

Pharmacokinetics and Metabolism
of [^{14}C]-SC-19129 in the Rhesus Monkey

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

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**Pharmacokinetics and Metabolism
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I. Abstract

The pharmacokinetics and metabolism of [U-¹⁴C-Phe]-SC-19129 were determined following administration of an oral solution to three female rhesus monkeys. The dose was 10 mg/kg body weight.

Mean peak plasma concentrations (C_{max}) of total ¹⁴C were 5.66 ± 1.00 mcg/ml (expressed as mcg SC-19129 equivalents/ml) \pm standard error of the mean) and the time of peak plasma concentrations (T_{max}) was 7.3 ± 2.4 hours. The concentrations of total ¹⁴C declined to 2.84 mcg/ml at 48 hours but there was little, if any, further decline from 48 to 96 hours. The percentage of plasma radioactivity recovered in the supernatant from ethanol precipitation of plasma proteins was 94% at 0.25 hour, 13% at 12 hours and less than 5% at 24 hours. Little, if any (<0.5% of radiolabel) intact SC-19129 was found in plasma. However β -aspartyl phenylalanine N-acetyl- β -AP, phenylalanine (Phe) and phenylacetylglutamine (PAG) were found to be metabolites of SC-19129 in plasma profiles. The mean C_{max} value (expressed as mcg equivalents of SC-19129/ml) for β -AP was 0.615 ± 0.094 , and the mean T_{max} value (hours) was 4.0 ± 1.2 . The mean areas under the plasma concentration-time curves (AUC, expressed as [mcg equivalents SC-19129/ml]hours) from 0 to 12 hours for total ¹⁴C and β -AP, were 48.3 ± 9.7 and 2.92 ± 0.33 , respectively. The AUC for β -AP was 6% of the total ¹⁴C AUC from 0 to 12 hours. The AUC values for Phe and N-Acetyl- β -AP were similar to that of β -AP

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while the AUC for PAG was about twice that of β -AP. The elimination half-life of β -AP in plasma was estimated to be between 2 and 6 hours.

The recovery of radioactivity in urine and feces (expressed as percent of dose) from 0 to 96 hours was $56.2 \pm 3.6\%$ and $4.58 \pm 1.03\%$ respectively. The low fecal excretion indicates high absorption ($>95\%$) of the radiolabel from the oral dose. The recovery of $^{14}\text{CO}_2$ expired in the breath was $4.82 \pm 0.57\%$ of dose from 0 to 7 hours. Thus the total recovery of radioactivity in urine, feces and breath was 65.6% . The vast majority (94%) of the radiolabel that was recovered in the urine from 0 to 96 hours was excreted in the first 12 hours. Radiolabeled β -AP, N-acetyl- β -AP, Phe and PAG were present in urine profiles and accounted for 1.7% , 5.9% , 0.8% and 41% of the dose respectively in the 0 to 12 hour urine samples. Thus at least 7.6% of the administered dose reached the systemic circulation with the β -aspartyl peptide bond intact (β -AP plus N-acetyl- β -AP in urine). The large percent of dose excreted as PAG (which is not normally a major mammalian metabolite of Phe), and the existence of a 2 hour lag time in the appearance of PAG in plasma and the appearance of $^{14}\text{CO}_2$ in breath, suggests that a major part of the administered dose is metabolized in the lower gastrointestinal tract by bacteria. The results suggest that Phe and phenylacetic acid produced by this metabolism was then absorbed, with at least part of the former further metabolized to $^{14}\text{CO}_2$ and the latter conjugated with glutamine to form PAG.

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II. Introduction

SC-19129 (N-L- β -aspartyl-L-phenylalanine, l-methyl ester, β -APM) and its free acid, SC-19200 (β -AP, N-L- β -aspartyl-L-phenylalanine), have been identified as conversion products of aspartame (SC-18862, N-L- α -aspartyl-L-phenylalanine methyl ester, APM) in sweetened soft drinks. The objectives of this study were to determine the pharmacokinetics and metabolism of [¹⁴C]-SC-19129 in the rhesus monkey.

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III. Materials and Methods

A. Overview of Study Design:

[U-¹⁴C-Phe]-SC-19129 was administered as an oral solution, at a dose of 10 mg/kg, to three female rhesus monkeys. Plasma, breath, urine and feces were collected at selected times after dosing. Total radioactivity was determined for all samples. Selected plasma and urine samples were further analyzed by high performance liquid radiochromatography (HPLRC).

B. Test Article and Dosage Form:

[U-¹⁴C-Phe]-SC-19129 was prepared by Amersham Corp. (Arlington Hts. IL). The specific activity was 1.4 mCi/mmol (approximately 4.8 mCi/mg) and the radiochemical purity was 95%. The dosage form was prepared by dissolving 2.5 mg of [¹⁴C]-SC-19129 and 2.5 mg of unlabelled SC-19129 (lot CD-103-112A) per ml of distilled water to give a final concentration of 5.0 mg/ml and approximately 12 mCi/ml. The dosing solution was prepared immediately before use and the radiochemical purity of the dosing solution was determined by thin layer radiochromatography (TLRC).

C. Animals, Animal Treatment and Test Article Administration:

Three female rhesus monkeys, which weighed 4.25 kg (#378), 5.1 kg (#383) and 4.9 kg (#396) were used. Housing, diet and fasting of these animals were as described in the protocol (Appendix 1, Section 10). The solution dose was administered

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by nasogastric tube in a volume of 2 ml/kg, to provide a dose of 10 mg/kg, as described in the protocol (Appendix 1, Section 9.D).

D. Sample Collection:

1. Plasma:

Blood samples were obtained from a saphenous vein as described in the protocol (Appendix 1, Section 11.A). Plasma was prepared by centrifugation and an aliquot was taken for total radioactivity determination (Section III.E.2). The remainder of each plasma sample was stored frozen at approximately -20°C until analysis. [¹⁴C]-SC-19129 was added to control monkey plasma (approximately 16 mcg/ml plasma) which had been pre-treated with 1x10⁻⁴ molar diethyl-p-nitrophenyl phosphate (Appendix I, Section 11.A) to inhibit esterase activity. Aliquots of the control plasma containing [¹⁴C]-SC-19129 were analyzed following frozen storage.

2. Urine and Feces:

Urine and fecal samples were collected as described in the protocol (Appendix 1, Sections 11.B and 11.C) and stored frozen at approximately -20°C until analysis. Control urine was spiked with [¹⁴C]-SC-19129 (50 mcg/ml urine) and stored frozen until analysis.

3. Breath:

¹⁴CO₂ eliminated in the breath was collected as described in the protocol (Appendix 1, Section 11.D) except that the ¹⁴CO₂ eliminated from 1-2 hours after dosing was collected as a single sample rather than as separate 1-1.5 hour and 1.5-2 hour samples (see Section III.J).

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E. Sample Analysis:

1. Dose Solution:

Aliquots of the dosing solution or a solution of unlabelled SC-19129 standard were spotted on 5X20 cm silica gel GF plates (Analtech, Newark, DE, catalog #26031). The plates were developed for 15 cm with a mobile phase consisting of chloroform:methanol:water:formic acid, 64:30:4:2 (by volume). The distribution of radioactivity on the dried plates was measured with a Model 200 Imaging Scanner (Bioscan, Inc., Washington, D.C.). Unlabelled standard was visualized by exposing the dried plates to t-butylhypochlorite vapor followed by spraying with an aqueous solution of starch and potassium iodide (5 mg of each per ml of distilled water).

2. Plasma:

Total ^{14}C was determined by liquid scintillation spectrometry (LSC, Section III.H) using duplicate 0.05 ml aliquots of samples collected from 0 to 24 hours and duplicate 0.10 ml aliquots of the 48, 72 and 96 hour samples.

Extracts of plasma samples were prepared for high performance liquid radiochromatographic analysis (HPLRC, Section III.F) by mixing 1 ml of plasma with 2 ml of ethanol. The mixture was centrifuged and the supernatant was removed and saved. The plasma protein pellet was mixed with an additional 1 ml of ethanol, centrifuged, and the supernatant combined with the supernatant from the first ethanol treatment. The combined supernatants were evaporated to dryness under a stream of nitrogen. The dried extract was either 1) dissolved in HPLRC mobile phase (Section III.F) or 2) dissolved in 1 ml of water and applied to a Bond Elut C18

column (Catalog #607303; Analytichem, International, Inc., Harbor City, CA) which had been preconditioned by washing sequentially with methanol and with 0.1 molar phosphate buffer, pH 7.5. Labelled compounds were eluted from the column with 1 ml of water followed by 1 ml of methanol. The water eluates and methanol eluates were dried separately and each dried sample was dissolved in HPLRC mobile phase (Section III.F). The Bond Elut[™] procedure was used initially but was omitted when it became clear that the ethanol extracts could be profiled directly without adverse effects on resolution of metabolites in the HPLRC system (Section III.F).

3. Urine:

Total ¹⁴C in duplicate aliquots of each urine sample was determined by LSC (Section III.H). Aliquots of the 0-6 and 6-12 hour samples were analyzed by HPLRC (Section III.F) after filtering through a 0.45 micron filter (Gelman Acrodisc[®]; Gelman Sciences, Inc., Ann Arbor, MI).

4. Feces:

Fecal samples were suspended in a volume (ml) of 1% Carbopol[™] 941 (B.F. Goodrich Co., Cleveland, OH) in water equal to the sample weight (g) using a blender (Stomacher Lab-Blender 400; Tekmar Co., Cincinnati, OH). Aliquots (0.4-0.6 ml) of suspension were oxidized with a Packard Tri-Carb Sample Oxidizer (Packard Model 306, Packard Instruments, Co., Downers Grove, IL). Total ¹⁴C in the combustion products was determined by LSC (Section III.H).

5. Breath:

Total ¹⁴C in 1 ml aliquots of CO₂ trapping solution (Appendix 1, Section III.D) was determined by LSC (Section III.H).

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F. High Performance Liquid Radiochromatography (HPLRC)
System for Profiles of Radioactivity in Plasma and Urine:

Extracts of plasma (dissolved in mobile phase) and urine (Section III.E.2-3) were profiled by HPLRC on a Waters C18 Nova radial compression module (RCM; Waters Associates, Medford, MA). The composition of the mobile phase was 0.18 N Na_2HPO_4 :methanol (68:32, v/v) where the 0.18 N Na_2HPO_4 in water also contained 0.02 N heptane sulfonic acid sodium salt, and was adjusted to pH 2 with phosphoric acid (isocratic ion pairing system). The system was run at ambient temperature with a flow rate of 1.5 ml/min. Unlabelled standards of SC-19129, β -AP, phenylalanine and tyrosine, used to calibrate the system, were detected by absorbance at 210 nm using a model 480 variable wavelength detector (Waters Associates, Medford, MA). Fractions were collected in 0.4 minute (0.6 ml) aliquots using either a Foxy™ or Golden Retriever™ fraction collector (Instrument Specialties Co. Inc., Lincoln, NE), and total radioactivity was determined by LSC (Section III.H).

G. Isolation and Identification of Metabolites from Urine:

Ten 1 ml aliquots of urine (0-6 hour sample) were extracted with the Bond Elut™ procedure described for plasma (Section III.E.2). The methanol eluants were combined, evaporated to dryness and reconstituted in water. Aliquots of the reconstituted extract were applied to the C18 RCM column (Section III.F) which had been equilibrated with water. The column was then eluted with a linear gradient of 100% water to 70% water:30% methanol (v/v) over 30 minutes at a flow rate of 1 ml/min. Fractions were collected in 0.4 min aliquots with a fraction collector and radioactive compounds were detected by LSC (Section III.H) of an aliquot of each fraction. Two

broad peaks were observed which were found to contain metabolites A and B by rechromatography of aliquots in the isocratic ion pairing HPLRC system (Section III.F).

Derivatives were prepared by 1) treatment with thionyl chloride in methanol, evaporation to dryness, treatment with pentafluoropropionic anhydride (PFPA) and evaporation to dryness or 2) treatment with bis (trimethylsilyl) trifluoroacetamide (BSTFA) in acetonitrile at 150°C in a sealed tube.

Mass spectra were obtained with a Finnegan 4000 GCMS (Finnegan MAT, San Jose, CA) with electron impact ionization (EI) or chemical ionization using isobutane reagent gas at 0.3 torr source pressure and 200°C source temperature. The samples were chromatographed on a 15 meter X 25 mm DB-1 fused silica capillary column (J & W Scientific Inc., Rancho Cordova, CA) coupled directly to the mass spectrometer. The column was kept at 15 psi helium and temperature programmed from 150°C to 290°C at 25°C/min post sample injection.

H. Radioactivity Measurements:

Samples of 0.050 ml plasma were mixed with 10 ml of PCS* (Amersham Corp., Arlington Heights, IL). Samples larger than 0.050 ml (plasma, urine, CO₂ 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* to form a stable gel. The combustion products from oxidized fecal samples were mixed with 9 ml of Carbosorb[®] and 12 ml of Permafluor V (both from Packard Instruments Co., Downers Grove, IL). Radioactivity was measured with liquid

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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.

I. 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 (1). The terminal half-life of β -AP in plasma was estimated by a.) log-linear regression (2) of the mean plasma concentrations versus time from 6-12 hours or b.) estimation of the rate constants describing the mean plasma concentration-time curve using the CSTRIP computer program (3) which were then further refined by use of the NONLIN computer program (4).

J. Protocol Deviations:

1. The samples of $^{14}\text{CO}_2$ eliminated in the breath were intended to include samples from 1-1.5 and from 1.5-2 hours (Appendix 1, Section 11.E). The $^{14}\text{CO}_2$ eliminated during these times was inadvertently collected as a single, 1-2 hour, sample.
2. Fecal samples were not extracted and analyzed by TLRC or HPLRC (Appendix 1, Section 12.B) since only a relatively small amount of the total dose was excreted in the feces (<5% of dose in 96 hours).

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IV. Results

A. Radiochemical Purity of Dosage Form:

Aliquots of the [^{14}C]-SC-19129 dose solution (Section III.B) analyzed by TLRC (Section III.D.1) either immediately after dosing (within 1 hour of preparation), or approximately 5 hours after preparation did not differ appreciably. The radiochemical purity of the dose solution was 93.3% (N=6). Approximately 3% of the radiolabel present had the TLRC Rf of β -AP.

B. Recovery of [^{14}C]-SC-19129 from Control Plasma and Urine:

The recovery of radiolabel from [^{14}C]-SC-19129 added to control plasma was 100% in the supernatant from the ethanol precipitation of plasma proteins. The percentage of the extracted radiolabel present in the appropriate peak in HPLRC profiles was 95.1% when analyzed immediately, and 95.2% when the extraction and HPLRC were performed after frozen storage of the spiked plasma for 6 days at -20°C . The recovery of [^{14}C]-SC-19129 from the Bond Elut[™] procedure was 106%. The percentage of [^{14}C]-SC-19129 added to control urine which was present in the appropriate HPLRC peak after frozen storage at -20°C was 95.6%.

C. Plasma:

Plasma concentrations of total radioactivity, expressed as mcg equivalents/ml, are given in Table 1. The mean concentrations for the 3 monkeys are given in Table 1 and shown in Figure 2. The highest mean plasma concentration occurred at 6 hours and was 5.46 ± 0.91 mcg/ml (\pm standard

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error of mean, SEM). The mean concentration of total radioactivity in plasma declined to 2.84 mcg/ml at 48 hours but was nearly constant from 48 to 96 hours. The percentage of plasma radioactivity recovered in the supernatant from the ethanol precipitation of the 0.25 hour plasma samples was $94 \pm 8\%$ but this percentage declined with time after dosing and was $13 \pm 1\%$ at 12 hours and less than 5% at 24 hours.

HPLRC profiles of plasma extracts revealed very little, if any, intact SC-19129 (Figures 3,4). However β -AP, Phe (identified by coincidence of retention time with authentic standard) and two other major metabolite peaks (peaks A and B, Figures 3 and 4) were present in plasma profiles. The metabolites present in these were identified as N-acetyl- β -AP and Phenylacetyl glutamine (PAG) (Figure 1) by isolation and identification of the same metabolite peaks from urine (Section III.G). The amounts of metabolites present in plasma samples taken from 0 to 12 hours were calculated to be the product of the total ^{14}C present, the recovery of radioactivity in the ethanol extract (and where used, the bond-elut extracts), and the percentage of radioactivity present in the appropriate peak in the HPLRC profile. The amount of extractable radioactivity in the plasma at 24 hours or later was insufficient for HPLRC profiling. The results are given in Tables 2-5 and shown in Figures 5-6.

Pharmacokinetic calculations derived from the plasma concentration data in Tables 1-5 are summarized in Table 6. The mean (\pm SEM) peak plasma concentrations (expressed as mcg equivalent SC-19129 per ml) of total radioactivity, β -AP, N-acetyl- β -AP, Phe, and PAG were 5.66 ± 1.00 , 0.615 ± 0.094 , 0.617 ± 0.212 , 0.376 ± 0.063 and 1.12 ± 0.20 respectively. The mean times of peak plasma concentrations (hours) were 7.3 ± 2.4 , 4.0 ± 1.2 , 4.0 ± 1.2 , 4.7 ± 0.7 and 6.7 ± 0.7 for total

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radioactivity, β -AP, N-acetyl- β -AP, Phe and PAG respectively. The mean areas under the plasma concentration-time curves (AUC) from 0 to 12 hours for total radioactivity, β -AP, N-acetyl- β -AP, Phe and PAG [(mcg equivalents SC-19129) hours] were 48.3 ± 9.7 , 2.92 ± 0.33 , 2.82 ± 0.87 , 2.26 ± 0.06 and 5.85 ± 0.80 respectively. The AUC for β -AP was thus 6% of the AUC for total ^{14}C during the first 12 hours after dosing. The terminal elimination half-life of total ^{14}C could not be determined from the available data but was apparently greater than 48 hours. The terminal elimination half-life of [^{14}C]- β -AP was estimated (Section III.I) to be between 2 hours (log-linear regression of mean plasma concentrations from 6-12 hours versus time) and 6 hours (estimate obtained from NONLIN computer program analysis of 0-12 hour plasma concentration data).

D. Elimination of $^{14}\text{CO}_2$ in Breath:

The cumulative excretion of $^{14}\text{CO}_2$ in the breath from 0 to 7 hours is given in Table 7 and shown in Figure 7. Following a lag time of approximately 2 hours, excretion of $^{14}\text{CO}_2$ in the breath was nearly linear from 3-7 hours (approximately 1% of dose excreted per hour) with a cumulative excretion from 0 to 7 hours of $4.82 \pm 0.57\%$ of dose.

E. Urine and Feces:

The cumulative excretion of radioactivity in urine and feces is given in Table 8 and shown in Figure 8. Cumulative excretion in the urine was $56.2 \pm 3.6\%$ of dose from 0 to 96 hours and 94% of this amount was excreted in the first 12 hours. Excretion in the feces was very low ($4.58 \pm 1.03\%$ of dose from 0 to 96 hours) and was less than one tenth the amount excreted in the urine.

HPLRC profiles of urine samples (Figures 9-11) were qualitatively similar to plasma profiles with four discernable metabolite peaks but little or no intact SC-19129. A peak with the approximate retention time of SC-19129, representing approximately 0.35% of the respective dose, was observed in the 0-6 hour urine sample from monkey #396 (Figure 11) but was not seen in the 6-12 hour sample from monkey #396 or samples from the other monkeys. $[^{14}\text{C}]\text{-}\beta\text{-AP}$ and $[^{14}\text{C}]\text{-Phe}$ (identified by their retention times compared to authentic standards) were minor components of the urinary radioactivity. The net excretion of $\beta\text{-AP}$ and Phe from 0-12 hours was approximately 1.7% and 0.8% of dose respectively.

The two major components in urine (peaks A and B, Figures 9-11) were isolated as described in Section III.G. Peak B was found to be N-acetyl- $\beta\text{-AP}$ (Figure 1) by GCMS analysis (Section III.G). This metabolite was prepared for GCMS analysis by sequential treatment with thionyl chloride in methanol and pentafluoropropionic anhydride. However the mass spectrum (Figure 12) indicated that the product had a molecular weight consistent with the N-acetyl, dimethyl ester of $\beta\text{-AP}$. There was no formation of a N-pentafluoropropionyl derivative further indicating that metabolite B lacked a free amine

group. The metabolite was confirmed to be N-acetyl- β -AP by comparison of its HPLC retention time with that of a standard prepared by acetylation of β -AP with acetic anhydride (in pyridine) and comparison of the GC retention time and mass spectrum of the methylated metabolite to that of the dimethyl ester of the N-acetyl- β -AP standard. The spectra of both the standard and metabolite (Figure 12) contain a molecular ion of 350 m/z, a base peak of 162 m/z, and peak of 113 m/z. The peak at 113 m/z is not present in a mass spectrum (not shown) of the N-acetyl, dimethyl ester of N-L- α -aspartyl-Phenylalanine (APM, SC-18862) and the GC retention time of this latter standard differed from that of metabolite B and the derivatized β -AP standard. An average of 5.9% of the radiochemical dose was excreted as N-acetyl- β -AP by the three monkeys.

Peak A was tentatively identified as Phenylacetylglutamine (PAG, Figure 1) by examination of the mass spectrum (Figure 13) of the trimethylsilyl (TMS) derivative (Section III.G). No authentic standard was available for a comparison. However the molecular ion (M^{+}) at 408 m/z and the peaks at 393 m/z ($M-CH_3^{+}$), 317 m/z ($M-C_7H_7^{+}$), 291 m/z ($M-CO_2TMS^{+}$) and 91 m/z ($C_7H_7^{+}$) are consistent with the proposed structure. The molecular weight was confirmed by the chemical ionization mass spectrum which contained an MH^{+} ion at 409 m/z as the base peak. Further evidence supporting the identification of metabolite A as PAG was obtained by comparison of its infrared and nuclear magnetic resonance spectra to published spectra for an authentic standard (5).

An average of 41% of the radiochemical dose was excreted as PAG between 0 to 12 hours after dose administration.

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V. Discussion and Conclusions

The absorption of total radioactivity from the oral solution dose of [^{14}C]-SC-19129 was 95% or greater based on the observed excretion of less than 5% of the dose in the feces from 0 to 96 hours (Table 8). Little, if any, intact SC-19129 was found in plasma (<0.5% of radiolabel at appropriate HPLRC retention time) or urine (0.35% of dose found in urine from 1 of 3 monkeys). However both β -AP and the N-acetylated metabolite of β -AP were present in plasma and urine profiles. It can be estimated that at least 8% of the administered test article was absorbed with the β -aspartyl peptide bond intact since 1) absorption of total radioactivity was nearly complete and 2) the urinary excretion of β -AP plus N-acetyl- β -AP was approximately 8% of the dose (approximately 2% and 6% respectively). Likewise the areas under the plasma concentration-time curves for [^{14}C]- β -AP and [^{14}C]-N-acetyl- β -AP were both approximately 6% of the total ^{14}C AUC. Thus the methyl ester bond appears to be nearly completely metabolized pre-systemically by enzymes in intestine and/or liver, but the β -aspartyl peptide bond is relatively stable to enzymatic hydrolysis.

Hydrolysis of a portion of the administered dose to release free phenylalanine (and presumably free aspartic acid) was demonstrated by the presence of [^{14}C]-phenylalanine in both plasma and urine. The majority of the radiolabel excreted in the urine (approximately 40% of the administered dose) was in the form of PAG (Figure 1). This compound is a normal urinary metabolite in higher primates and man (6,7) and is formed by conjugation of phenylacetic acid, arising from phenylalanine metabolism, with glutamine. However

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the major route of metabolism of phenylalanine is normally via hydroxylation to tyrosine (8), and phenylacetylglutamine is usually only a minor metabolite in mammals (8). The high proportion of the phenylalanyl moiety of SC-19129 metabolized via Phenylacetic acid, to PAG may be the results of bacterial metabolism in the lower gastrointestinal (GI) tract. Figure 5 shows that PAG is very low in plasma at early times but, after a 2 hour lag period, rises to become the major ethanol-soluble constituent in plasma with a C_{max} at 6.7 hours (Table 6). The results suggest that absorption of β -AP is slow and that a large fraction reaches the lower GI tract where it is metabolized by the gut bacteria to phenylalanine and phenylacetic acid. The two hour lag period in $^{14}CO_2$ excretion in the breath (Table 7 and Figure 7) followed by a linear rate of $^{14}CO_2$ excretion for at least 4 hours, probably reflects the absorption and metabolism of [^{14}C]-Phe liberated in the lower GI tract. The phenylacetic acid is absorbed and conjugated with glutamine following absorption.

Aspartame (APM), the α -aspartyl peptide from which SC-19129 is formed, is rapidly and completely metabolized to its constituent amino acids and methanol during the absorption process, and intact APM is not observed systemically (9). The metabolism of each moiety of APM (e.g. the phenylalanyl subunit) was found to be the same as that of its free counterpart (e.g. phenylalanine) in experimental animals including the rhesus monkey (9,10). Absorption of radiolabel from either [^{14}C]-SC-19129 or [^{14}C]-APM (both labeled with [^{14}C]-Phe) following oral administration to the rhesus monkey is nearly complete, as evidenced by low fecal excretion (<5% of dose), but metabolism of the two peptides differs in

several respects. Excretion of radiolabel in urine is very low for [^{14}C]-APM (3.1% of dose in 48 hours) compared to [^{14}C]-SC-19129 (55.9% of dose in 48 hours). However excretion of radiolabel as $^{14}\text{CO}_2$ in the breath (0 to 7 hours) was much higher for [^{14}C]-APM (17.9% of dose) than for [^{14}C]-SC-19129 (4.8% of dose). The low net excretion for [^{14}C]-APM from 0 to 48 hours is consistent with extensive formation of free [^{14}C]-Phe which is largely incorporated (as Phe or tyrosine) into plasma and tissue proteins. The major route of excretion, as $^{14}\text{CO}_2$ in the breath, is the same for [^{14}C]-APM and [^{14}C]-Phe. Comparison of the percents of dose excreted as $^{14}\text{CO}_2$ for [^{14}C]-SC-19129 and [^{14}C]-APM suggests that at least 25% of the phenylalanyl moiety of SC-19129 reaches the systemic circulation as free Phe. An upper limit of 40% of the dose reaching the systemic circulation as free Phe may be assumed based on the recovery of approximately 60% of the radiochemical dose from [^{14}C]-SC-19129 in urine and feces versus an expected recovery of approximately 3% (10) for free Phe.

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VI. References

1. Gibaldi, M. and D. Perrier. Pharmacokinetics. Marcel Dekker, Inc. New York. 1975. pp. 293-296.
2. Ryan, T.A., B.L. Joiner and B.F. Ryan. Minitab Student Handbook. Duxbury Press, North Scituate, MA, 1976, pp. 152-157.
3. Sedman, A.J. and J.G. Wagner. CSTRIP, a Fortran IV Computer Program for Obtaining Initial Polyexponential Parameter Estimates. J. Pharm. Sci. 65:1006-1010, 1976.
4. Metzler, C.M., G.K. Elfring and A.J. McEwen. A Package of Computer Programs for Pharmacokinetic Modeling. Biometrics 30:562-563, 1974.
5. Library of Infra-Red Spectra, Sadtler Research Laboratory, Philadelphia, PA, #6381.
6. Stein, W.H., A.C. Paladini, C.H.W. Hirs and S. Moore. Phenylacetylglutamine as a Constituent of Normal Human Urine. J. Amer. Chem. Soc. 76 2848-2849 (1954).
7. James, M.O., R.L. Smith, R.T. Williams, and M. Reidenberg. The Conjugation of Phenylacetic Acid in Man, Sub-Human Primates and Some Non-Primate Species. Proc. R. Soc. Lond. B. 182, 25-35 (1972).
8. Harper, A.E. Phenylalanine Metabolism. In: Stegink, L.D. and L.J. Filer, Jr. (Editors) Aspartame: Physiology and Biochemistry. Marcel Dekker, Inc. New York, 1984, pp. 77-109.
9. Oppermann, J.A. Aspartame Metabolism in Animals. In: Stegink, L.D. and L.J. Filer, Jr. (Editors) Aspartame: physiology and Biochemistry. Marcel Dekker, Inc., New York, 1984, pp. 141-159.
10. Oppermann, J.A., E. Muldoon and R.E. Ranney. Metabolism of Aspartame in Monkeys. J. Nutr. 103, 1454-1459 (1973).

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VII. Tables

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-VII.1-

Table 1

Plasma Concentrations of Total ^{14}C
in Monkeys Given ^{14}C -SC-19129

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME	#378	#383	#396	MEAN	SEM ^a
0.25	0.204	0.114	0.0642	0.127	0.0408
0.50	0.431	0.475	0.565	0.491	0.0394
1.0	0.677	1.95	1.52	1.38	0.373
2.0	1.01	4.84	2.62	2.83	1.11
3.0	1.46	4.95	3.53	3.31	1.01
4.0	3.14	6.90	4.41	4.82	1.10
6.0	3.68	6.63	6.08	5.46	0.906
8.0	2.96	4.55	5.98	4.50	0.873
12.	2.34	5.10	6.41	4.61	1.20
24.	1.79	4.74	4.60	3.71	0.959
48.	1.42	3.56	3.55	2.84	0.711
72.	1.30	3.39	3.18	2.62	0.662
96.	1.56	3.87	3.51	2.98	0.718

^a Standard error of the mean.

Table 2

Plasma Concentrations of ^{14}C - β -AP
in Monkeys Given ^{14}C -SC-19129

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME	ANIMAL NUMBER			MEAN	SEM ^a
	#378	#383	#396		
0.25	0.0343	0.0212	0.00340	0.0196	0.00895
0.5	0.143	0.110	0.109	0.121	0.0112
1.	0.146	0.711	0.518	0.458	0.166
2.	0.0530	0.787	0.507	0.449	0.214
3.	0.0717	0.413	0.239	0.241	0.0985
4.	0.111	0.150	0.595	0.285	0.155
6.	0.463	0.261	0.332	0.352	0.0592
8.	0.279	0.124	0.234	0.212	0.0460
12.	0.0476	0.0465	0.0125	0.0355	0.0115

^a Standard error of the mean.

Table 3

Plasma Concentrations of ^{14}C -N-Acetyl- β -AP
in Monkeys Given ^{14}C -SC-19129

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME	ANIMAL NUMBER			MEAN	SEM ^a
	#378	#383	#396		
0.25	0.0381	0.0127	0.00380	0.0182	0.0103
0.5	0.140	0.0648	0.0991	0.101	0.0217
1.	0.0974	0.481	0.426	0.335	0.120
2.	0.0449	0.838	0.625	0.503	0.237
3.	0.0577	0.495	0.322	0.292	0.127
4.	0.0981	0.461	0.820	0.460	0.208
6.	0.192	0.265	0.428	0.295	0.0698
8.	0.0811	0.139	0.152	0.124	0.0218
12.	0.0462	0.0330	0.0428	0.0407	0.00396

^a Standard error of the mean.

Table 4

Plasma Concentrations of ^{14}C -Phenylalanine
in Monkeys Given ^{14}C -SC-19129

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME	ANIMAL NUMBER			MEAN	SEM ^a
	#378	#383	#396		
0.25	0.0264	0.00930	0.00150	0.0124	0.00735
0.5	0.0557	0.0356	0.0326	0.0413	0.00725
1.	0.0395	0.115	0.118	0.0908	0.0257
2.	0.0369	0.193	0.128	0.119	0.0453
3.	0.0941	0.115	0.114	0.108	0.00681
4.	0.441	0.436	0.237	0.371	0.0672
6.	0.430	0.191	0.250	0.290	0.0719
8.	0.174	0.212	0.194	0.193	0.0110
12.	0.0505	0.110	0.184	0.115	0.0386

^a Standard error of the mean.

Table 5

Plasma Concentrations of ^{14}C -Phenylacetylglutamine
in Monkeys Given ^{14}C -SC-19129

Plasma Concentration (micrograms/ml) for Animals vs. Time (hours)

TIME	ANIMAL NUMBER			MEAN	SEM ^a
	#378	#383	#396		
0.25	0.0215	0.0185	0.00770	0.0159	0.00419
0.5	0.0225	0.0363	0.0205	0.0264	0.00497
1.	0.0134	0.0530	0.0348	0.0337	0.0114
2.	0.0125	0.0723	0.0183	0.0344	0.0190
3.	0.110	0.223	0.0117	0.115	0.0610
4.	0.979	0.995	0.124	0.699	0.288
6.	1.50	1.05	0.641	1.06	0.247
8.	0.818	0.522	0.806	0.715	0.0967
12.	0.0963	0.147	0.272	0.172	0.0522

^a Standard error of the mean.

Table 6

Pharmacokinetic Parameters of Radioactive Compounds
in Plasma Following Oral Administration
of [^{14}C]-SC-19129 to Rhesus Monkeys^a

Parameter	Total Radioactivity	β -AP	N-Acetyl β -AP	Phenyl- alanine	Phenylacetyl glutamine
Peak Concentration ^b (C _{max} , mcg/ml)	5.66 \pm 1.00	0.615 \pm 0.094	0.617 \pm 0.212	0.376 \pm 0.063	1.12 \pm 0.20
Time of Peak Concentration ^b (T _{max} , hours)	7.3 \pm 2.4	4.0 \pm 1.2	4.0 \pm 1.2	4.7 \pm 0.7	6.7 \pm 0.7
AUC [(mcg/ml) hours]	48.3 \pm 9.7 (310 \pm 75) ^d	2.92 \pm 0.33	2.82 \pm 0.87	2.26 \pm 0.14	5.85 \pm 0.80

^a Values are the mean \pm standard error of the mean of 3 animals.

^b Obtained by inspection of the plasma concentration data in Tables 1-5.

^c AUC values calculated from 0 to 12 hour plasma concentration data, Tables 1-5.

^d The value in parentheses is the AUC from 0 to 96 hours for total ^{14}C .

Table 7

Mean Cumulative Excretion of $^{14}\text{CO}_2$
in Expired Air of Monkeys
Given [U- ^{14}C -Phe]-SC-19129

Individual Cumulative Percent Recoveries of Radioactivity

B R E A T H

TIME ^a	#378	#383	#396	MEAN	SEM ^b
0 - 30	0.0133	0.00842	0.00879	0.0102	0.00156
0 - 60	0.0409	0.0541	0.0546	0.0499	0.00448
0 - 120	0.138	0.391	0.316	0.281	0.0751
0 - 180	0.544	1.09	0.609	0.748	0.173
0 - 240	1.62	2.56	1.04	1.74	0.443
0 - 300	2.76	4.32	1.62	2.90	0.782
0 - 360	3.89	5.24	2.57	3.90	0.770
0 - 420	4.93	5.74	3.79	4.82	0.565

^a Time after dose administration in hours.

^b Standard error of the mean.

Table 8

Mean Urinary and Fecal Excretion of Total ^{14}C
Following Administration of ^{14}C -SC-19129 to Monkeys

Individual Cumulative Percent Recoveries of Radioactivity

U R I N E

TIME ^a	ANIMAL NUMBER			MEAN	SEM ^b
	#378	#383	#396		
0 - 6	31.5	41.3	16.8	29.9	7.12
0 - 12	58.7	52.1	46.9	52.6	3.43
0 - 24	61.8	53.9	50.2	55.3	3.43
0 - 48	62.9	54.1	50.7	55.9	3.63
0 - 72	63.1	54.2	50.9	56.1	3.65
0 - 96	63.2	54.3	51.2	56.2	3.61

F E C E S

TIME	ANIMAL NUMBER			MEAN	SEM
	#378	#383	#396		
0 - 24	1.08	5.71	2.58	3.12	1.36
0 - 48	2.75	6.30	3.70	4.25	1.06
0 - 72	3.01	6.45	3.95	4.47	1.03
0 - 96	3.06	6.55	4.12	4.58	1.03

U R I N E & F E C E S

TIME	ANIMAL NUMBER			MEAN	SEM
	#378	#383	#396		
0 - 6	31.5	41.3	16.8	29.9	7.12
0 - 12	58.7	52.1	46.9	52.6	3.43
0 - 24	62.9	59.6	52.8	58.4	2.97
0 - 48	65.6	60.4	54.4	60.1	3.24
0 - 72	66.1	60.7	54.8	60.5	3.26
0 - 96	66.3	60.8	55.3	60.8	3.17

^a Time after dose administration in hours.

^b Standard error of the mean.

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VIII. Figures

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-VIII.1-

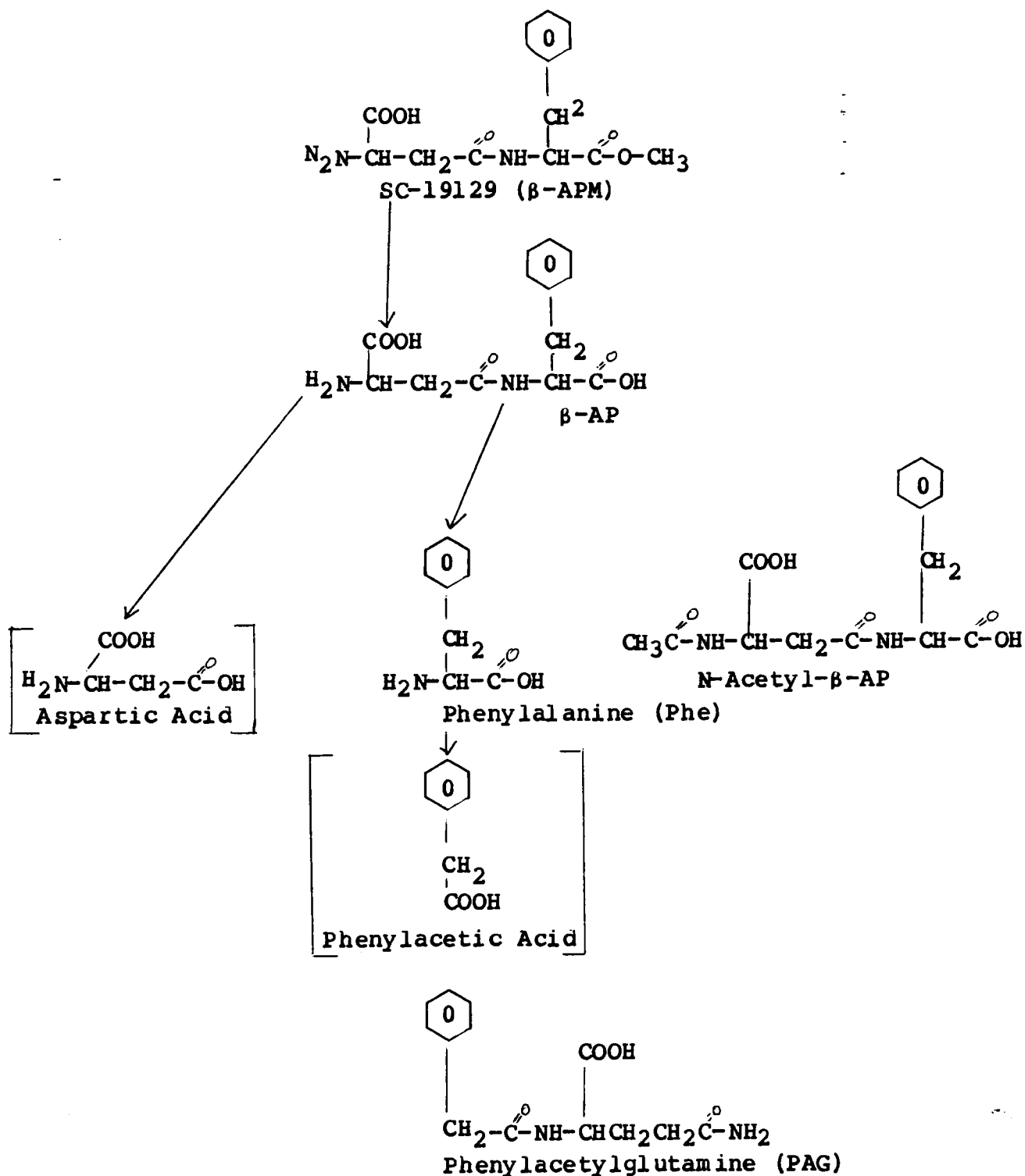


Figure 1. Structures and proposed metabolic pathway of SC-19129. Structures in brackets are hypothetical metabolites which were not identified in this study.

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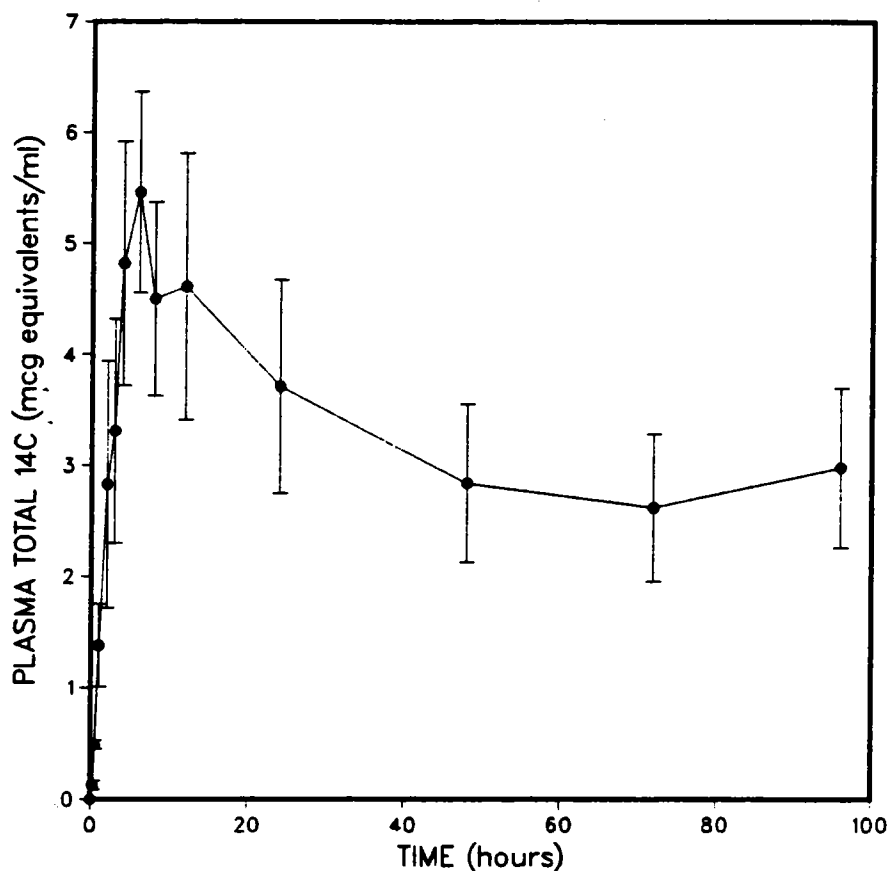


Figure 2. Mean plasma concentrations of total radioactivity following oral administration of 10 mg [^{14}C]-SC-19129 per kg body weight to 3 female rhesus monkeys. Abscissa: time after dose administration in hours. Ordinate: concentration in plasma expressed as mcg equivalents of SC-19129 per ml. The vertical bars indicate the standard errors of the means.

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-VIII.3-

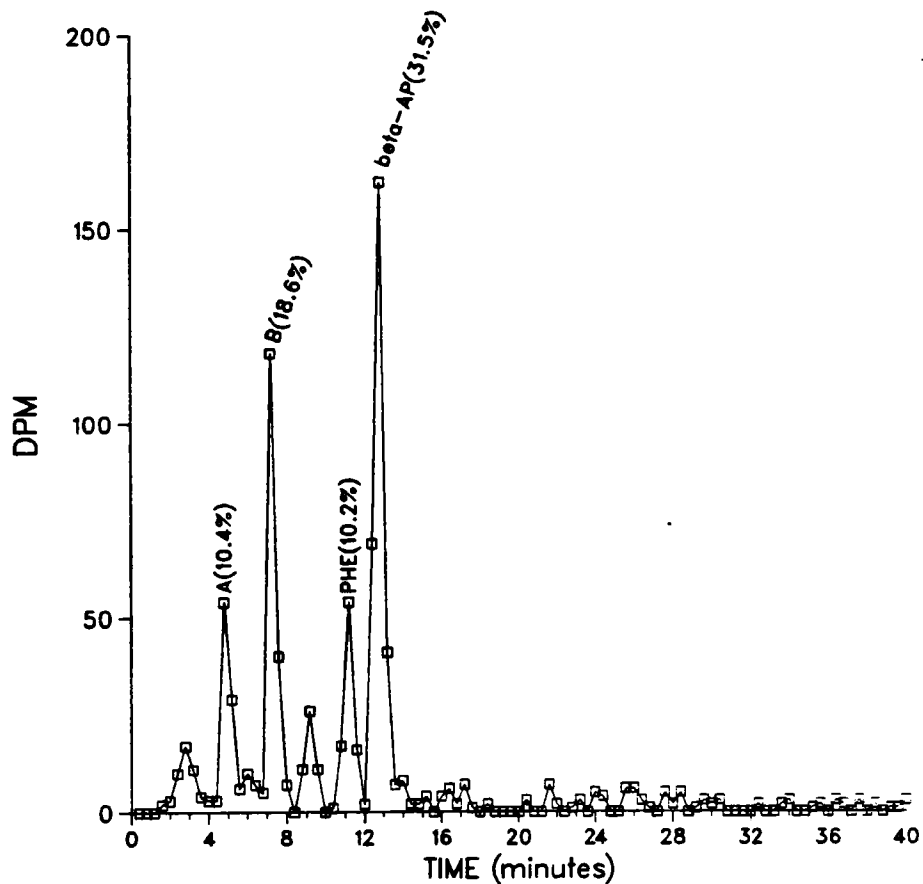


Figure 3. High performance liquid radiochromatogram of the ethanol extract from the 0.5 hour plasma sample from monkey #383 following oral administration of 10 mg [^{14}C]-SC-19129 per kg. The locations of the reference standards of β -AP and phenylalanine (Phe), and of metabolites A and B, are marked on the chromatogram. The percentages of radioactivity applied to the column that are associated with the above 4 peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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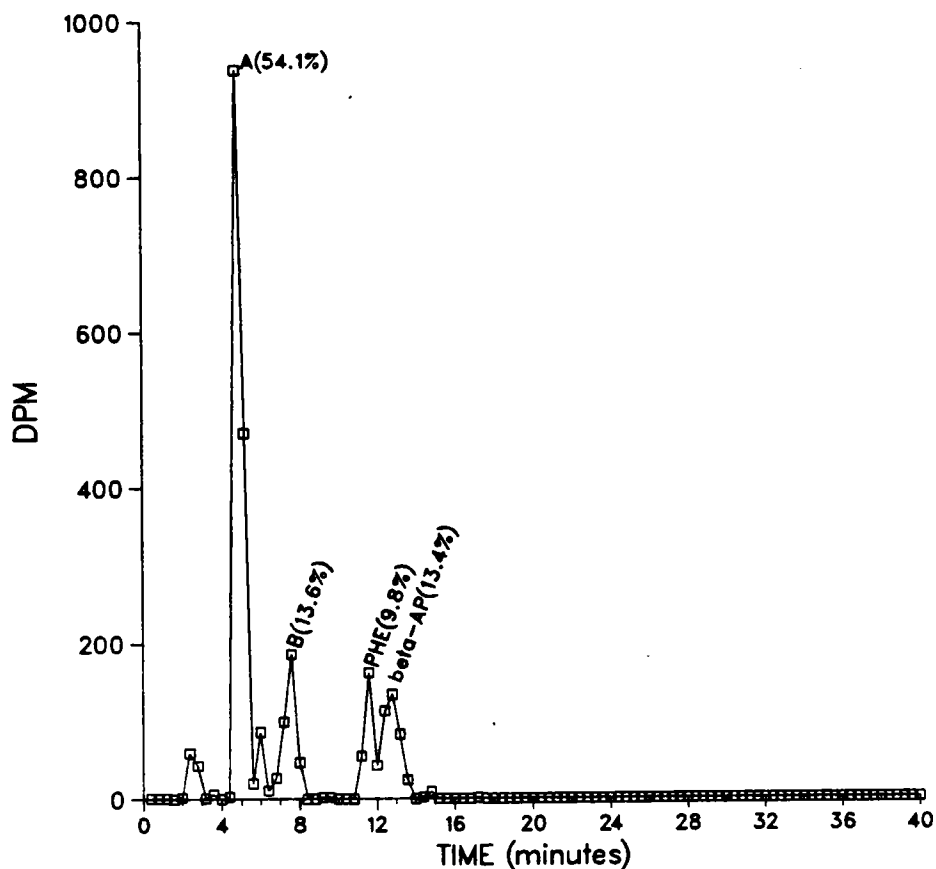


Figure 4. High performance liquid radiochromatogram of the ethanol extract from the 6 hour plasma sample from monkey #383 following oral administration of 10 mg [14 C]-SC-19129 per kg. The locations of the reference standards of β -AP and phenylalanine (Phe), and of metabolites A and B, are marked on the chromatogram. The percentages of radioactivity applied to the column that are associated with the above 4 peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

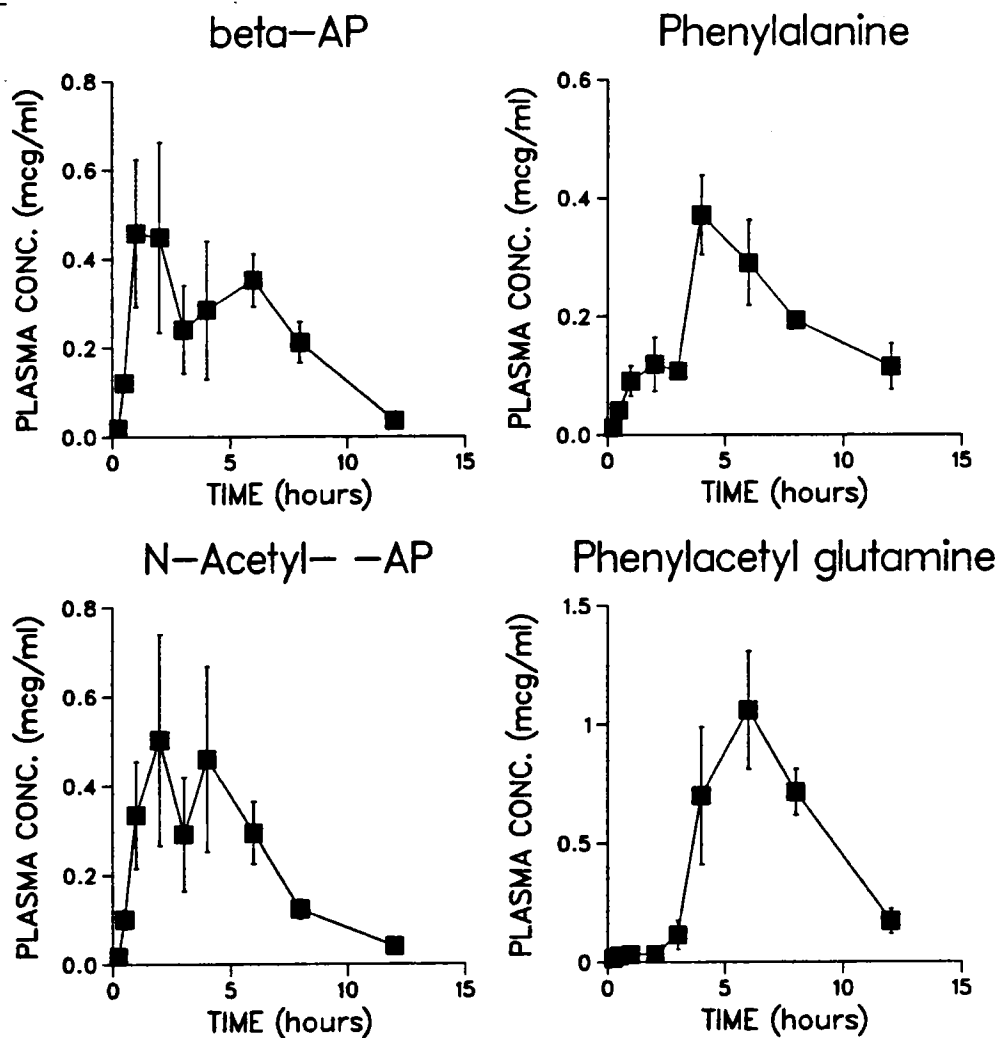


Figure 5. Mean plasma concentrations of A) β -AP, B) Phenylalanine, C) N-acetyl- β -AP and D) phenylacetylglutamine following oral administration of 10 mg [14 C]-SC-19129 per kg to 3 female rhesus monkeys. Abscissa: time after dose administration in hours. Ordinate: concentration in plasma expressed as mcg equivalents of SC-19129 per ml.

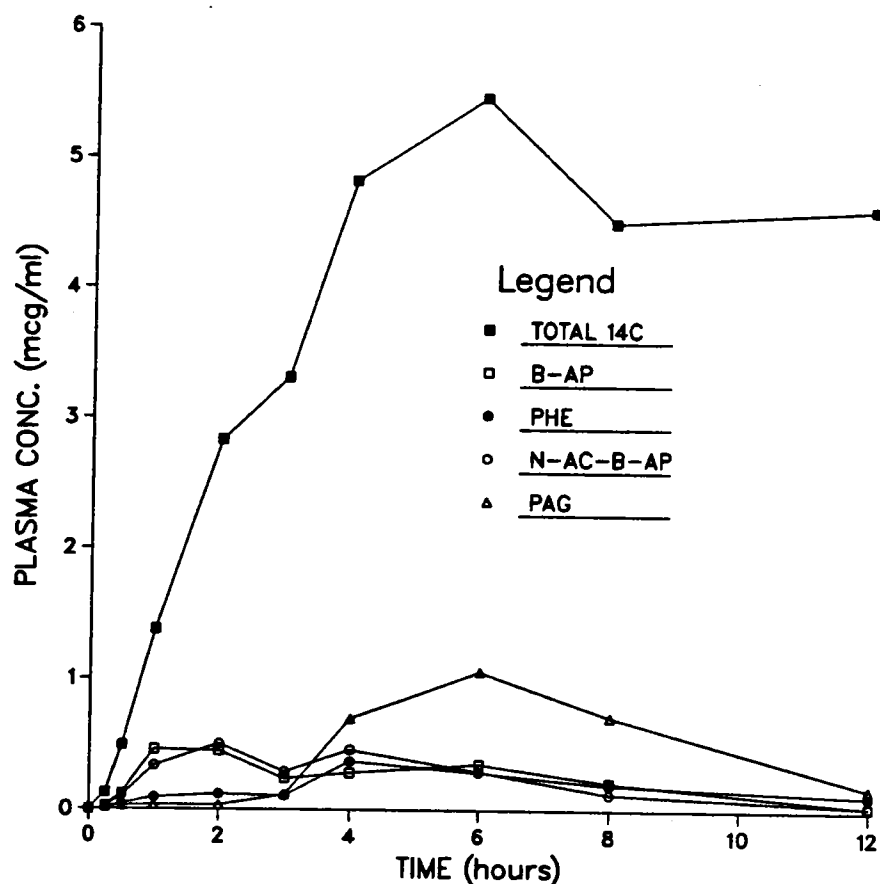


Figure 6. Plasma concentrations of total ^{14}C (■), [^{14}C - β -AP (□), [^{14}C]-phenylalanine (Phe, ●), N-acetyl- β -AP (N-Ac- β -AP, ○) and phenylacetylglutamine (PAG, △) following oral administration of 10 mg [^{14}C]-SC-19129 per kg to 3 female rhesus monkeys. Abscissa: time after administration in hours. Ordinate: concentration in plasma expressed as mcg equivalents SC-19129 per ml.

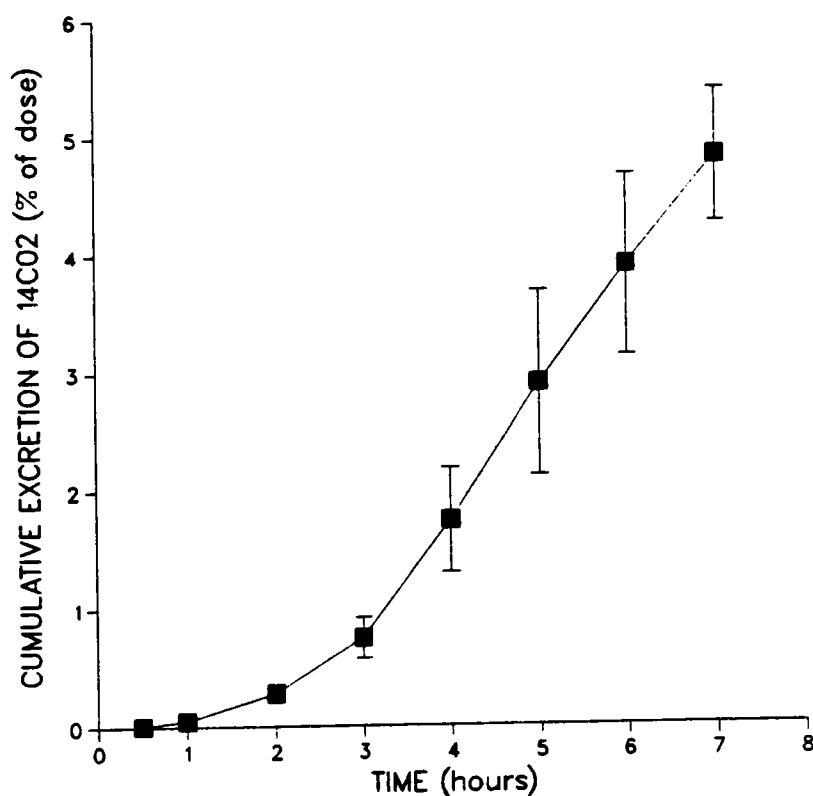


Figure 7. Mean cumulative excretion of $^{14}\text{CO}_2$ in the breath following oral administration of 10 mg [^{14}C]-SC-19129 per kg to 3 female rhesus monkeys. Abscissa: time after dose administration in hours. Ordinate: cumulative excretion of radioactivity as a percentage of dose. The vertical bars indicate the standard errors of the means.

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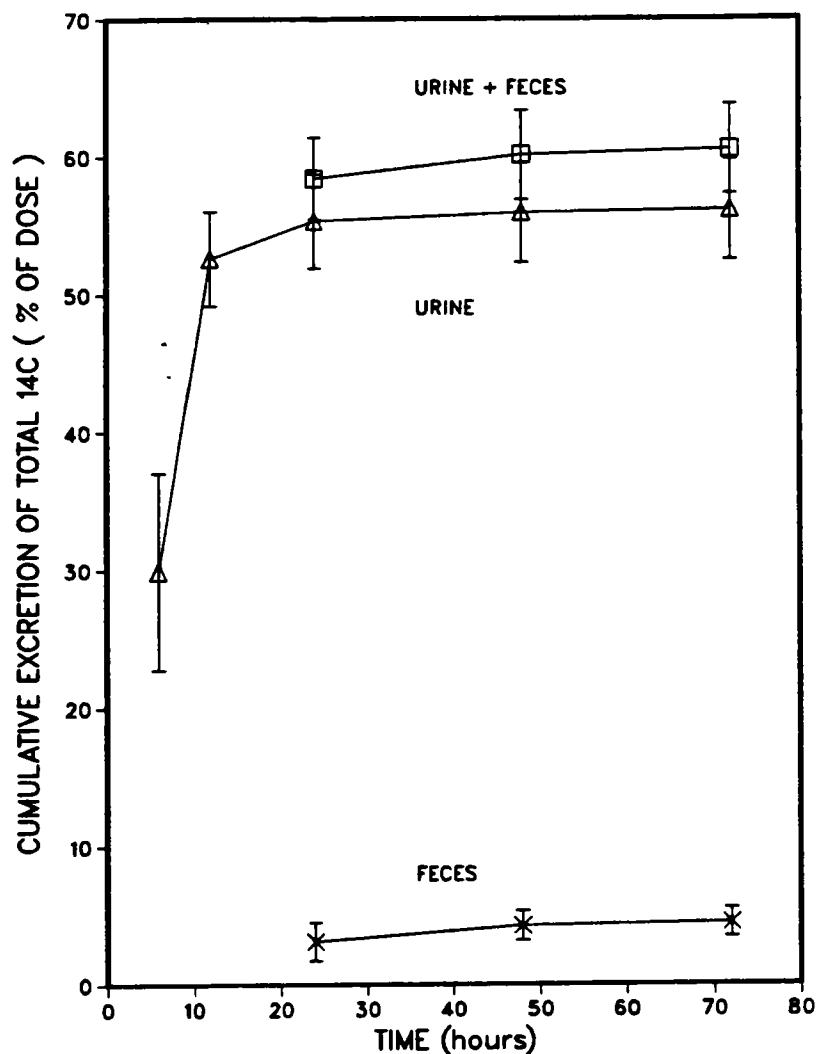


Figure 8. Mean cumulative excretion of radioactivity in the urine (Δ), feces (X) and urine and feces combined (\square) following oral administration of 10 mg [^{14}C]-SC-19129 per kg to 3 female rhesus monkeys. Abscissa: time after dose administration in hours. Ordinate: cumulative excretion of radioactivity as a percentage of dose. The vertical bars indicate the standard errors of the means.

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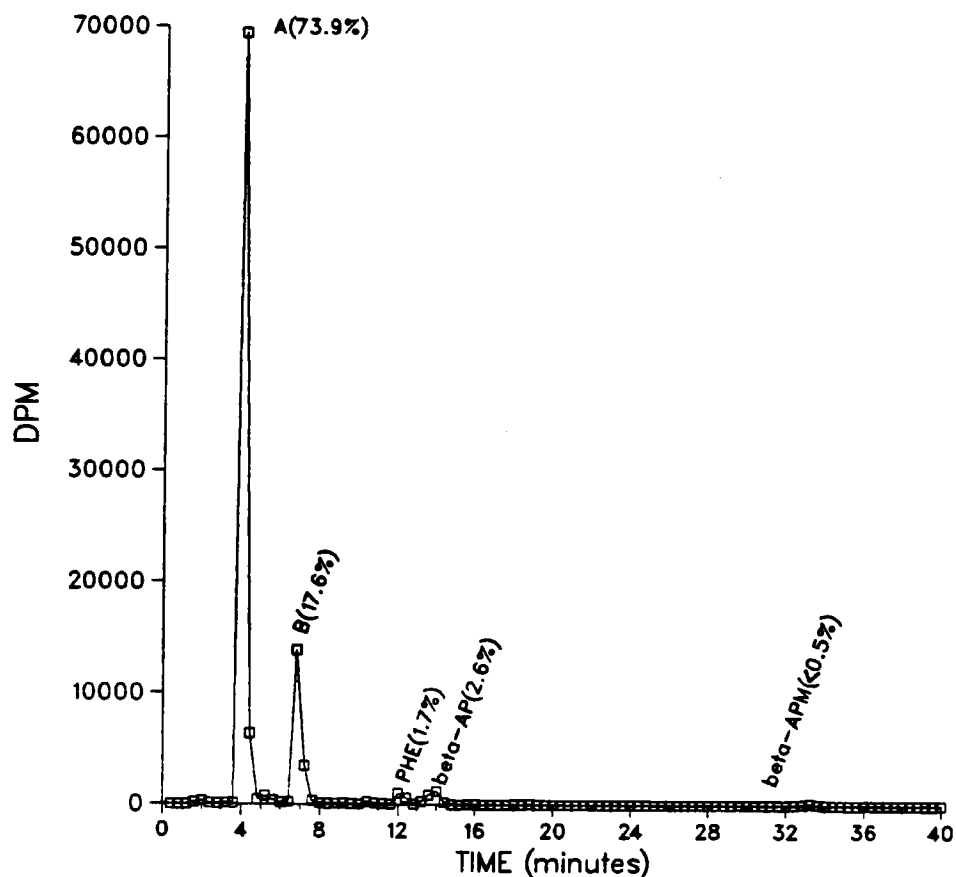


Figure 9. High performance liquid radiochromatogram of the 0-6 hour urine sample from monkey #383 following oral administration of 10 mg [14 C]-SC-19129 per kg. The locations of the reference standards of β -AP and phenylalanine (Phe), and of metabolites A and B, are marked on the chromatogram. The percentages of radioactivity applied to the column that are associated with the above 4 peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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-VIII.10-

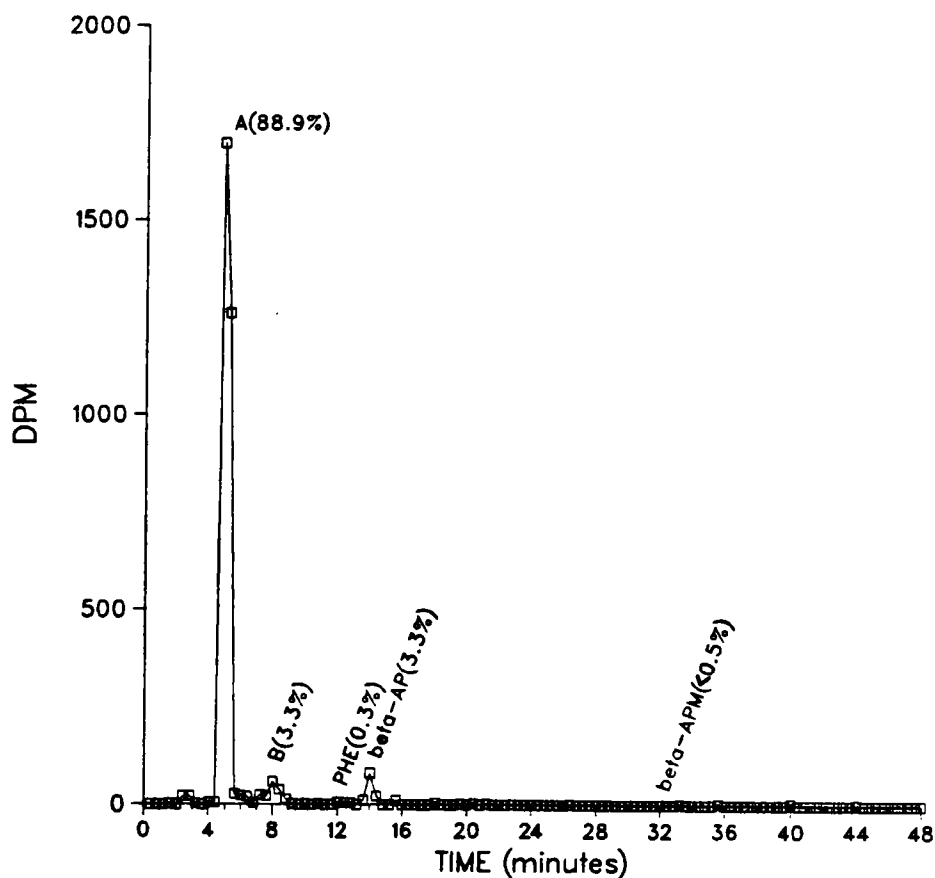


Figure 10. High performance liquid radiochromatogram of the 6-12 hour urine sample from monkey #383 following oral administration of 10 mg [^{14}C]-SC-19129 per kg. The locations of the reference standards of β -AP and phenylalanine (Phe), and of metabolites A and B, are marked on the chromatogram. The percentages of radioactivity applied to the column that are associated with the above 4 peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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-VIII.11-

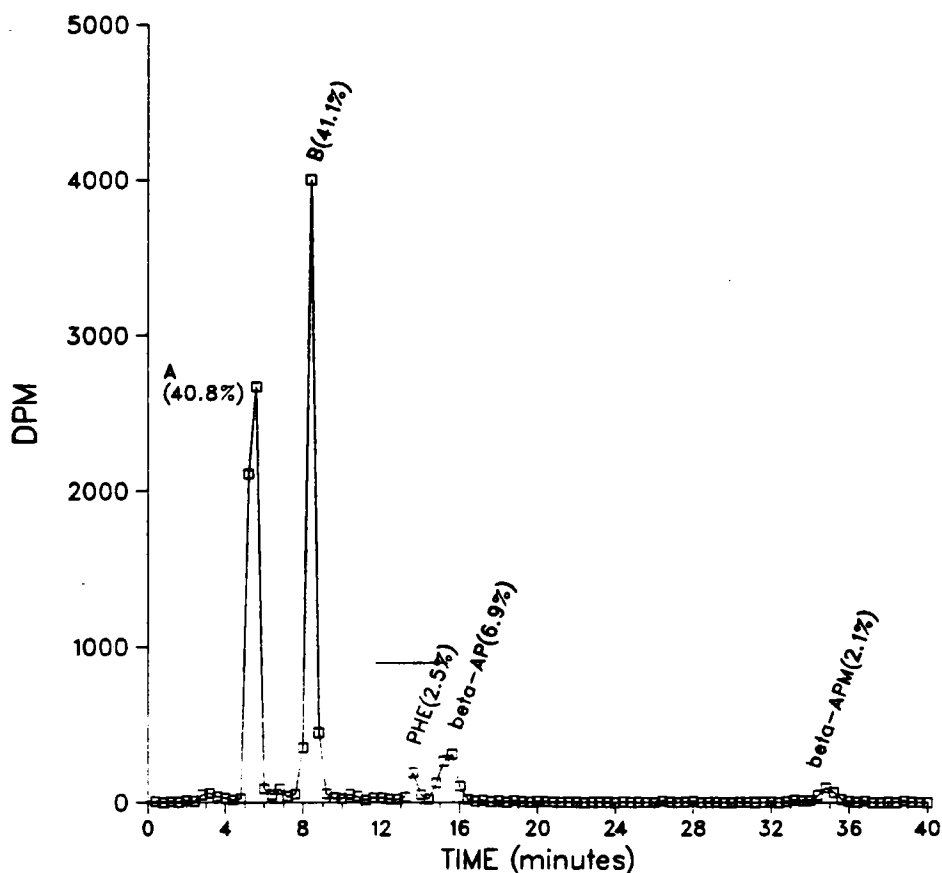


Figure 11. High performance liquid radiochromatogram of the 0-6 hour urine sample from monkey #396 following oral administration of 10 mg [^{14}C]-SC-19129 per kg. The locations of the reference standards of β -AP and phenylalanine (Phe), and of metabolites A and B, are marked on the chromatogram. The percentages of radioactivity applied to the column that are associated with the above 4 peaks are also shown on the chromatogram. Abscissa: elution time in minutes. Ordinate: disintegrations per minute (DPM).

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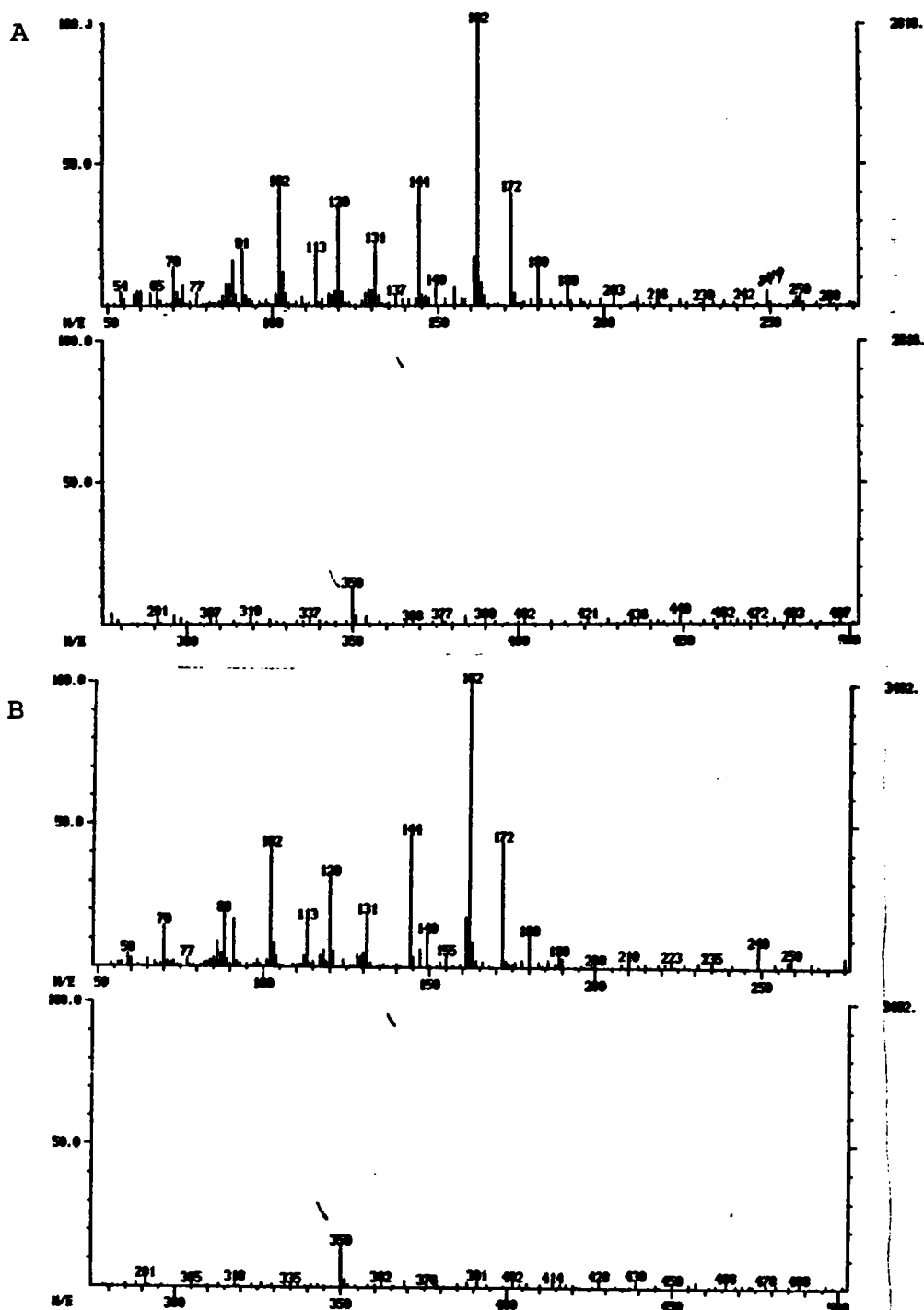


Figure 12. The electron impact mass spectra of (A) N-acetyl-β-AP dimethyl ester standard and (B) the methylated derivative of metabolite B.

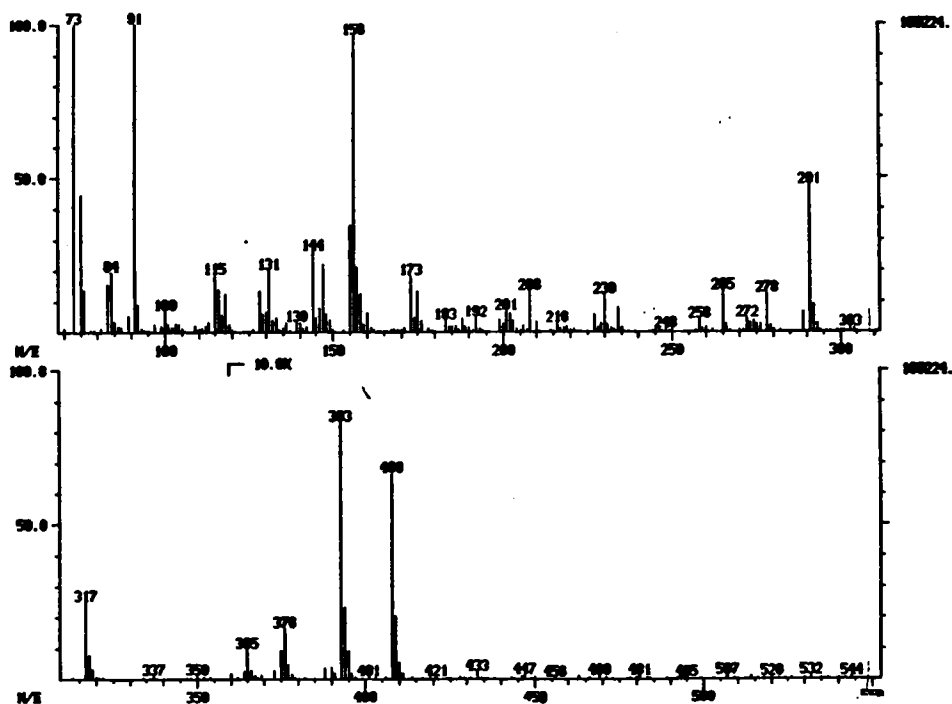


Figure 13. The electron impact mass spectrum of the trimethylsilyl (TMS) derivative of metabolite A.

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-VIII.14-

IX. Appendix 1: Protocol

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-IX.1-

Protocol

1. Study Title:

Pharmacokinetics and Metabolism of [^{14}C]- β -Aspartyl Phenylalanine Methyl Ester, β -APM, in the Rhesus Monkey.

2. Study Sponsor:

G.D. Searle and Co.

3. Facility:

G.D. Searle and Co., 4901 Searle Parkway,
Skokie, IL 60077

4. Proposed Date:

First Dosing: July 17, 1984

5. Introduction:

β -APM has been identified as a conversion product of aspartame (SC-18862, N-L- β -aspartyl-L-phenylalanine methyl ester, APM) in sweetened soft drinks.

6. Purpose:

The purpose of this study is to determine the pharmacokinetics and metabolism of orally administered [^{14}C]- β -APM in the rhesus monkey.

7. Overview of Study Design:

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[U-¹⁴C-Phe]-β-APM will be administered as an oral solution to three rhesus monkeys. Plasma, breath, urine and feces will be collected at selected times after dosing. Total radioactivity will be determined for all samples. Plasma and urine concentrations of β-APM (if present), β-AP (the free acid of β-APM) and other major metabolites will be determined by a high performance liquid radiochromatographic (HPLRC) procedure.

8. Laboratory Procedure:

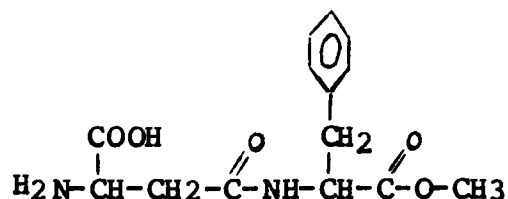
This study is not within the scope of Good Laboratory Practice Regulations.

9. Test Article:

A. Chemical Name:

β-APM (SC-19129) is N-L-β-Aspartyl-L-phenyl-alanine, 1-methyl ester.

B. Chemical Structure:



C. Formulation:

1. [U-¹⁴C-Phe]-β-APM with a specific activity of 1.4 mCi/mmol (approximately 4.8 mCi/mg) prepared by Amersham Corp. (Arlington Heights, IL) will be supplied by the Radiochemistry

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Group, G.D. Searle and Co.

2. The dosage form will be prepared by dissolving 2.5 mg of [^{14}C]- β -APM and 2.5 mg of unlabelled β -APM (lot #CD103-112A) per ml of distilled water to give a final concentration of 5.0 mg/ml and approximately 12 mCi/ml.

D. Administration:

1. **Route:** The test article will be administered intragastrically, by nasogastric or stomach tube.
2. **Frequency:** Each animal will receive a single dose of the test article.
3. **Volume and Dosage:** Each dose will consist of 2 ml/kg body weight. This will provide a dose of 10 mg/kg and approximately 24 mCi/kg. Each dose will be followed by approximately 10 ml of distilled water administered in such a way as to rinse the stomach tube.

E. Analyses:

The radiochemical purity of the test article in the dose solution will be determined by thin layer radiochromatography (TLRC) within 4 hours of dose administration.

F. Storage:

[^{14}C]- β -APM will be stored at approximately 4°C; the unlabelled β -APM will be stored at room temperature. The test article dosing solution will be prepared fresh on the day of administration.

10. **Test System, Housing and Diet:**

A. Test System:

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Three female rhesus monkeys weighing 4-6 kg will be used. Each animal will be identified by a unique tatoo on its chest.

B. Housing and Restraint:

The animals will be restrained in primate chairs prior to, and until approximately 12 hours following, dose administration. They will then be transferred to individual stainless steel metabolism cages and maintained in these cages until approximately 96 hours after dosing. The animals will be located in room J-286 during dosing and sample collection. They will be housed in the LAR primate colony prior to and following the study.

C. Diet:

1. ~~Food~~: The animals will be maintained on a diet of Purina Biscuit Chow #5037 (Ralston Purina, St. Louis, MO) supplemented with bread and/or fruit. They will be fasted from at least 16 hours prior to dosing until at least 7 hours after dosing.
2. ~~Water~~: Tap water will be available ad libitum while the animals are housed in metabolism cages.
3. Special analyses of food and water will not be performed since no contaminants known to be capable of interfering with the study are reasonably expected to be present.

11. Sample Collection, Times and Storage and Fluid Replacement:

A. Blood:

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Approximately 3 ml of blood will be collected from a saphenous or cephalic vein, into chilled heparinized tubes, at or near 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96 hours. The blood collection tubes for the samples taken up to 6 hours after dosing will contain 0.05 ml of 0.006 molar diethyl-p-nitrophenyl phosphate to inhibit esterase activity. Plasma will be prepared by centrifugation. The plasma will be frozen and stored at $-20 \pm 10^{\circ}\text{C}$ if not analyzed within one hour.

B. Urine:

Urine will be collected from the urinary bladder via a catheter from approximately 0-6 and 6-12 hours after dosing. Urine will be collected by free catch from 12-24, 24-48, 48-72 and 72-96 hours after dosing. The urine samples will be frozen and stored at $-20 \pm 10^{\circ}\text{C}$ until analysis.

C. Feces:

Feces will be collected at approximately 24, 48, 72 and 96 hours and stored frozen until analysis.

D. Breath:

$^{14}\text{CO}_2$ eliminated in the breath will be collected for approximately 7 hours after dosing. A plastic helmet will be placed over each monkey's head after dosing and expired air will be drawn, by means of a vacuum, through gas washers containing ethanalamine:2-methoxyethanol (1:2, v/v). Samples will be collected from approximately 0-0.5, 0.5-1, 1-1.5, 1.5-2, 2-3, 3-4, 4-5, 5-6 and 6-7 hours.

E. Control Plasma, Urine and Feces:

Plasma, urine and feces will be collected from control animals which have not been treated with

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the test article. Aliquots of the plasma, urine and feces will be spiked with [^{14}C]- β -APM prior to frozen storage. The spiked samples will be used to determine stability and efficiency of extraction with each matrix.

F. Fluid Replacement:

Prior to dosing a slow intravenous infusion of isotonic saline will be administered via a cannulated cephalic vein. The saline infusion will be continued until approximately 5 hours after dosing.

12. **Sample Analysis:**

A. Plasma and Urine:

Total ^{14}C will be measured by direct liquid scintillation counting (LSC). Based on the results from the total ^{14}C analysis, plasma and urine samples from appropriate time points will be selected from sample extraction followed by analysis by high performance liquid radiochromatography (HPLRC) for [^{14}C]- β -APM (if present), [^{14}C]- β -AP (the free acid of β -APM) and related compounds.

B. Feces:

Total ^{14}C will be measured by sample combustion and LSC of the trapped products. Based on the results of the total ^{14}C analysis, selected samples will be extracted and analyzed by TLRC or HPLRC.

C. Breath:

Total ^{14}C in each sample will be measured by LSC of aliquots of the ethanolamine:2-methoxyethanol

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trapping solutions.

13. Statistical Procedure:

Individual data for total radioactivity determinations will be tabulated. Plasma total ^{14}C concentration-time curves will be prepared from the mean ^{14}C plasma concentrations of the animals at each time point and the related pharmacokinetic parameters determined. The recovery of ^{14}C will be calculated from the urinary, fecal, and breath data. Plasma concentration-time curves will be prepared from the [^{14}C]- β -APM (if present), [^{14}C] β -AP or other major metabolite concentration data obtained from the metabolic profiles of pooled samples.

14. Archiving of Materials:

A report will be written and submitted to the R&D Central File. The raw data will be submitted to the R&D Central File after completion of the report.

15. Study Participants:

Study Director	E. Burton
Test article administration, specimen collection	M. DalCorobbo
Dosage form preparation and analysis and sample analysis	I. Dressler, K. Hoglund
Report	E. Burton

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16. Protocol Review:

T. Hutsell
J. Oppermann
S. Smeach
C. Tschanz


17. Protocol Approval:

 7/17/84

J. Oppermann Date

 7/17/84

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E. Burton Date
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Table/Figure Number/Section	Book Number & Pages and/or Worksheet #
TABLE 1	NTB MRC-0523, pg. 15
TABLE 2	MRC-0523 LL, 7 Pg. 1 A, B, C, 2 D
TABLE 3	MRC-0523 LL, 7 Pg. 1 A, B, C, 4 C
TABLE 4	MRC-0523 LL, 7 Pg. 1 A, B, C, 3 C
TABLE 5	MRC-0523 LL, 7 Pg. 1 A, B, C, 5 C
TABLE 6	MRC-0523 LL, 7 Pg. 6 and 7
TABLE 7	NTB MRC-0523, pg. 29
1	MRC-0523 LL, A Pg. 59 A-N, 61 A-C
TABLE 8 (urine)	NTB MRC-0523, pg. 15, 20
1	MRC-0523 LL, A Pg. 45 thru 49, 59 (O-T)
TABLE 8 (Feces)	NTB MRC-0523, pg. 15, 22
	MRC-0523 LL, A Pg. 50-59 (59: U-Z, AA-DD)

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Table/Figure Number/Section	Book Number & Pages and/or Worksheet #
FIGURE 2	MRC-0523 LL, A Pg. 59-MM thru QQ
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FIGURE 6	MRC-0523 LL, A Pg. 59-MM, LL-J: 2-D, 3-C, 4-C, 5-C
FIGURE 7	MRC-0523 LL, A Pg. 59-G
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FIGURE 9	MRC-0523 LL, B Pg. 26, 27, -LL-J pg 17, 22
FIGURE 10	MRC-0523 LL, B Pg. 30, 31, -LL-J pg 18, 24
FIGURE 11	MRC-0523 LL, B Pg. 28, 31, -LL-J pg 25, 29

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D. Sample Collections	
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