

A Pharmacokinetic Study of [ $^{14}\text{C}$ ]SC-19129  
([ $^{14}\text{C}$ ]Beta-Aspartame) in Man

Department of Drug Metabolism  
Research and Development Division - G.D. Searle & Co.

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A Pharmacokinetic Study of [ $^{14}\text{C}$ ]SC-19129  
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I. Abstract

Six male human subjects received a single oral dose of [ $^{14}\text{C}$ ]SC-19129, dissolved in water. One subject was part of a pilot study and provided samples used to develop methods and generate data to design the study with the additional five subjects. The pilot subject received 40.0 mg of [ $^{14}\text{C}$ ]SC-19129. The five other subjects received  $31.9 \pm 0.1$  mg (mean  $\pm$  SEM) of [ $^{14}\text{C}$ ]SC-19129. The results summarized below are for the five subject study unless otherwise indicated.

1. More than 90% of the orally administered radioactive dose of [ $^{14}\text{C}$ ]SC-19129 was absorbed by man based on the low recovery of compound-related radioactivity in the feces ( $9.6 \pm 5.4\%$  of the dose).
2. Minimal amount of [ $^{14}\text{C}$ ]SC-19129 were absorbed with the methyl ester bond intact. [ $^{14}\text{C}$ ]SC-19129 could not be detected in plasma and very small amounts (less than 0.15% of the dose) were excreted into the urine. [ $^{14}\text{C}$ ]SC-19200, the free acid of [ $^{14}\text{C}$ ]SC-19129, was a major metabolite observed in plasma and urine.
3. Peak concentrations ( $\text{C}_{\text{max}}$ ) of total radioactivity ( $0.448 \pm 0.071$  mcg equivalents of [ $^{14}\text{C}$ ]SC-19129) occurred  $5.5 \pm 0.7$  hours ( $\text{T}_{\text{max}}$ ) after dosing. In contrast, maximal concentrations of [ $^{14}\text{C}$ ]SC-19200 (0.072 mcg/ml) were measured in pooled plasma samples at 1.5 hours after oral dosing. The

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differences in the Cmaxs and Tmaxs for total radioactivity and [ $^{14}\text{C}$ ]SC-19200 probably are a consequence of the incorporation of  $^{14}\text{C}$ -phenylalanine, released during the metabolism of [ $^{14}\text{C}$ ]SC-19129 and [ $^{14}\text{C}$ ]SC-19200, into plasma proteins.

4. Plasma total radioactivity was eliminated in two phases with half-lives of  $6.2 \pm 0.7$  hours and  $587 \pm 99$  hours. The area under the plasma concentration-time curve (AUC) for total radioactivity was  $154 \pm 24$  (mcg/ml) hours. By contrast, the elimination half-life of [ $^{14}\text{C}$ ]SC-19200 was 1.1 hours and the AUC was 0.22 (mcg/ml) hours. The large AUC and prolonged elimination half-life for total radioactivity also are consistent with the incorporation of  $^{14}\text{C}$ -phenylalanine into plasma proteins.
5. Concentrations of total radioactivity increased gradually in red blood cells. The ratios of total radioactivity in red blood cells (mcg equivalents/g) to total radioactivity in plasma (mcg equivalents/ml) were approximately 0.9 on day 7 and approximately 1.5 on day 36 for the pilot subject. These data also indicate the incorporation of  $^{14}\text{C}$ -phenylalanine into protein, in this case protein of erythrocytes.
6. The major route of elimination of total radioactivity following orally administered [ $^{14}\text{C}$ ]SC-19129 was the kidney. By 168 hours  $42.0 \pm 4.0\%$  of the radioactive dose of [ $^{14}\text{C}$ ]SC-19129 was

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excreted in the urine;  $9.6 \pm 5.4\%$  of the dose was recovered in the feces.

7. [ $^{14}\text{C}$ ]SC-19200 and  $^{14}\text{C}$ -phenylacetylglutamine ( $^{14}\text{C}$ -PAGln) were the major metabolites recovered in the 0-24 hour urine, accounting for more than 95% of the radioactivity in the urine and  $6.97 \pm 2.05$  and  $30.8 \pm 3.4\%$  of the radioactive dose of [ $^{14}\text{C}$ ]SC-19129, respectively. Therefore, based on the urinary excretion of [ $^{14}\text{C}$ ]SC-19200, 6.97% of the orally administered dose of [ $^{14}\text{C}$ ]-SC-19129 was absorbed with the peptide bond intact.

In addition, to [ $^{14}\text{C}$ ]SC-19200, phenylalanine and PAGln were the only other metabolites observed in plasma. By 8 hours after dosing PAGln was the sole metabolite found in plasma.

[ $^{14}\text{C}$ ]-Phenylalanine was the only metabolite recovered from feces. When [ $^{14}\text{C}$ ]SC-19200 was added to control feces, it was completely converted to  $^{14}\text{C}$ -phenylalanine, suggesting extensive bacterial metabolism in the lower gastrointestinal tract capable of converting [ $^{14}\text{C}$ ]SC-19200 to phenylalanine. Released phenylalanine could then either be absorbed or converted to PAGln by bacterial metabolism. The large percentage of PAGln (normally a minor metabolite of phenylalanine in mammals, including man) excreted into the urine suggests that bacterial metabolism in the lower gastrointestinal tract, with the release of



phenylalanine and its subsequent conversion to phenylacetic acid and PAGln, is an important pathway for the metabolism of [ $^{14}\text{C}$ ]SC-19200 (and [ $^{14}\text{C}$ ]SC-19129) in man.

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([ $^{14}\text{C}$ ]Beta-Aspartame) in Man

II. Introduction

SC-19129 (N-L- $\beta$ -aspartyl-L-phenylalanine, 1-methyl ester, beta-aspartame,  $\beta$ -APM) and SC-19200 (N-L- $\beta$ -aspartyl-L-phenylalanine,  $\beta$ -AP) are formed (1) during storage of beverages containing aspartame (SC-18862, N-L- $\alpha$ -aspartyl-L-phenylalanine methyl ester, APM). The structures of SC-19129 and its free acid, SC-19200, are shown in Figure 1. The pharmacokinetics and metabolism of orally administered [ $^{14}\text{C}$ ]SC-19129 have been examined in the rhesus monkey (2), rat (3), rabbit (4) and dog (5). In addition, plasma and urinary concentrations of total radioactivity and major metabolites were determined in pregnant rats administered [ $^{14}\text{C}$ ]SC-19129 in dietary admix (6) and the tissue distribution of total radioactivity was determined in male rats following oral administration of [ $^{14}\text{C}$ ]SC-19129 (7). SC-19200 has been shown to be an endogenous constituent in human plasma and urine in the absence of aspartame consumption (8). The major conclusions reached in the previous studies with orally administered [ $^{14}\text{C}$ ]SC-19129 were:

- The ester bond of SC-19129 is extensively hydrolyzed in the gastrointestinal tract or liver and little, if any, SC-19129 reaches the systemic circulation intact.
- Approximately 7% to 20% of orally administered SC-19129 reaches the systemic circulation in the form of its free acid, SC-19200.

- Peak plasma concentrations of [ $^{14}\text{C}$ ]SC-19200 following an oral dose of 10 mg [ $^{14}\text{C}$ ]SC-19129 per kg are 0.4 to 0.7 mcg/ml in rats, rabbits and monkeys and approximately 1.6 mcg/ml in the dog.
- SC-19200 is eliminated from plasma with a terminal half life of approximately 1 hour or less.
- Absorption of [ $^{14}\text{C}$ ]SC-19200 and other metabolites from orally administered [ $^{14}\text{C}$ ]SC-19129, estimated from total radioactivity, reaches approximately 90% of dose.
- The major metabolites of [ $^{14}\text{C}$ ]SC-19200 are phenylalanine, N-acetyl-SC-19200 and either the glycine (rats, rabbits) or glutamine (rhesus monkey) conjugate of phenylacetic acid (Figure 1).
- The terminal plasma half-life of total radioactivity ranges from approximately 80 hours in the rat to approximately 340 hours in the dog. The slow elimination of plasma radioactivity appears to be due to incorporation of [ $^{14}\text{C}$ ]phenylalanine into plasma and tissue proteins (9-11).
- The major routes of elimination of total radioactivity are in urine and in breath (expired  $^{14}\text{CO}_2$ ).
- SC-19200 is found endogenously in urine in healthy human subjects who have not consumed aspartame. The daily urinary excretion is approximately 0.8 mg.

The objective of this study was to determine the pharmacokinetics and metabolism of orally administered [ $^{14}\text{C}$ ]SC-19129 in man.

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### III. Materials and Methods

#### A. Overview of Study Design:

Six healthy male subjects received single, oral doses of [ $^{14}\text{C}$ ]SC-19129, administered as a solution in water, during this study. The subjects were divided into two groups. The first group consisted of one subject (pilot subject) given a dose of 40.0 mg. Urine and plasma samples were collected to obtain a data base to develop the methodology and study design for the remaining subjects. The second group of 5 subjects (five subject study) were administered  $31.9 \pm 0.1$  mg (mean  $\pm$  standard error; see Section IV.A and Table 1A) of [ $^{14}\text{C}$ ]SC-19129. Blood, urine and fecal samples were collected from the 5 men for 168 hours. An additional blood sample was obtained from each subject at 504 hours (day 22) to increase the accuracy of determination of the terminal half-life of total radioactivity in plasma.

A complete description of the clinical aspects of the study, is given in the clinical protocol (12).

#### B. Test Article and Dosage Form:

[U- $^{14}\text{C}$ -Phe]SC-19129 (lot MRC-332-43-1), having a specific activity of 1.51 mCi/mmol (5.13 mCi/mg) and a radiochemical purity of 99.0%, was prepared by the Radiochemistry Group, G.D. Searle & Co. (13). Twelve bottles, each containing  $200 \pm 5$  mCi and  $40 \pm 1$  mg of [ $^{14}\text{C}$ ]SC-19129 were prepared by the Radiochemistry Group, G.D. Searle & Co., and shipped to the study site. The dosage form was prepared by dissolving the [ $^{14}\text{C}$ ]SC-19129 in a bottle in water immediately before dosing.

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C. Sample Collection and Storage:

A. Plasma and Red Blood Cells:

Blood samples were collected, as described in the protocol (12), in heparinized tubes containing the esterase inhibitor, paraoxon (diethyl p-nitrophenyl phosphate). Plasma and red blood cells were separated by centrifugation. Plasma was stored on dry ice or at approximately -20°C, and red blood cells were stored on ice or at approximately 4°C, until analysis.

B. Urine and Feces:

Urine and fecal samples were collected and stored frozen as described in the protocol (12).

D. Sample Analysis:

1. Dosage Form:

Aliquots of unused dosing solution remaining after dose administration to subjects 2 through 6 (main group) were taken for determinations of total radioactivity by liquid scintillation spectrometry (LSC; Section III.H). The radiochemical purity of [ $^{14}\text{C}$ ]SC-19129 in two of the six unused bottles remaining after dose administration to the 6 subjects was determined by high performance liquid radiochromatography on a C8 reversed-phase column (13).

2. Plasma:

Total  $^{14}\text{C}$  was determined by LSC (Section III.H) using duplicate 0.05 or 0.10 ml aliquots.

Pooled plasma samples from selected time points

were prepared for HPLRC analysis (Section III.E) by dividing 1.0 ml into two 0.5 ml aliquots and mixing each 0.5 ml aliquot with 1.0 ml of acetonitrile:phosphoric acid (99:1, v/v) in a 1.5 ml micro test tube (Brinkman Instruments Co., Westbury, NY). The tubes were centrifuged for 5 minutes at 15,600 x g (Eppendorf Model 5414 Microcentrifuge, Brinkman Instruments Co., Westbury, NY) and the supernatants from the duplicate aliquots were combined and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.30 ml of HPLRC mobile phase (Section III.E), and 0.050 ml of 0.20 M heptanesulfonic acid, and an aliquot (0.15 ml) was analyzed by HPLRC (Section III.E).

3. Urine:

Total  $^{14}\text{C}$  in duplicate aliquots of each urine sample was determined by LSC (Section III.H). Aliquots (1.0 ml) of selected urine samples were mixed with 0.15 ml of 0.2 M heptanesulfonic acid and 0.01 ml of concentrated (85%, w/v) phosphoric acid:water (1:1, v/v). The solution was filtered through a 0.45 micron filter (Millex<sup>R</sup> -HA, Millipore Corp., Bedford, MA) and a 0.50 ml aliquot was analyzed by HPLRC (Section III.E).

4. Red Blood Cells:

Packed red blood cells (approximately 0.5 to 1.0 g) were placed in Combustococones<sup>TM</sup> (Packard Instruments Co., Downers Grove, IL) and allowed to dry at room temperature. Duplicate samples were oxidized with a Packard Tri-Carb Sample Oxidizer (Packard Model 306, Packard Instruments Co., Downers Grove, IL). Total  $^{14}\text{C}$  in the combustion products was determined by LSC (Section III.H).

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5. Feces:

Fecal samples were homogenized, without added water, using a blender (Stomacher Lab-Blender 80, Tekmar Co., Cincinnati, OH). Triplicate aliquots (0.75 - 1.2 g) of homogenate were dried in Combustocoones<sup>TM</sup> and total <sup>14</sup>C was determined by sample oxidation as described for red blood cells (Section III.D.4). A 10 ml aliquot of fecal homogenate and 25 ml of water were combined and mixed for 15 minutes (Rugged Rotator<sup>TM</sup>, Kraft Apparatus, Inc., Mineola, NY). The mixture was centrifuged at 3000 x g for 5 minutes and an aliquot of the supernatant was centrifuged at 15,600 x g for 15 minutes. The supernatant from the second centrifugation was filtered as described for urine (Section III.D.3) and an aliquot was analyzed by HPLRC (Section III.E).

E. High Performance Liquid Radiochromatography (HPLRC):

The standard system used to determine the distribution (profile) of radioactivity in plasma urine and fecal extracts consisted of a NOVA Pak<sup>TM</sup> C18 column (15 cm x 3.9 mm; Waters Associates, Milford, MA) and a mobile phase consisting of 0.020 M heptane sulfonic acid containing 0.001 M dimethyloctylamine and 0.040 M phosphoric acid:acetonitrile (90:10; v/v). The column was eluted at a flow rate of 1 ml/min. The eluate was collected as 0.3 minute aliquots (Foxy<sup>TM</sup> fraction collector, ISCO Inc., Lincoln, NE) and total <sup>14</sup>C in the fractions was determined by LSC (Section III.H). Unlabeled standards of SC-19129, SC-19200, phenylalanine and phenylacetylglutamine, used to calibrate the HPLRC system, were detected by absorbance at 222 nm (Model 480 detector, Waters Associates, Milford, MA).

Selected samples were also analyzed, to examine specificity of the standard system described above, using a Zorbax ODS column (15 cm x 4.6 mm, Dupont Instruments, Wilmington, DE) and a mobile phase consisting of 0.020 M sodium phosphate, pH 3.0:methanol (80:20; v/v). The column was maintained at a temperature of 40°C and a flow rate of 1 ml/min.

F. Isolation of [<sup>14</sup>C]SC-19129 from Urine:

A 500 mg C18 Bond Elut<sup>TM</sup> column (Analytichem International, Inc., Harbor City, CA) was conditioned by washing with 1 column volume each of acetonitrile and 0.02 M phosphoric acid. A 10 to 20 ml aliquot (depending on concentration of radioactivity) of a 0-1 hour urine sample was applied to the column which was then eluted sequentially with 1) 6 ml of 0.02 M phosphoric acid, 2) 0.5 ml of acetonitrile, 3) 0.5 ml of acetonitrile:0.02 M phosphoric acid (80:20, v/v) and 4) 0.5 ml of acetonitrile:0.02 M phosphoric acid (80:20, v/v). The acetonitrile eluate and the two acetonitrile:0.02 M phosphoric acid eluates were combined and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.25 ml of mobile phase (see below) and 0.20 ml of the solution was chromatographed on a RCM-C18 NOVA cartridge column (4  $\mu$ ; Waters Associates, Milford, MA) with a mobile phase of 0.010 M heptafluorobutyric acid:acetonitrile (90:10, v/v) at a flow rate of 1.5 ml/min. The fraction corresponding to SC-19129 was collected and evaporated to dryness under a stream of nitrogen.



G. Identification of [ $^{14}\text{C}$ ]SC-19129 from Urine by Gas Chromatography-Coupled Mass Spectrometry (GC-MS):

The residue containing [ $^{14}\text{C}$ ]SC-19129 isolated from urine (Section III.F) was dissolved in acetonitrile:bis (trimethylsilyl)-trifluoroacetamide (1:1, v/v) to give an intended concentration of approximately 10 to 15 ng/ml and heated for 15 minutes at 60°C in a closed tube. The N-trimethyl silyl, cyclic imide derivative which was formed by this procedure was analyzed by gas chromatography-coupled mass spectrometry (GC-MS). The samples were chromatographed on a DB-1 fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA) coupled directly to the mass spectrometer. The column was maintained at 15 psi helium and temperature programmed from 150°C to 290°C at 25°C per minute post sample injection. The mass spectra were obtained with a Finnegan- 4500 GCMS (Finnegan, MAT, San Jose, CA) with electron impact ionization.

H. Liquid Scintillation Counting (LSC):

Samples of 0.010 ml or 0.050 ml plasma were mixed with 10 ml of PCS<sup>R</sup> (Amersham Corp., Arlington Heights, IL). Samples larger than 0.050 ml (plasma, urine, CO<sub>2</sub> trapping solution or HPLRC mobile phase) were mixed with sufficient water to give approximately 4 ml total aqueous volume and then mixed with 5 ml of PCS<sup>R</sup> to form a stable gel. The combustion products from oxidized red blood cells or oxidized fecal samples were mixed with 9 ml of Carbosorb<sup>TM</sup> and 12 ml of Permafluor V (both from Packard Instruments Co., Downers Grove, IL). Radioactivity was measured with liquid scintillation spectrometers (Mark II or Mark III,

Tracor Analytic, Elk Grove Village, IL). Counting efficiency was determined by the automatic external standard channels ratio method.

Values for radioactivity disintegrations per minute (DPM) for individual plasma, urine, red blood cell and fecal samples were corrected by subtracting DPM values obtained for the appropriate control (pre-dose) samples treated in the same manner. DPM in HPLRC fractions were corrected by subtracting 2 times the background DPM determined in control HPLRC fractions.

### I. Calculations:

The concentration of [ $^{14}\text{C}$ ]SC-19200 in pooled plasma samples was calculated as follows:

$$\left[ \begin{array}{l} [^{14}\text{C}]\text{SC-19200} \\ \text{in Plasma} \\ (\text{mcg/ml}) \end{array} \right] = \left[ \begin{array}{l} \text{DPM per ml of Pooled} \\ \text{Plasma Recovered in} \\ \text{HPLRC Peak} \\ \hline \text{Fraction of DPM from} \\ \text{Spiked Control} \\ \text{Plasma Recovered in} \\ \text{HPLRC Peak} \end{array} \right] \times \left[ \begin{array}{l} 1 \\ \hline [^{14}\text{C}]\text{SC-19200} \\ \text{Specific} \\ (\text{DPM/mcg}) \end{array} \right]$$

The percentage of the dose excreted in the urine as metabolites was calculated as follows:

$$\left[ \begin{array}{l} \text{Metabolite} \\ \text{Excreted} \\ (\% \text{ of Dose}) \end{array} \right] = \left[ \begin{array}{l} \text{Radioactivity} \\ \text{Excreted in} \\ \text{Urine} (\% \text{ of} \\ \text{Dose}) \end{array} \right] \times \left[ \begin{array}{l} \text{Fraction Eluted} \\ \text{from HPLRC} \\ \text{as Metabolite} \end{array} \right]$$

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J. Pharmacokinetic Calculations:

Maximum plasma concentrations (Cmax) and the times at which Cmax values occurred (Tmax) were determined by inspection of the plasma concentration-time curves. Areas under the plasma concentration-time curves were calculated using the trapezoidal rule (14).

Plasma concentration versus time curves of total  $^{14}\text{C}$  (individual subjects) and [ $^{14}\text{C}$ ]-SC-19200 (pooled plasma from 5 subjects) were analyzed using the CSTRIP computer program (15). The initial parameters estimated from CSTRIP were then used in the NONLIN computer program (16).

#### IV. Results and Discussion

##### A. Amount and Radiochemical Purity of Administered Dose:

The pilot subject received a dose of 40.0 mg [ $^{14}\text{C}$ ]SC-19129 and a radiochemical dose of 205 mCi (Table 1A). Following analysis of the plasma and red blood cells from the pilot study it was decided to reduce the dose to the remaining subjects to approximately 80% of the dose received by the pilot subject (see protocol amendment; reference 12). The five main group subjects received a mean ( $\pm$  SEM) dose of  $31.9 \pm 0.1$  mg and a radiochemical dose of  $164 \pm 1$  mCi, calculated from the difference between the original measured dose per bottle and the unused (returned) portion of each dose solution (Table 1A). The radiochemical purities of the [ $^{14}\text{C}$ ]SC-19129 in two of the unused bottles remaining after dose administration to the six subjects were 98.6% (bottle #7) and 98.4% (bottle #12).

##### B. Recovery of [ $^{14}\text{C}$ ]SC-19129 and [ $^{14}\text{C}$ ]SC-19200 from Control Plasma, Urine and Feces:

The recoveries of added [ $^{14}\text{C}$ ]SC-19200 and [ $^{14}\text{C}$ ]SC-19129 from control plasma in the acetonitrile extract were 84.9% and 94.4% respectively (Table 1). The respective percentages of the extracted radiolabel present in the appropriate peak in HPLRC profiles following frozen storage for 119 days, were 86.6% and 80.3% (Table 2). The recovery of radiolabel added to control urine was approximately 99% (Table 1) during the filtration procedure (Section III.D.3). The percentages of radiolabel from [ $^{14}\text{C}$ ]SC-19200 and [ $^{14}\text{C}$ ]SC-19129 present in the appropriate HPLRC peaks, following frozen storage in urine for 104 days, were 85.3%

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and 91.8%, respectively (Table 2). The recovery of radiolabel from [ $^{14}\text{C}$ ]SC-19200 added to control feces was 93.3% (Table 1). HPLRC analysis indicated that the extracted radioactivity was almost completely in the form of [ $^{14}\text{C}$ ]phenylalanine.

Application of the Bond Elut<sup>TM</sup> isolation procedure (Section III.F) to a 20 ml sample of control urine, to which approximately 0.2 mcg of [ $^{14}\text{C}$ ]SC-19129 had been added, resulted in recovery of 76% of the radiolabel. The concentration of [ $^{14}\text{C}$ ]SC-19129 in this sample (approximately 0.01 mcg/ml) was equivalent to the concentration that would be present in a 0-1 hour urine collection (average volume of 220 ml for the 5 subjects) in which 0.007% of a 32 mg dose was excreted.

#### C. Total Radioactivity in Plasma:

Mean plasma concentrations of total radioactivity for the 5 subject study are shown in Figure 2. Individual values are given in Table 2A for the pilot and the 5 subject studies. The pharmacokinetic parameters are summarized in Table 3. The absorption half-life of total radioactivity was  $3.76 \pm 0.77$  hours. The mean peak plasma concentration ( $C_{\text{max}}$ ) of  $0.448 \pm 0.071$  mcg equivalents (expressed as equivalents of SC-19129) per ml was reached at  $5.5 \pm 0.7$  hours after dose administration. Total radioactivity was eliminated from plasma in two phases with mean half-lives of  $6.21 \pm 0.70$  hours and  $587 \pm 99$  hours. The area under the plasma concentration time curve ( $\text{AUC}_0^\infty$ ) from time zero to infinity was  $154 \pm 24$  (mcg/ml) hours.

These values for total radioactivity in plasma are comparable with the results of animal studies (2-5), allowing for the difference between the doses given to

animals (10 mg/kg) and man (approximately 32 mg or approximately 0.5 mg/kg). In particular, the C<sub>max</sub> (10.4 mcg equivalent/ml) and AUC<sub>0</sub><sup>∞</sup> [3100 (mcg/ml)hours] for orally administered [<sup>14</sup>C]SC-19129 in the dog (5) are proportional to the values observed for man in the present study. The terminal elimination half-life in man (Table 3) is somewhat longer than that observed for the dog (approximately 340 hours, reference 5) and markedly longer than that observed for the rat (85 hours, reference 3) and the rabbit (148 hours, reference 4). The long terminal half-lives of total radioactivity in animal studies have been attributed to release of [<sup>14</sup>C]phenylalanine ([<sup>14</sup>C]Phe) from [<sup>14</sup>C]SC-19129 and incorporation of the [<sup>14</sup>C]Phe into plasma and tissue proteins (2-5). The longer terminal half-life for man compared to animal species may reflect relative rates of protein turnover in tissue. For example, the turnover rate of total protein, measured during intravenous infusion of L-[<sup>14</sup>C]lysine, was approximately 10-times faster in the rat (25-30 g/kg/day; reference 17) than in man (1.6 - 3.2 g/kg/day; reference 18). The terminal half-life of plasma total radioactivity in the present study (587 hours, or 24.5 days; Table 3) was similar to the reported elimination half-life of radiolabel from plasma protein following oral administration of L-[U-<sup>14</sup>C]Phe (19.7 days; reference 9) to a phenylketonuric patient. The plasma free Phe concentration was adjusted by dietary means in this latter study (9) to minimize recycling of [<sup>14</sup>C]Phe and maximize the apparent turnover rate of protein.

#### D. Distribution of Plasma Radioactivity:

The radioactive compounds in pooled plasma were

extracted using acetonitrile precipitation of plasma proteins. The recovery of [ $^{14}\text{C}$ ]SC-19129 and [ $^{14}\text{C}$ ]SC-19200 using this procedure is given in Table 1. Total radioactivity concentrations (DPM/ml; 11,400 DPM/mcg) in pooled plasma and in the acetonitrile extract are shown in Figure 3. The acetonitrile extract contained approximately 100% of the radioactivity in plasma for samples taken up to 1 hour after dose administration. This percentage declined in samples taken after 1 hour and was less than 5% by 12 hours. The radioactivity not extracted by acetonitrile may include [ $^{14}\text{C}$ ]Phe incorporated into plasma proteins.

The only metabolites in HPLRC profiles (Figure 4) of the acetonitrile extract were SC-19200 and phenylacetylglutamine (PAGln), based on comparison of retention times to authentic standards. Chromatography of the 3 hour pooled plasma sample using an alternative HPLRC system (Section III.E) also indicated that [ $^{14}\text{C}$ ]SC-19200 and [ $^{14}\text{C}$ ]PAGln were the major plasma metabolites, with approximately the same percentages of radioactivity eluted at the appropriate retention times (Figure 5). However, a small peak at the retention time of phenylalanine (Phe) was also present in the HPLRC profile using the alternate system (Figure 5). SC-19200 was present at higher concentrations than PAGln up to 3 hours after dose administration but at later times PAGln was higher than SC-19200 (Figure 4).

Total radioactivity in the acetonitrile extracts was very low (maximum of approximately 1300 DPM/ml). Recovery of SC-19200 from control plasma (N=3), spiked with approximately 400 DPM (approximately 0.04 mcg/ml) of [ $^{14}\text{C}$ ]SC-19200, in the HPLRC elute was approximately  $32.8 \pm 4.8\%$  (due in part to the 2 times background criterion for judging peak significance - see Section III.H). The

concentrations of [ $^{14}\text{C}$ ]SC-19200 in pooled plasma, calculated by application of this recovery factor to the HPLRC eluates (see equation in Section III.I) are given in Table 4. The  $C_{\text{max}}$  of 0.072 mcg/ml was reached at 1.5 hours. The 0 to 8 hour AUC was 0.22 (mcg/ml) hours and the terminal elimination half-life was 1.1 hours (Table 4). The  $C_{\text{max}}$  in this study is approximately proportional to dose compared to the  $C_{\text{max}}$  values determined in animals at approximately 20 times higher doses (0.4-0.7 mcg/ml in rats, monkeys and rabbits and approximately 1.6 mcg/ml in dogs; references 2-5).

#### E. Total Radioactivity in Red Blood Cells:

Total radioactivity was determined in red blood cells from the pilot study and from subject TWC in the five subject study. The results are shown in Figure 6 and given in Table 3A. Total radioactivity (expressed as mcg equivalents of [ $^{14}\text{C}$ ]SC-19129 per g) increased slowly throughout the 168 hour sample collection period and concentrations at 168 hours were approximately 90% of the plasma radioactivity concentrations (Table 2A and Table 3A). The pilot subject plasma and red blood cell total radioactivity concentrations on day 36 (not shown in Tables) were 45% and 150% of the respective day 7 values given in Tables 2A and 3A.

Incorporation of a labeled amino acid into red blood cell protein has been shown to increase gradually, with a peak between 20 and 30 days (19). The half-life of the human red blood cell is approximately 120 days (19). The red blood cell radioactivity concentration-time curve in the present study is consistent with labeling of the proteins with [ $^{14}\text{C}$ ]Phe.



**F. Urinary and Fecal Excretion of Total Radioactivity:**

The mean cumulative excretion of radioactivity in urine and feces for the 5 subject study is shown in Figure 7. Individual data is also given in Table 4A. The mean percentages of dose excreted in urine and feces from 0 to 168 hours were  $42.0 \pm 4.0\%$  and  $9.56 \pm 5.44\%$  respectively. The total recovery of radioactivity in the 5 subject study in urine and feces was  $51.6 \pm 6.8\%$ . Fecal excretion varied markedly between individuals in this study (Table 4A). This variation may have been due in part to the failure of three of the five subjects to produce fecal samples during the first 2-3 days of the study (Table 4A).

Total urinary and fecal excretion of radioactivity following oral administration of [ $^{14}\text{C}$ ]SC-19129 in animal studies varied from 32.4% in the dog (5) to 74.9% in the rabbit (4). The values for urinary and fecal excretion in man (Table 4A) were intermediate to the above values and resembled the total excretion by the rat (63.0%; reference 3) and rhesus monkey (60.8%; reference 2).

**G. Distribution of Urinary and Fecal Radioactivity:**

The distribution of radioactivity in HPLRC profiles of urine from subject JSV is shown in Figure 8. The major metabolites present were [ $^{14}\text{C}$ ]SC-19200 and [ $^{14}\text{C}$ ]PAGln. Chromatography of the 0-1 hour urine sample on an alternate HPLRC system (Section III.E) also indicated [ $^{14}\text{C}$ ]SC-19200 and [ $^{14}\text{C}$ ]PAGln to be the major metabolites present. The percentages of radioactivity eluted in the standard and alternate HPLRC systems at the retention times of [ $^{14}\text{C}$ ]PAGln

(16.4% and 19.5% respectively) and [ $^{14}\text{C}$ ]SC-19200 (71.0% and 79.5% respectively) are in reasonable agreement. A radioactive peak eluting at the retention time of [ $^{14}\text{C}$ ]Phe in the standard system (Figure 8) was shown not to be phenylalanine by its absence in the alternate system (Figure 9). This peak appeared to elute with the same retention time as SC-19200 in the alternate system, accounting for the somewhat higher percentage of radioactivity eluting in this peak with this system. A peak with the appropriate retention time for intact [ $^{14}\text{C}$ ]SC-19129 was present in 0-1 hour urine profiles using either HPLRC system. The lower percentage eluting as [ $^{14}\text{C}$ ]SC-19129 using the alternate system (Figure 9) compared to the standard system (Figure 8) resulted at least in part from the increased peak width associated with the longer retention time of this compound in the former system (retention time 23.5 minutes; recovered in 6 fractions) compared to the latter system (retention time 13.5 minutes; recovered in 3 fractions).

It is concluded that the standard HPLRC is the more appropriate system to use for metabolite distribution in human urine. The percentages of dose recovered as [ $^{14}\text{C}$ ]SC-19200, [ $^{14}\text{C}$ ]PAGln and intact [ $^{14}\text{C}$ ]SC-19129 using this method are given in Table 5. [ $^{14}\text{C}$ ]PAGln accounted for  $30.8 \pm 3.4\%$  of the administered radiochemical dose, and approximately 80% of the radioactivity excreted in urine, during the period from 0-24 hours. A mean of  $6.97 \pm 2.05\%$  of the dose was excreted as [ $^{14}\text{C}$ ]SC-19200. This value may be taken as an estimate of oral absorption (bioavailability) with the  $\beta$ -aspartyl bond intact. [ $^{14}\text{C}$ ]SC-19129 was detected only in the 0-1 hour urine samples and accounted for less than 0.005% to 0.015% of the dose (see Section IV.H).

The major radiolabeled constituent in fecal extracts was

[<sup>14</sup>C]Phe (Figure 10). [<sup>14</sup>C]SC-19200 or [<sup>14</sup>C]SC-19129 was not detected. When [<sup>14</sup>C]SC-19200 was added to a control fecal homogenate and carried through the extraction and HPLRC procedures it was found to be completely converted to [<sup>14</sup>C]Phe.

#### H. [<sup>14</sup>C]SC-19129 in Urine:

[<sup>14</sup>C]SC-19129 was present in analytical HPLRC profiles of the 0-1 hour urine sample obtained from the pilot subject and 3 of the subjects in the 5 subject study. The radiolabeled compound eluting at the appropriate retention time was verified to be [<sup>14</sup>C]SC-19129 for the pilot subject by GC-MS analysis (see Section III.G). Larger aliquots of urine (10-20 ml) from subjects MMM and TWC (see Table 5) were concentrated by a Bond Elut<sup>TM</sup> column procedure and profiled by HPLRC (see Section III.F. for methods). Significant amounts of radioactivity were found at the appropriate retention time for [<sup>14</sup>C]SC-19129 for both subjects. The [<sup>14</sup>C]SC-19129 fraction for subject MMM was submitted to GC-MS analysis to verify the presence of the intact compound. The mass spectra of the sample and of an authentic standard are shown in Figure 11. The molecular ion (M<sup>+</sup>) at 348 m/z and the base peak at 333 m/z (M-CH<sub>3</sub>) indicate the presence of the intact methyl ester rather than the free acid.

Evidence for the absorption of trace amounts of [<sup>14</sup>C]SC-19129 with the ester bond intact was seen in studies in the rhesus monkey (2) and rabbit (4) as well as in the present study. However, the amounts absorbed appear to be very low (e.e., less than 0.05% of dose) both in animals and in man.

## V. Conclusions

- The radiolabel in an oral dose of [ $^{14}\text{C}$ ]SC-19129 was extensively absorbed as judged by the fact that the mean recovery in feces was less than 10% of dose.
- [ $^{14}\text{C}$ ]SC-19129 was not detected in plasma, but small amounts (0.015% of dose or less) were excreted in urine. Thus, the methyl ester bond appears to be almost completely hydrolyzed by enzymes in the intestines or liver.
- [ $^{14}\text{C}$ ]SC-19200, the free acid of [ $^{14}\text{C}$ ]SC-19129, was a major metabolite in plasma and urine. At least 6.97% of the dose of [ $^{14}\text{C}$ ]SC-19129 was absorbed with the  $\beta$ -aspartyl bond intact based on recovery of [ $^{14}\text{C}$ ]SC-19200 in urine.
- The major metabolites, in addition to SC-19200, were PAGln in plasma and urine and Phe in feces. Formation of phenylacetic acid, and subsequent conjugation with glutamine to form PAGln, is a minor pathway of Phe metabolism in mammals, including man (20, 21). The metabolic profile in man suggests that bacterial metabolism in the lower gastrointestinal tract, with the formation of Phe and phenylacetic acid, is an important pathway for SC-19200 metabolism.
- [ $^{14}\text{C}$ ]SC-19200 reached a peak mean concentration (pooled plasma) of 0.072 mcg/ml and was cleared rapidly from plasma with a half-life of 1.1 hours. Total radioactivity reached a mean maximum plasma concentration of  $0.448 \pm 0.071$  mcg equivalents/ml and was eliminated with a terminal half-life

of  $587 \pm 99$  hours. At two hours and later plasma total radioactivity was largely precipitable by acetonitrile. This precipitable, slowly cleared, radioactivity probably consists of [ $^{14}\text{C}$ ]Phe incorporated biosynthetically into plasma proteins, as discussed in previous reports (2-5).

The major route of excretion of total radioactivity was in urine ( $42.0 \pm 4.0\%$  of dose), with lesser amounts excreted in feces ( $9.56 \pm 5.44\%$  of dose). The urinary excretion consisted almost exclusively of [ $^{14}\text{C}$ ]SC-19200 and [ $^{14}\text{C}$ ]PAGln (6.97% of dose and 30.8% of dose respectively, compared to total radioactivity excretion of 38.1% of dose, from 0 to 24 hours). Fecal excretion consisted almost exclusively of [ $^{14}\text{C}$ ]Phe.

Plasma concentrations of total radioactivity and [ $^{14}\text{C}$ ]-SC-19200, and urinary and fecal excretions of total radioactivity and major metabolites, are consistent with the range of values seen in previous studies with the monkey, rat, rabbit and dog (2-5).

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## VII. Tables

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Table 1  
Extractability of [ $^{14}\text{C}$ ]-SC-19200  
from Human Plasma, Urine and Feces

<u>Sample Type</u>	<u>Mean Percent Recovered</u>	
	[ $^{14}\text{C}$ ]-SC-19200	[ $^{14}\text{C}$ ]-SC-19129
Plasma	84.9 $\pm$ 3.5 <sup>a</sup>	94.4 $\pm$ 2.3 <sup>b</sup>
Urine	98.8 $\pm$ 0.8 <sup>b,c</sup>	76.0 <sup>d</sup>
Feces	93.3 $\pm$ 7.3 <sup>a</sup>	e

- a Mean  $\pm$  standard error of 6 samples.
- b Mean  $\pm$  standard error of 3 samples.
- c Recovery following filtration, Section III.D.3.
- d Recovery following Bond Elut <sup>TM</sup> isolation procedure, Section III.F.
- e Not done.

Table 2  
Stability of [<sup>14</sup>C]SC-19129 and [<sup>14</sup>C]SC-19200  
in Biological Samples

<u>Sample Type</u>	<u>Conditions</u>	<u>Compound Added</u>	<u>Percent Eluted from HPLRC as Compound Added</u>
Human Plasma <sup>a</sup>	Stored at -20°C for 119 days	[ <sup>14</sup> C]SC-19129	80.3%
	Stored at -20°C for 119 days	[ <sup>14</sup> C]SC-19200	86.6%
Human Urine	Stored at -20°C for 104 days	[ <sup>14</sup> C]SC-19129	91.8%
	Stored at -20°C for 104 days	[ <sup>14</sup> C]SC-19200	85.3%

<sup>a</sup> Control plasma was pretreated with  $2.3 \times 10^{-3}$  M diethyl-p-nitrophenyl phosphate to inhibit esterase activity.

Table 3

Pharmacokinetic Parameters of Total Radioactivity  
After an Oral Dose of [ $^{14}$ C]-SC-19129 in Five Male Human Subjects

Parameter	Individual Subjects					
	<u>TWC</u>	<u>EML</u>	<u>LTD</u>	<u>MMM</u>	<u>JSV</u>	Mean $\pm$ SEM <sup>a</sup>
Peak concentration of total plasma radioactivity (mcg/ml) <sup>b</sup>	0.438	0.300	0.316	0.492	0.693	0.448 $\pm$ 0.071
Time to reach peak plasma radioactivity concentration (hrs)	6.0	3.5	5.0	5.0	8.0	5.5 $\pm$ 0.7
Absorption half-life (hrs) <sup>c</sup>	2.67	1.72	3.94	4.25	6.24	3.76 $\pm$ 0.77
Elimination half-life (hrs) <sup>c</sup> of first phase	6.93	4.23	5.82	5.63	8.44	6.21 $\pm$ 0.70
Elimination half-life (hrs) <sup>c</sup> of second phase	660	329	901	619	428	587 $\pm$ 99
AUC <sub>0-120</sub> [(mcg/ml)hr]	22.7	16.2	14.1	25.4	40.5	23.8 $\pm$ 4.7
AUC <sub>0-504</sub> [(mcg/ml)hr]	70.3	49.3	46.2	78.3	127	74.3 $\pm$ 14.6
AUC <sub>0-∞</sub> [(MCG/ML)HR]	173	76.3	143	159	221	154 $\pm$ 24

a Mean and standard error of the mean of individual parameter values, calculated prior to rounding to significant figures.

b Same molecular weight was assumed for SC-19129 and its metabolites.

c Half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$C_p = A e^{-\alpha t} + B e^{-\beta t} + C e^{-\gamma t}$$

Table 4  
Concentrations of [<sup>14</sup>C]SC-19200 in Pooled Plasma,  
and Associated Pharmacokinetic Parameters,  
Following Oral Administration of [<sup>14</sup>C]SC-19129  
to Five Male Human Subjects

<u>Parameter</u>	<u>[<sup>14</sup>C]SC-19200 in Pooled Plasma<sup>a</sup></u>
Plasma Concentration (mcg/ml) at Indicated Time (hours)	
0.5	<0.005 <sup>b</sup>
0.75	0.017
1.0	0.036
1.5	0.072
2	0.058
3	0.065
4	0.023
6	0.012
8	<0.005 <sup>b</sup>
AUC <sub>0-8</sub> · [(mcg/ml)hours]	0.22
Absorption Half-Life (hours) <sup>c</sup>	0.80
Elimination Half-Life (hours) <sup>c</sup>	1.1

<sup>a</sup> Plasma concentrations were determined by HPLRC analysis of pooled plasma from the 5 subject study.

<sup>b</sup> Peak not detected in chromatogram at 2 times background.

<sup>c</sup> Half-lives calculated by the NONLIN program by fitting the plasma concentrations to the equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

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Table 5

Urinary Excretion of [<sup>14</sup>C]SC-19129, [<sup>14</sup>C]SC-19200  
and [<sup>14</sup>C]Phenylacetylglutamine ([<sup>14</sup>C]PAGln)  
Following Oral Administration of [<sup>14</sup>C]SC-19129 to Five Male Human Subjects

Compound Excreted in Urine <sup>a</sup>	Subject Identification					Mean	SEM
	EML	JSV (Percent of Dose Excreted)	LTD	MMM <sup>a</sup>	TWC		
[ <sup>14</sup> C]SC-19129 <sup>b</sup>	0.015	0.011	0.005	<0.005 <sup>c</sup>	<0.005 <sup>c</sup>	d	d
[ <sup>14</sup> C]SC-19200	5.62	15.0	3.89	3.98	6.40	6.97	2.05
[ <sup>14</sup> C]PAGln	36.8	28.3	38.4	19.5	30.7	30.8	3.4
Total <sup>14</sup> C <sup>e</sup>	43.0	43.7	42.6	24.0	37.5	38.1	3.7

<sup>a</sup> Percentage of dose recovered in urine from 0-24 hours as the indicated metabolite.

<sup>b</sup> [<sup>14</sup>C]SC-19129 was detected only in the 0-1 hour urine samples.

<sup>c</sup> [<sup>14</sup>C]SC-19129 concentrations could not be determined from the analytical HPLC results (peak less than 2 times background) but [<sup>14</sup>C]SC-19129 was shown to be present in the 0-1 hour urine by isolation from a larger volume of the sample (Sections III.F and IV.H).

<sup>d</sup> Not calculated.

<sup>e</sup> Mean percentage of total radioactivity recovered in urine from 0-24 hours (Table 4A).

## VIII. Figures

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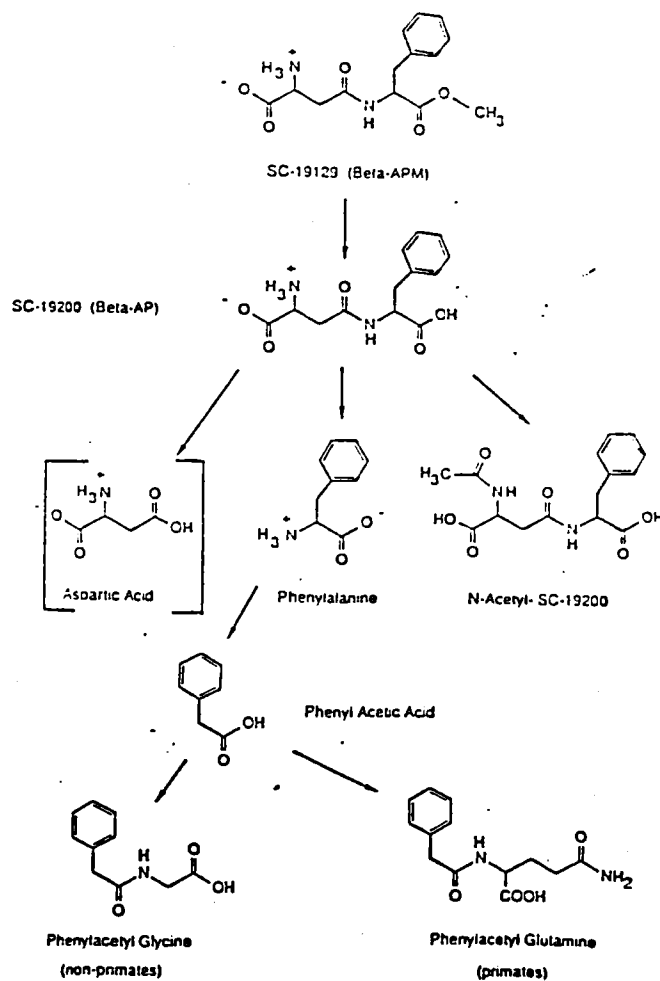


Figure 1. Structures and proposed metabolic pathway of SC-19129. Aspartic acid [in brackets] is a hypothetical metabolite which was not identified in this study. N-Acetyl-SC-19200 was not detected in this study in man, but has been identified in animal studies.

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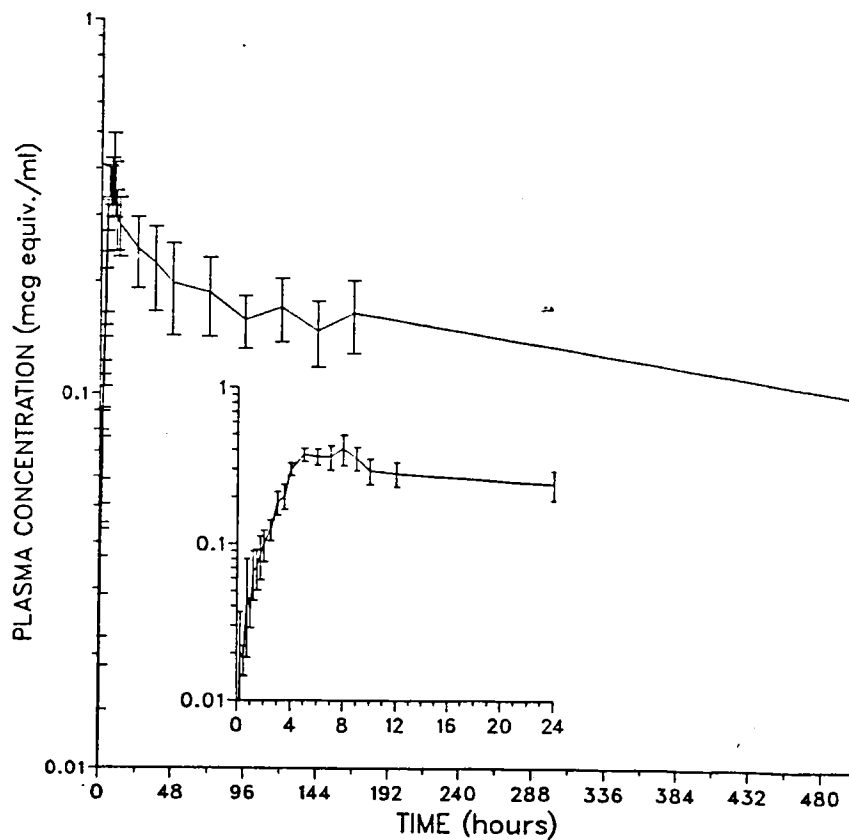


Figure 2. Mean plasma concentrations of total radioactive compounds following oral administration of [ $^{14}$ C]SC-19129 ( $31.9 \pm 0.1$  mg) to five human male subjects. The vertical bars indicate the standard errors of the means. The insert shows the mean plasma concentrations for the first 24 hours with the time axis expanded for clarity. Abscissa: time in hours after administration. Ordinate: plasma concentration of total radioactive compounds expressed as mcg equivalents of SC-19129 per ml.

TOTAL 14C AND SOLUBLE 14C  
IN HUMAN PLASMA FOLLOWING  
[14C]B-APM ADMINISTRATION

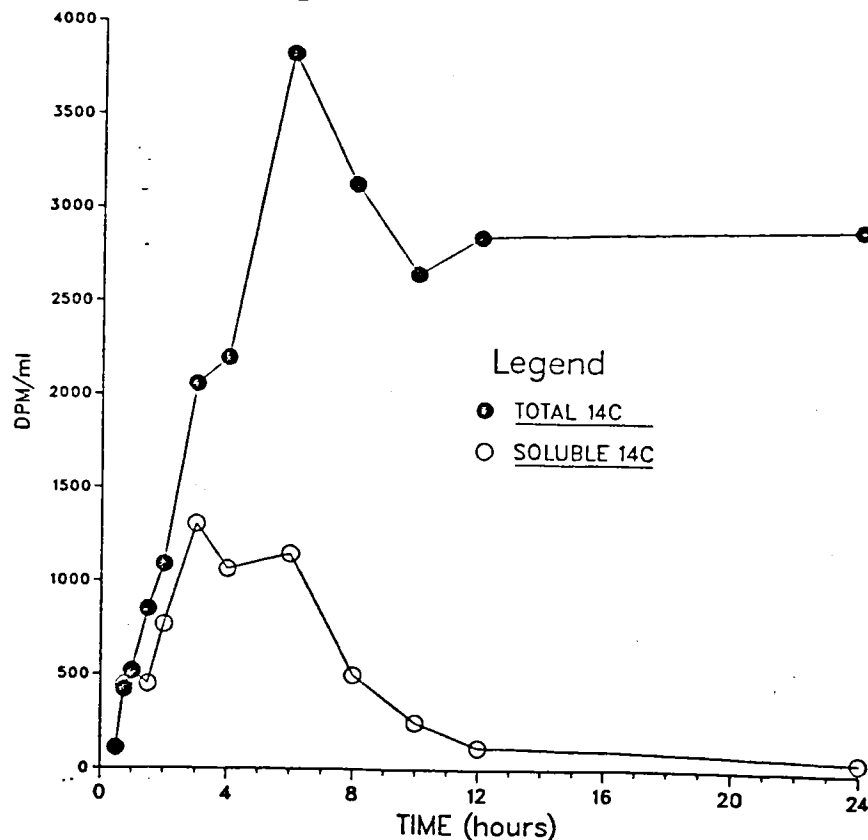


Figure 3. Concentrations of total radioactive compounds in pooled plasma (●) and the acetonitrile extract (Section III.D2) of pooled plasma (○) following administration of [14C]SC-19129 ( $31.9 \pm 0.1$  mg) to five human male subjects. Abscissa: time after administration in hours. Ordinate: concentration of total radioactive compounds expressed as disintegrations per minute (DPM) per ml of pooled plasma.

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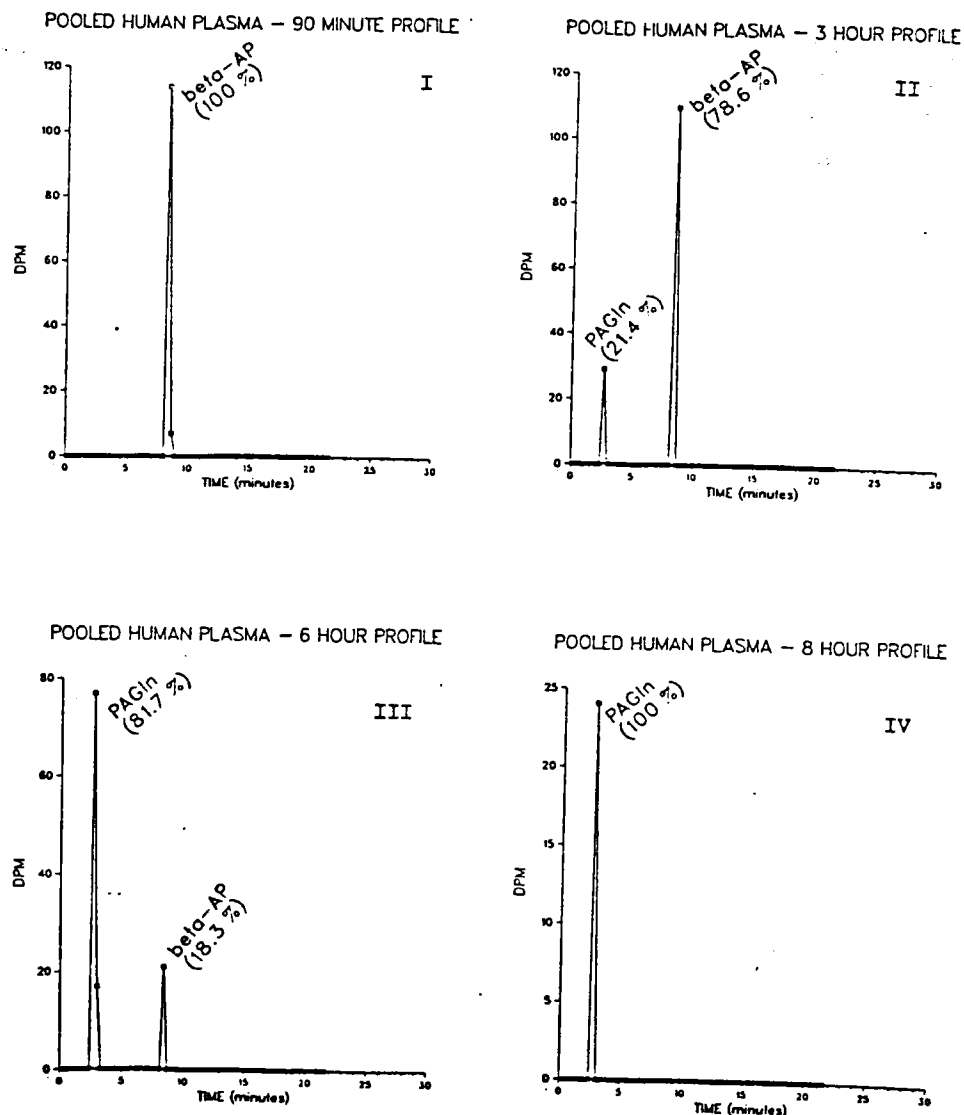


Figure 4. High performance liquid radiochromatograms of the acetonitrile extract of pooled plasma collected at 1.5 hours (I), 3 hours (II), 6 hours (III) and 8 hours (IV) after oral administration of [ $^{14}$ C]SC-19129 to five male human subjects. The locations of reference standards are marked on the chromatograms. The percentages of radioactivity eluted from the column that are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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### 3 HR. POOLED PLASMA PROFILE

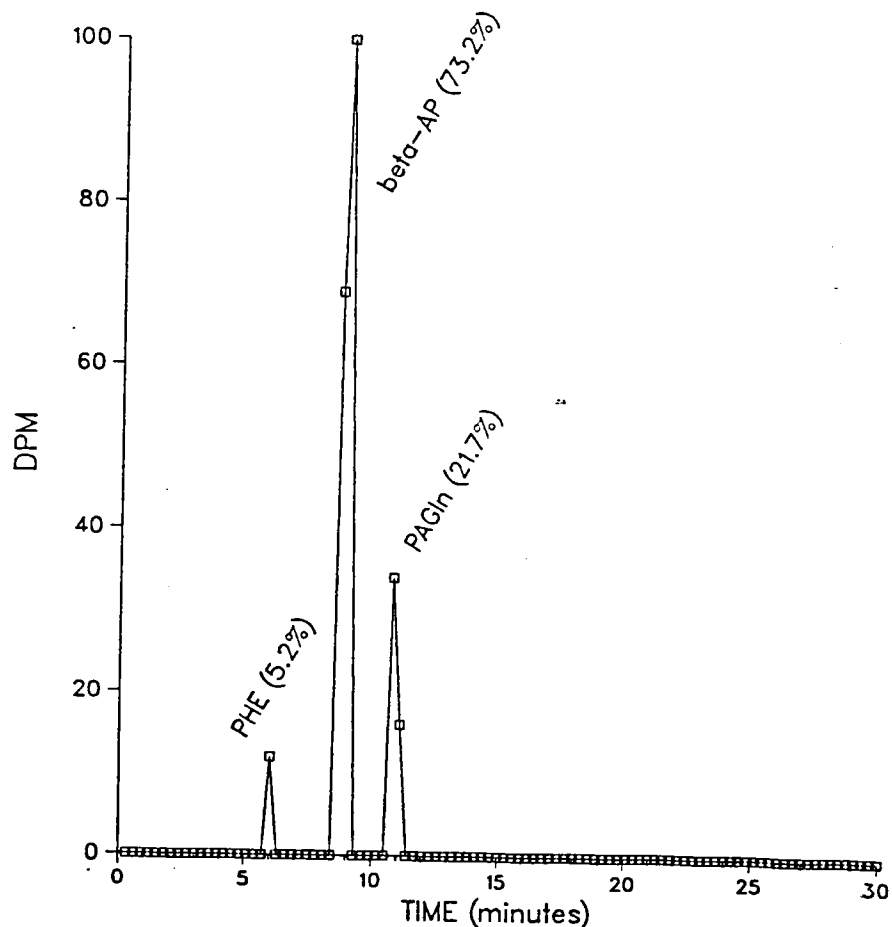


Figure 5. High performance liquid radiochromatogram, using an alternate HPLRC system (Section III.E), of the acetonitrile extract of pooled plasma collected at 3 hours after oral administration of [ $^{14}$ C]SC-19129 ( $39.1 \pm 0.1$  mg) to five male human subjects. The locations of reference standards are marked on the chromatogram. The percentages of radioactivity eluted from the column which are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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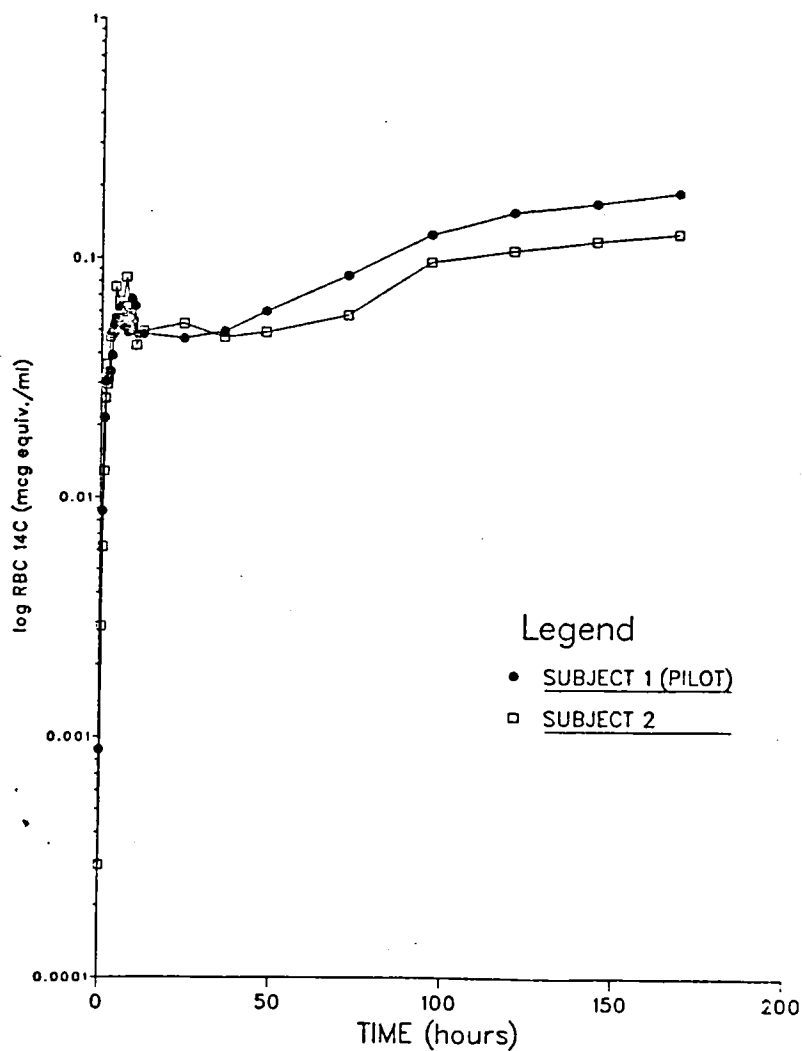


Figure 6. Red blood cell concentrations of total radioactive compounds following oral administration of [ $^{14}\text{C}$ ]SC-19129 to a pilot subject (40.0 mg) and subject TWC (32.2 mg). Abscissa: time in hours after administration. Ordinate: concentration of total radioactive compounds in packed red blood cells expressed as mcg equivalents of SC-19129 per g.

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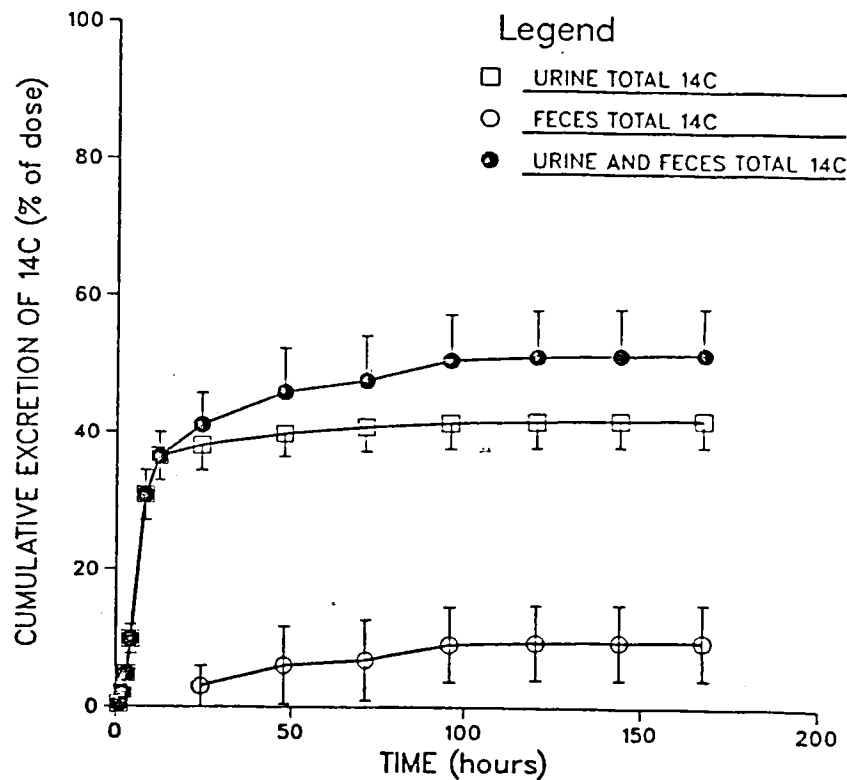


Figure 7. Mean cumulative excretion of total radioactive compounds in urine ( $\square$ ), feces (o) and urine and feces combined ( $\bullet$ ) following oral administration of [ $^{14}\text{C}$ ]SC-19129 ( $31.9 \pm 0.1$  mg) to five male human subjects. The vertical bars indicate the standard errors of the means. Abscissa: time after administration in hours. Ordinate: cumulative excretion of radioactivity as a percentage of dose.

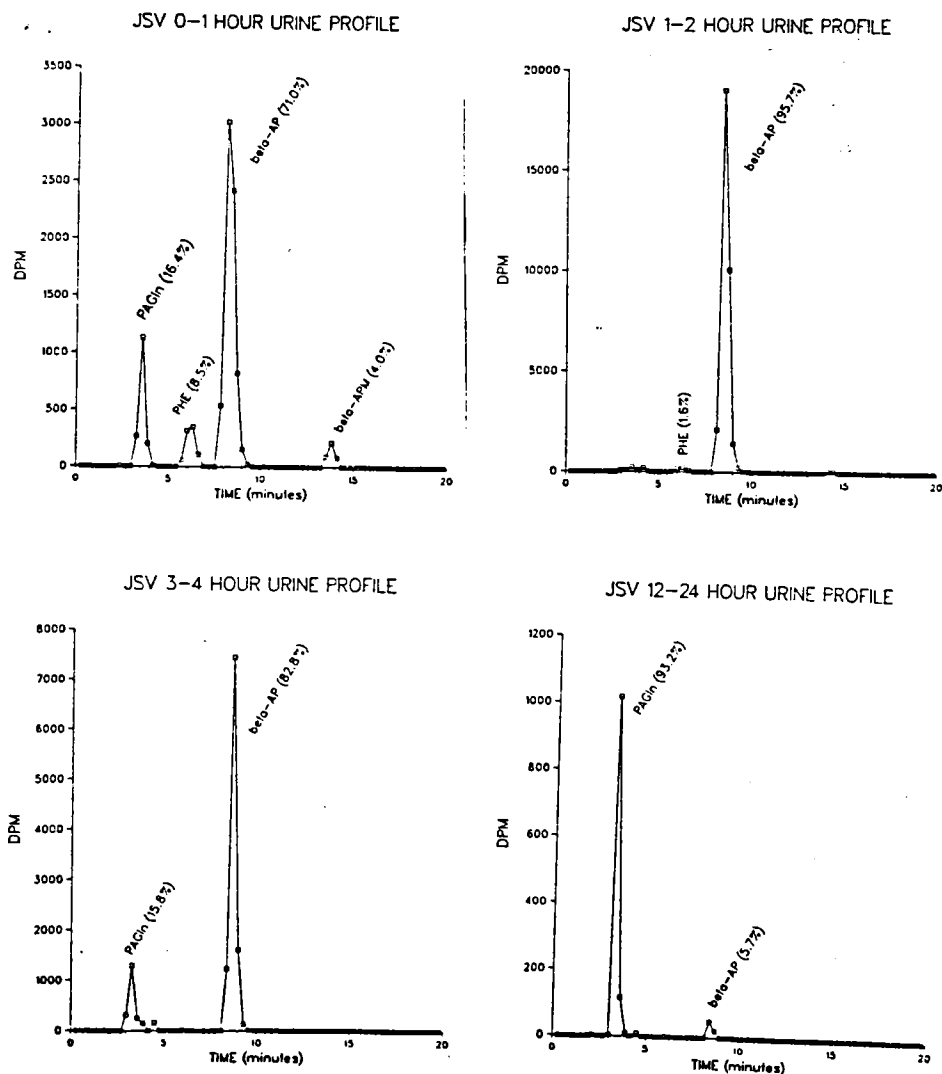


Figure 8. High performance liquid radiochromatograms of urine collected from 0-1 hour (I), 1-2 hours (II), 3-4 hours (III) and 12-24 hours (IV) after oral administration of [<sup>14</sup>C]SC-19129 (31.9 mg) to subject JSV. The locations of reference standards are marked on the chromatograms. The percentages of radioactivity eluted from the column that are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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# JSV 0-1 HOUR URINE PROFILE

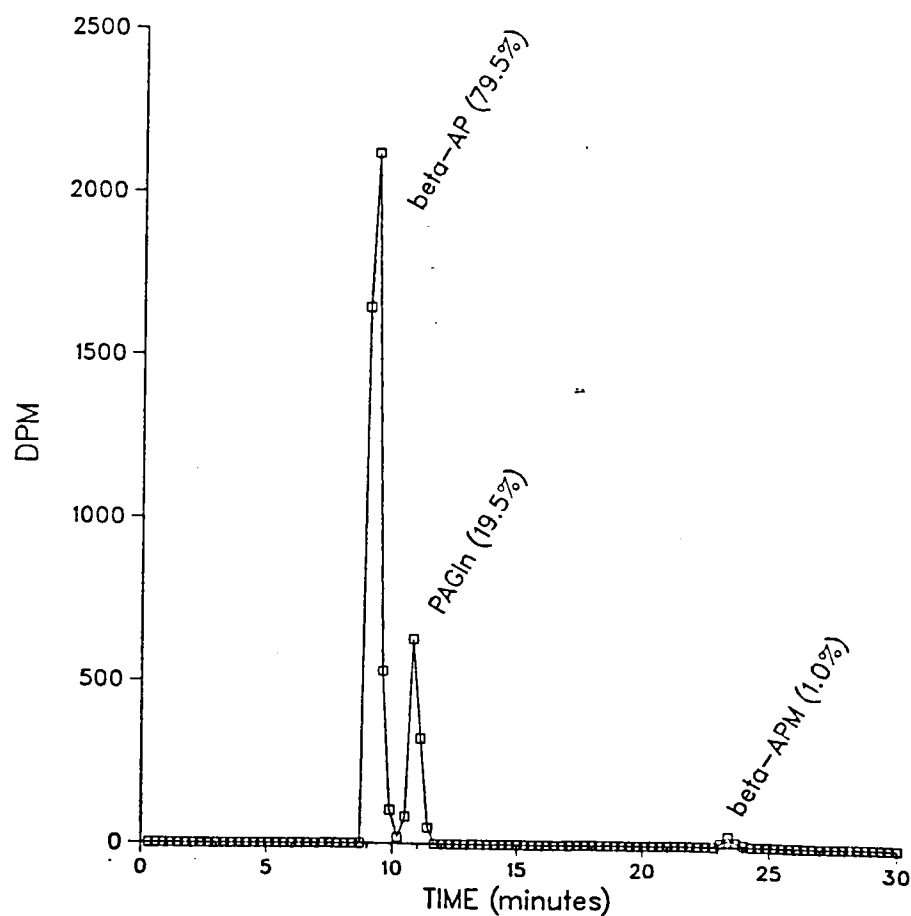


Figure 9. High performance liquid radiochromatogram, using an alternate HPLRC system (Section III.E), of urine collected from 0-1 hour after oral administration of [ $^{14}$ C]SC-19129 (31.9 mg) to subject JSV. The locations of reference standards are marked on the chromatograms. The percentages of radioactivity eluted from the column which are associated with the standards peaks are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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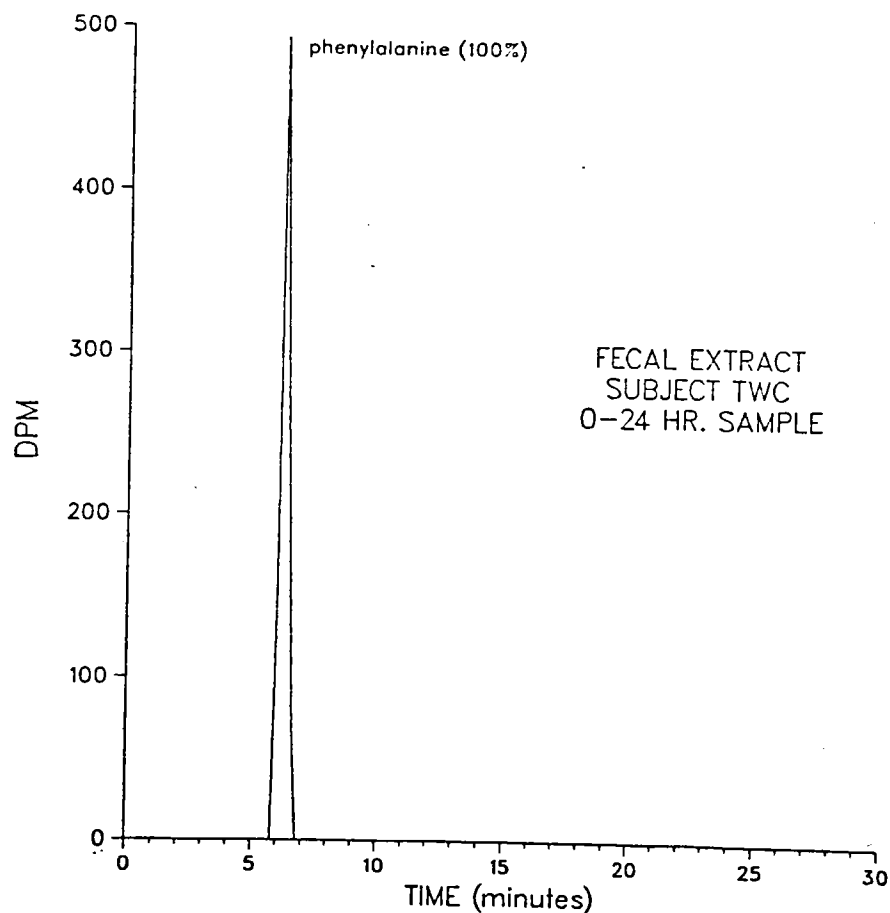


Figure 10. High performance liquid radiochromatogram of a water extract of feces collected from 0-24 hours following oral administration of [ $^{14}\text{C}$ ]SC-19129 (32.1 mg) to subject TWC. The location of the phenylalanine reference standard and the percentage of radioactivity eluted from the column associated with this standard peak are shown. Abscissa: elution time in minutes. Ordinate: disintegrations per minute.

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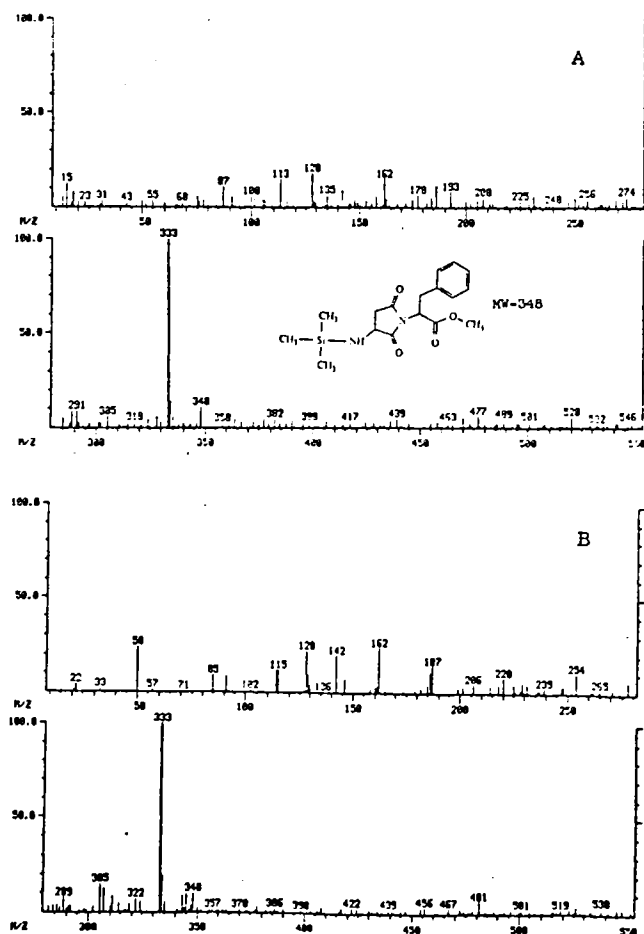


Figure 11. The electron impact mass spectra of the trimethylsilyl, cyclic imide derivatives of (A) SC-19129 standard and (B) [ $^{14}\text{C}$ ]SC-19129 isolated from urine collected from 0-1 hour following oral administration of [ $^{14}\text{C}$ ]SC-19129 (31.8 mg) to subject MMM. The inset structure is the N-trimethylsilyl, cyclic imide derivative of SC-19129.

**IX. Appendix A - Tables**

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Table 1A  
Amount of Administered Dose<sup>a</sup>

Subject	Bottle #	Amount of <sup>14</sup> CJSC-19129 in Indicated Bottle		Radioactivity in Returned Portion of Dose Solution (mCi)		Calculated Dose Received	
		mg	mCi			mg	mCi
RCS (Pilot)	1	40.0	205	c	c	c	c
TWC	2	40.3	207	41.5	32.2	165	165
EML	3	39.9	205	39.5	32.2	165	165
LTD	4	39.8	204	42.7	31.5	161	161
MMM	5	40.0	206	41.9	31.9	164	164
JSV	6	40.1	206	41.9	31.9	164	164

<sup>a</sup> Values are rounded to 3 significant digits.

<sup>b</sup> Calculated from the difference between the amount of [<sup>14</sup>CJSC-19129 originally present in the dose bottle and the amount of radioactivity recovered in the returned (unused) portion of the respective dose solution. Values were calculated using unrounded data and then rounded to 3 significant digits.

<sup>c</sup> Not applicable to this subject.

Table 2A

Plasma Concentrations of Total Radioactivity<sup>a</sup>  
 in a Pilot Human Subject and Five Human Subjects  
 Following a Single Oral Dose  
 (40 mg, Pilot Subject and 31.9 + 0.1 mg, Five Subjects)  
 of [<sup>14</sup>C]SC-19129

		Plasma Concentration (mcg equivalents/ml)						
TIME								
(hours)	PILOT	EML	JSV	LTD	MMM	TWC	MEAN	SEM
0.13	c	0.002	0.003	0.003	0.003	0.006	0.003	0.001
0.25	c	0.082	0.003	0.004	0.012	0.001	0.020	0.015
0.50	0.038	0.033	0.013	0.016	0.011	0.017	0.018	0.004
0.75	c	0.166	0.013	0.016	0.014	0.030	0.048	0.030
1.0	0.119	0.055	0.018	0.027	0.025	0.054	0.056	0.008
1.25	c	0.135	0.022	0.020	0.049	0.098	0.065	0.022
1.5	0.164	0.122	0.022	0.032	0.063	0.106	0.069	0.020
1.75	c	0.161	0.025	0.043	0.062	0.125	0.083	0.026
2.0	0.194	0.157	0.056	0.052	0.076	0.142	0.097	0.022
2.5	0.212	0.138	0.074	0.078	0.148	0.160	0.120	0.018
3.0	0.248	0.257	0.118	0.096	0.214	0.210	0.179	0.031
3.5	0.317	0.300	0.148	0.110	0.163	0.264	0.197	0.036
4.0	0.349	0.288	0.264	0.212	0.378	0.318	0.292	0.027
5.0	0.395	0.283	0.372	0.316	0.492	0.345	0.362	0.036
6.0	0.438	0.255	0.349	0.267	0.451	0.438	0.352	0.041
7.0	0.497	0.237	0.585	0.246	0.332	0.355	0.351	0.063
8.0	0.450	0.215	0.693	0.241	0.474	0.359	0.396	0.087
9.0	0.527	0.245	0.554	0.222	0.354	0.354	0.346	0.059
10.	0.412	0.162	0.453	0.188	0.295	0.339	0.287	0.053
12.	0.418	0.128	0.389	0.196	0.358	0.307	0.275	0.049
24.	0.362	0.153	0.390	0.105	0.304	0.235	0.237	0.051
36.	0.330	0.162	0.434	0.147	0.136	0.206	0.217	0.056
48.	0.299	0.134	0.401	0.104	0.147	0.178	0.193	0.053
72.	0.274	0.125	0.335	0.089	0.216	0.147	0.183	0.043
96.	0.246	0.101	0.235	0.105	0.177	0.153	0.154	0.025
120.	0.227	0.113	0.276	0.100	0.200	0.145	0.167	0.032
144.	0.215	0.083	0.229	0.083	0.189	0.137	0.144	0.029
168.	0.220	0.112	0.292	0.091	0.171	0.136	0.161	0.036
504.	c	0.057	0.152	0.075	0.090	0.108	0.096	0.016

- a Plasma concentrations were calculated assuming the same molecular weight for SC-19129 and its metabolites. Values are rounded to the nearest 0.001 mcg/ml.
- b Mean + standard error (SEM) of five subjects (not including pilot subject), calculated prior to rounding of individual values.
- c No sample taken.

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Table 3A

Red Blood Cell Concentrations of Total Radioactivity<sup>a</sup>  
 in a Pilot Human Subject and in Subject TWC  
 Following a Single Oral Dose  
 (40 mg, Pilot Subject and 32.1 mg, Subject TWC)  
 of [<sup>14</sup>C]SC-19129

TIME (hours)	Red Blood Cell Concentration (mcg equivalents/g)	
	PILOT	TWC
0.5	0.009	0.003
0.75	b	0.006
1.	0.021	0.013
1.25	b	0.026
1.5	0.030	0.036
1.75	b	0.030
2.	0.031	0.031
2.5	0.034	0.047
3.	0.039	0.048
3.5	0.053	0.050
4.	0.056	0.076
4.5	0.054	b
5.	0.062	0.066
5.5	0.061	b
6.	0.052	0.062
6.5	0.059	b
7.	0.060	0.083
7.5	0.049	b
8.	0.054	0.064
8.5	0.067	b
9.	0.051	0.054
9.5	0.063	b
10.	0.048	0.043
12.	0.048	0.049
24.	0.046	0.053
36.	0.049	0.047
48.	0.060	0.049
72.	0.085	0.058
96.	0.127	0.097
120.	0.157	0.108
144.	0.172	0.119
168.	0.191	0.129

a Red blood cell concentrations were calculated assuming the same molecular weight for SC-19129 and its metabolites. Values are rounded to the nearest 0.001 mcg/ml.

b No sample taken.

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Table 4A

Individual Cumulative Percent Recoveries of Radioactivity  
in the Urine and Feces of Five Human Subjects  
Following a Single Oral Dose of  $31.9 \pm 0.1$  mg [ $^{14}\text{C}$ ]SC-19129

U R I N E							
TIME	EML	JSV	LTD	MMH	TWC	MEAN	SEM <sup>a</sup>
0 - 1	0.659	0.277	0.265	0.389	0.312	0.380	0.0729
0 - 2	2.72	1.32	1.19	1.47	3.06	1.95	0.390
0 - 3	8.43	3.42	2.73	3.13	6.15	4.77	1.10
0 - 4	17.3	6.24	6.09	8.60	10.7	9.79	2.06
0 - 8	39.0	26.8	35.7	18.9	33.6	30.8	3.59
0 - 12	41.7	41.4	40.6	22.9	36.2	36.6	3.55
0 - 24	43.0	43.7	42.6	24.0	37.5	38.1	3.70
0 - 48	44.2	44.3	45.1	27.2	38.6	39.9	3.37
0 - 72	45.5	47.0	45.5	27.5	38.8	40.9	3.63
0 - 96	46.5	49.2	45.7	27.7	38.9	41.6	3.86
0 - 120	46.9	49.9	45.7	27.8	39.0	41.9	3.95
0 - 144	46.9	50.3	45.8	27.8	39.0	42.0	3.99
0 - 168	47.0	50.5	45.8	27.8	39.1	42.0	4.00

F E C E S							
TIME	EML	JSV	LTD	MMH	TWC	MEAN	SEM <sup>a</sup>
0 - 24	b	0.0820	b	b	14.7	2.96	2.94
0 - 48	b	1.67	b	b	28.6	6.05	5.65
0 - 72	b	2.68	0.781	0.558	30.0	6.80	5.82
0 - 96	6.11	7.21	1.18	0.738	30.3	9.11	5.45
0 - 120	6.60	8.42	1.28	0.764	30.3	9.47	5.41
0 - 144	6.74	8.42 <sup>b</sup>	1.34	0.776	30.4	9.54	5.42
0 - 168	6.74 <sup>b</sup>	8.42 <sup>b</sup>	1.36	0.776 <sup>b</sup>	30.5	9.56	5.44

U R I N E & F E C E S							
TIME	EML	JSV	LTD	MMH	TWC	MEAN	SEM <sup>a</sup>
0 - 1	0.659	0.277	0.265	0.389	0.312	0.380	0.0729
0 - 2	2.72	1.32	1.19	1.47	3.06	1.95	0.389
0 - 3	8.43	3.42	2.73	3.13	6.15	4.77	1.09
0 - 4	17.3	6.24	6.09	8.60	10.7	9.79	2.06
0 - 8	39.0	26.8	35.7	18.9	33.6	30.8	3.58
0 - 12	41.7	41.4	40.6	22.9	36.2	36.6	3.56
0 - 24	43.0	43.8	42.6	24.0	37.2	41.1	4.63
0 - 48	44.2	46.0	45.1	27.2	38.6	45.9	6.35
0 - 72	45.5	49.7	46.3	28.1	38.8	47.7	6.49
0 - 96	52.6	56.4	46.9	28.4	69.2	50.7	6.67
0 - 120	53.5	58.3	47.0	28.6	69.3	51.3	6.76
0 - 144	53.6	58.7	47.1	28.6	69.4	51.5	6.79
0 - 168	53.7	58.9	47.2	28.6	69.6	51.6	6.83

<sup>a</sup> Standard error of the mean.

<sup>b</sup> No sample available from subject.

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