine 32-hydroxy-rifabutin, 27-O-demethyl-rifabutin, 20-hydroxy-rifabutin, and two products that resulted from oxidation of the N-isobutyl-piperidyl group.

Five rifabutin metabolites were isolated from human urine and identified in this study as 25-O-deacetyl-rifabutin, 31-hydroxy-rifabutin, 32-hydroxy-rifabutin, 20-hydroxy-rifabutin, and 27-O-demethyl-rifabutin. The first three have been described previously (13, 14),
MAJOR URINARY METABOLITES OF RIFABUTIN

TABLE 4

Relative amounts* of rifabutin metabolites as a % of unchanged drug in human urine (0–48 hr) after a single dose of 300 mg of rifabutin

<table>
<thead>
<tr>
<th>Rifabutin</th>
<th>HU1</th>
<th>HU2</th>
<th>HU3</th>
<th>HU4</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ± 29</td>
<td>29 ± 16</td>
<td>9.4 ± 3.3</td>
<td>6.0 ± 2.0</td>
<td>36 ± 10</td>
</tr>
</tbody>
</table>

Urinary metabolites of rifabutin and unchanged drug were extracted with 1-chlorobutane and separated by HPLC in a gradient of 27–42% acetonitrile in aqueous phase A over 45 min as described in Materials and Methods.

* Values are means ± SD for 10 subjects.

About 5% of the dose is excreted in urine as rifabutin (11).

References


