Table IV—Percentage of Recovery of Anthralin and Its Decomposition Products [a]

Sample	Anthralin, %	Dimer, %	Quinone, %	Total Recovery, %
1	88.5	7.2	1	96.7
2	92	9	2	103.0
3	80.9	15.5	2.2	98.6
4	72.6	25	1.3	98.9
5	71.2	4.7	1.1	77.0
6	70.5	25.1	1.8	97.4
7	69.0	22.4	1.7	93.1
8	67.4	29.5	1	97.9
9	39.0	10.4	1.2	50.6
10	29.8	2.5	12.9	45.2
11	17.3	81.1	5.9	104.3
12	4.4	90.9	5.1	100.4

[a] Chloroform extraction; average of data obtained using both chromatographic systems.

formation of high molecular weight materials, and an HPLC method is currently being developed for the analysis of these products.

The surprising disparity between the USP and HPLC assay results can be rationalized when the data in Table IV are considered. Although both methods rely on UV absorption, the USP technique cannot quantify the dimer and merely includes it with the anthralin level. However, a discrepancy between the results is still evident after correcting the USP data for the presence of dimer.

The majority of these ointments contained ~70% (range 44–92%) of the theoretical amount of anthralin, the remaining 30% being various quantities of the dimer, quinone, and, perhaps, high molecular weight materials.

The need to check anthralin and its preparations must be emphasized, and the danger of further decomposition of samples in use cannot be ignored.

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High-Performance Liquid Chromatographic Determination of Stereoselective Disposition of Carprofen in Humans

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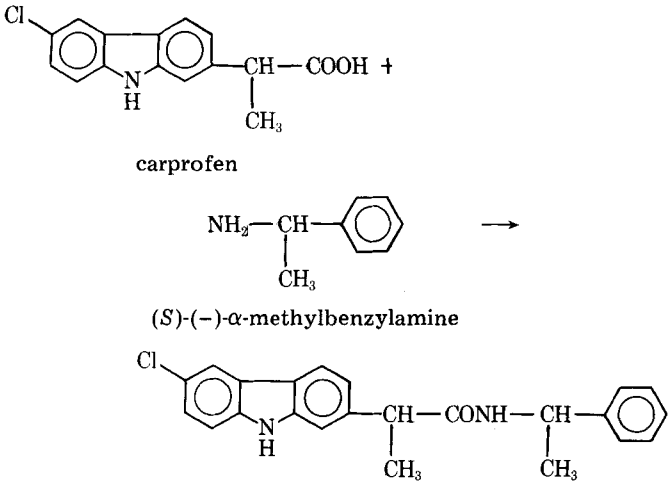
Received January 26, 1981, from the **Department of Biochemistry and Drug Metabolism and the* ‡*Department of Pharmacokinetics and Biopharmaceutics, Hoffmann-La Roche Inc., Nutley, NJ 07110.* Accepted for publication March 25, 1981.

Abstract □ A high-performance liquid chromatographic (HPLC) assay was developed for the determination of the ratios of the (*S*)-(+) and (*R*)-(−) enantiomers of the anti-inflammatory drug carprofen in blood, urine, and feces. The procedure relies on: (*a*) extraction and purification of carprofen from biological fluids, (*b*) reaction of carprofen with (*S*)-(−)-α-methylbenzylamine to form the two diastereomeric (S)-(−)-α-methylbenzylamides *via* the 1,1′-carbonyldiimidazole intermediate, (*c*) purification of the reaction mixture by extraction of the diastereomeric derivatives into hexane at pH 11, and (*d*) analysis of the diastereomeric derivatives by HPLC with UV detection. The (*S*)-(+):(*R*)-(−) ratios in the blood of three subjects receiving single 100-mg oral doses of carprofen were greater than unity up to 16 hr after dosing. The mean ± *SD* of the ratios in the early blood samples (0.5, 1, and 2 hr) was 1.21 ± 0.09, while the mean of the ratios in the later blood samples (4, 6, 8, 12, and 16 hr) was slightly higher (1.48 ± 0.17). The blood level fall-off curves for the (*S*)-(+) and (*R*)-(−) enantiomers were similar in each of the three subjects for the 4–16-hr period. The carprofen enantiomers were excreted stereoselectively by humans. An excess of the (*S*)-(+) enantiomer relative to the (*R*)-(−) enantiomer was excreted in the urine as the ester glucuronide, while unchanged (*R*)-(−) enantiomer predominated in the feces. The total urinary plus fecal excretion of the enantiomers (0–96 hr) revealed only a slight excess of the (*S*)-(+) enantiomer over the (*R*)-(−) enantiomer, which amounted to 2.1–4.9% of the dose. Since the amount of carprofen (free and glucuronide) excreted in 96 hr by the three subjects only accounted for 62–72% of the dose, no definitive statement could be made relative to the possible inversion of the carprofen chiral center.

Keyphrases □ Carprofen—stereoselective disposition determination in humans using high-performance liquid chromatography □ Anti-inflammatory agents—carprofen, metabolism in humans, and rats, high-performance liquid chromatography □ High-performance liquid chromatography—determination of stereoselective disposition of carprofen in humans

Carprofen [(D,L)-6-chloro-α-methylcarbazole-2-acetic acid] is presently undergoing extensive clinical evaluation as a nonsteroidal anti-inflammatory agent. The possible stereoselective disposition of carprofen is of interest since it is a racemic compound with a chiral center at the α-carbon position. Other α-methylarylacetic acids have been shown to undergo stereoselective disposition and inversion at the α-carbon (1–5).

A TLC procedure was previously developed (6) for the quantitation of the enantiomers of [^{14}C]carprofen as their diastereomeric (*S*)-(−)-α-methylbenzylamides. This TLC procedure has been used to study stereoselective elimi-



carprofen

(*S*)-(−)-α-methylbenzylamine

(*R,S*) and (*S,S*) diastereomeric amides of carprofen
Scheme I

nation of [^{14}C]carprofen in rats (6) and humans (7). In the rat, the (*R*)-(−) enantiomer is eliminated from the blood and secreted in the bile as the ester glucuronide at a rate approximately twice that of the (*S*)-(+) enantiomer. Concurrently, the (*S*)-(+) isomer, the pharmacologically more active enantiomer (8), persists longer in rat blood. Preliminary data suggested a preferred urinary elimination of the (*S*)-(+) enantiomer as the ester glucuronide and a preferred fecal elimination of the (*R*)-(−) enantiomer in humans (7).

This report describes a high-performance liquid chromatographic (HPLC) assay for the determination of the (*S*)-(+):(*R*)-(−) ratios of carprofen in biological fluids by measuring the diastereomeric (*S*)-(−)-α-methylbenzylamide derivatives of carprofen. Whereas the radio-TLC stereospecific assay depends on labeled carprofen for quantitation, the HPLC assay can be used to evaluate clinical specimens from studies using nonlabeled drugs. This stereospecific HPLC assay, in conjunction with other assays (9, 10) for total carprofen, was used to study the stereoselective disposition of carprofen in humans.

EXPERIMENTAL

Materials—The procedure relies on the reaction of racemic carprofen with (*S*)-(−)-α-methylbenzylamine[1] to form the (*R,S*) and (*S,S*) diastereomers (Scheme I). However, the synthetic analytical standards, I and II, were prepared by reacting the carprofen enantiomers with (*R*)-(+)-α-methylbenzylamine. Therefore, standards I and II are the antipodes, respectively, of the (*R,S*) and (*S,S*) diastereomers formed in the carprofen reaction procedure. A solution of 32.5 mg of 1,1′-carbonyldiimidazole[1]/ml of chloroform was prepared daily.

Carprofen—Carprofen ($C_{15}H_{12}ClNO_2$) has a molecular weight of 273.72. A stock solution of 1 mg/ml in ethanol was prepared. Carprofen[2] labeled with carbon 14 in the α-position was used in certain experiments as indicated. The original specific activity of 9.91 μCi/mg was diluted in solution with appropriate amounts of unlabeled compound.

For the (*S*)-(+) enantiomer the specific rotation in methanol was $[\alpha]_D^{23}$ +53.7°; for the (R)-(−) enantiomer, the specific rotation in methanol was $[\alpha]_D^{23}$ −54.2°.

Standard I[3] [(S,R)-N-(*2-Phenethyl*)-α-*methyl*(*6-chloro-9H-carbazol-2-yl*)*acetamide*]—Standard I ($C_{23}H_{21}ClN_2O$) has a molecular weight of 376.88, mp 238–239°. It dissolved slowly in the HPLC solvent system (0.75% CH_3OH in methylene chloride) at concentrations greater than 0.5 mg/ml.

Standard II[3] [(R,R)-N-(*2-Phenethyl*)-α-*methyl*(*6-chloro-9H-carbazol-2-yl*)*acetamide*]—Standard II ($C_{23}H_{21}ClN_2O$) has a molecular weight of 376.88, mp 191–193°. It was soluble in the HPLC solvent system at concentrations of 1.0 mg/ml.

Reagents—The following analytical reagent grade chemicals were used: 1 and 0.6 *N* NaOH, 0.1 and 6 *N* HCl, 1 and 0.2 *M* sodium acetate buffer (pH 5), 1 *M* potassium phosphate buffer (monobasic) (pH 7), 0.2 *N* NH_4OH (pH 11), acetic acid, formic acid, methanol, ethanol (anhydrous), chloroform (stabilized with 0.75% ethanol), methylene chloride, UV quality hexane, and ether[4] (anhydrous).

TLC—TLC plates were precoated with silica gel 60F-254[5]. Filter paper[6] was used for lining the TLC tanks. A shortwave UV lamp was used for visualization of spots.

Extraction and Purification of Carprofen from Biological Samples—*Blood*—Five-milliliter aliquots of blood were adjusted to pH 5 with 2 ml of 1 *M* acetate buffer and extracted twice with 12 ml of ether. The blood–ether mixtures were shaken for 10 min in a reciprocating shaker[7] at 60–80 strokes/min and centrifuged[8] at 2400 rpm for 5 min at room temperature. The combined ether layers were evaporated to dryness with nitrogen in a 30–40° water bath[9]. The extract residues were dissolved

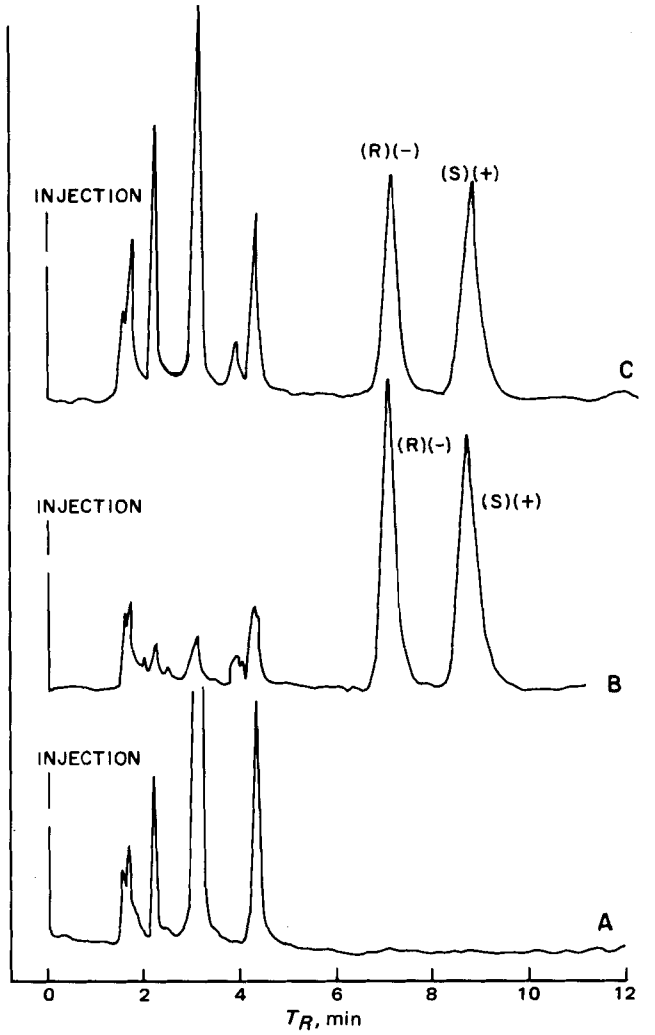


Figure 1—*HPLC (UV detection) of the products from the reaction with (S)(−)-α-methylbenzylamine. Key (A), reagent blank; B, standard carprofen; and C, control human urine spiked with carprofen. Peaks labeled (R)-(−) and (S)-(+) are the diastereomeric amides of the (R)-(−) and (S)-(+) enantiomers of carprofen, respectively.*

[1] Aldrich Chemical Co.
[2] ^{14}C-Labeled carprofen was provided by Dr. A. Liebman and Dr. R. Muccino, Chemical Research Department, Hoffmann–LaRoche.
[3] Mr. L. Berger and Mr. A. Corraz, Chemical Research Department, Hoffmann–LaRoche, synthesized the analytical standards I and II.
[4] J. T. Baker Chemical Co.
[5] Brinkmann Instruments.
[6] Whatman No. 1.
[7] Eberbach.
[8] Model K, IEC Corp.
[9] Organomation Associates N-EVAP.

Table I—HPLC ($N = 3$ [a]) Determination of the (*S*)-(+):(*R*)-(−) Values for Varying Amounts of Standard Carprofen

Amount of Carprofen Reacted, μg	(*S*)-(+):(*R*)-(−) Value Found	CV, %
5	1.00 ± 0.03	3.0
10	1.01 ± 0.01	1.0
15	1.01 ± 0.01	1.0
20	1.01 ± 0.01	1.0
Average		1.5%

[a] Number of separate reactions on different days.

in 100 μl of ethanol and spotted on the TLC plate as 3-cm wide streaks.

The plate was developed with chloroform—acetic acid (90:10) until the solvent reached 14 cm from the origin. The UV-absorbing band corresponding to carprofen (R_f 0.54) was well separated from biological impurities (R_f 0.61). The silica gel containing carprofen was scraped off the plate and, after addition of 3 ml of 0.2 *M* acetate buffer (pH 5), was extracted twice with 5 ml of ether. The combined ether layers were evaporated to dryness.

Urine—Aliquots of 2–5 ml of urine were adjusted to pH 11 by the addition of 1 ml of 1 *N* NaOH. After standing 2 hr at room temperature to cleave the ester glucuronides, the solution was adjusted to pH 5 and the deconjugated carprofen was extracted twice with 10 ml of ether as already described. The ethanolic extract was spotted on a TLC plate, and the plate was developed using chloroform–ethanol–formic acid (90:10:5). Carprofen (R_f 0.50) was extracted from the silica gel as previously described. When it was necessary to extract 10 ml of urine, two 5-ml aliquots were extracted in separate tubes and the extracts were combined before TLC analysis.

Feces—Feces were homogenized in ~10 volumes of ethanol–water (70:30), and a 10-ml aliquot of each homogenate was diluted with 5 ml of 1 *M* acetate buffer (pH 5). The resulting ethanolic suspension was extracted once with 20 ml of hexane–ether (3:2). The supernatant fraction was back-extracted with 5 ml of 0.6 *N* NaOH. After centrifugation, the hexane–ether phase was discarded. The alkaline layer was washed with 10 ml of ether and, after centrifugation, the ether was discarded. Then 0.5 ml of 6 *N* HCl and 2 ml of 1 *M* phosphate buffer (pH 7) were added to the alkaline extract. This solution was extracted twice with 10 ml of ether.

The residue of this ether extract was dissolved in 7.5 ml of ethanol–0.1 *N* HCl (4:1). This solution was washed twice with 10 ml of hexane, and the hexane was discarded after each washing. The ethanol–hydrochloric acid layer was diluted with 7.5 ml of water and extracted once with 15 ml of hexane–ether (3:2). The residue of the hexane–ether extract was dissolved in ethanol and spotted on a TLC plate. The plate was developed using chloroform–ethanol–acetic acid (90:10:1). Carprofen (R_f 0.40) did not resolve from endogenous biological impurities in this system, necessitating redevelopment in another solvent system; chloroform–methanol–concentrated ammonium hydroxide (70:30:1) separated carprofen (R_f 0.12) from the biological impurities. Carprofen was extracted from the silica gel as already described.

Derivatization Procedure (Scheme I)—The derivatization procedure is based on the method used by VanGiessen and Kaiser (11) for the GLC determination of the enantiomers of ibuprofen, an α-methylarylacetic acid. Carprofen standards were processed in duplicate with each series of unknowns in concentrations comparable to the expected carprofen concentrations in the unknowns (1.4–30 μg). The reaction was performed in 15-ml conical glass-stoppered centrifuge tubes. To the dried residues of the standards and the unknowns, 0.1 ml of the 1,1′-carbonyldiimidazole solution was added. Each tube was rotated to permit the reactant to contact the residue completely. The mixture was allowed to stand at room temperature for 5 min, then 10 μl of acetic acid and 25 μl of (*S*)-(−)-α-methylbenzylamine were added stepwise, and the solution was mixed well with a vibrating mixer. The solution was centrifuged briefly and then was allowed to react at room temperature for 20 min.

After completion of the reaction, 3 ml of 0.2 *N* NH_4OH (pH 11) and 5 ml of hexane were added. The tubes were stoppered tightly, shaken for 10 min in a reciprocating shaker at 60–80 strokes/min, and centrifuged for 5 min. A 4.5-ml aliquot of the hexane extract was transferred to a 15-ml conical centrifuge tube and evaporated to dryness under nitrogen in a 30–40° water bath. After drying thoroughly for 5 min in a desiccator *in vacuo*, the residue was dissolved in 0.5– 2.0 ml of the HPLC mobile phase and a 10-μl aliquot was injected into the liquid chromatograph.

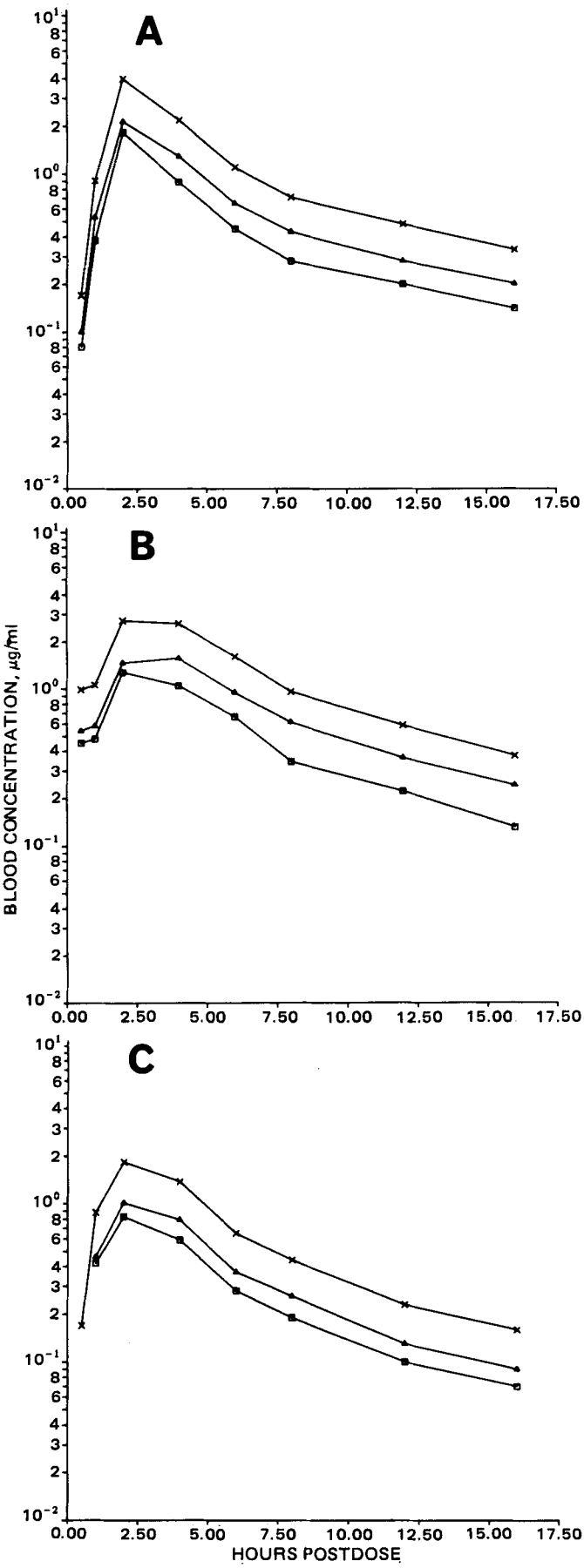


Figure 2—*Blood levels of carprofen (×) and of its (S)-(+) (△) and (R)-(−) (□) enantiomers in three subjects, each receiving 100 mg of carprofen. Key: A, Subject III/14; B, subject V/41; and C, subject V/42.*

Table II—HPLC Determination of the (*S*)-(+):(*R*)-(−) Values of Synthetic Mixtures of (*S*)-(+) and (*R*)-(−) Carprofen Enantiomers in Human Blood and Urine

Sample	(*S*)-(+):(*R*)-(−) Value Added	Total Added, μg	Volume of Biological Sample, ml	(*S*)-(+):(*R*)-(−) Value Found ± *SD*	*n*[a]	*CV*, %
External standards	0.34	17.5	—	0.33 ± 0.001	2	0.3
	1.07	19.4	—	1.11 ± 0.017	2	1.5
	1.99	29.8	—	2.00 ± 0.005	2	0.3
Human blood	1.07	10.1	5.0	1.11 ± 0.03	3	2.7
	1.50	10.5	5.0	1.5 ± 0.04	3	2.7
	1.99	10.8	5.0	2.02 ± 0.03	3	1.5
Human urine	0.49	21.6	1.0	0.48 ± 0.01	2	2.1
	1.07	23.3	1.0	1.08 ± 0.01	2	1.0
	1.50	24.2	1.0	1.39 ± 0.03	2	2.2

[a] *n* = number of replicate samples. Each reaction sample was analyzed twice by HPLC.

Table III—Comparison of the HPLC Method with UV Detection and the Radio-TLC Method in Determining (*S*)-(+):(*R*)-(−) Values in Human Urine (Subject II) after a Single 50-mg Oral Dose of [^{14}C]Carprofen

Excretion period, hour	HPLC–UV	Radio-TLC
0–2	1.25	1.30
2–4	1.26	1.34
4–6	1.27	1.27
6–9	1.16	1.16
9–12	1.19	1.20
12–24	1.27	1.24
24–48	1.01	0.986

[a] Average (*S*)-(+):(*R*)-(−) values are based on duplicate determinations of each specimen by each method.

HPLC Analysis of Diastereomeric Derivatives of Carprofen—*Column*—A 25-cm × 4.6-mm i.d. stainless steel column containing 10-μm silica gel[10] was used along with a guard column[11]. To maintain good performance, the column was sequentially washed (2.0 ml/min) with 200 ml of methanol, 200 ml of methylene chloride, 200 ml of *n*-heptane, and 60 ml of methylene chloride.

Instrumental Parameters—The liquid chromatograph[12] was equipped with a UV absorbance detector[13] operated at 254 nm and a solvent delivery system[14]. A fluorescence detector[15], operated at 240 nm for excitation and at wavelengths greater than 350 nm[16] for emission, was used for a limited number of HPLC analyses. The isocratic mobile phase was a mixture of 0.75% CH_3OH in methylene chloride at a head pressure of 450 psi and at a constant flow rate of 2.0 ml/min. Solvents were routinely filtered and degassed[17] immediately before use.

The UV detector sensitivity was 1×10^{-2} aufs, and the fluorescence detector sensitivity was 0.1 μA.F.s. The chart speed on the recorder[18] was 2.5 cm/min. A full-scale pen response was obtained with an injection of 75 ng of each diastereomer when operating in either detection mode. Under these conditions, the (*R*,*S*) diastereomer and standard I had the same retention time of 6.4 min with $k' = 5.1$. The (*S*,*S*) diastereomer and standard II had the same retention time of 7.6 min with $k' = 6.7$.

Analytical Standards—Separate stock solutions of standards I and II were prepared by dissolving 5.000 mg of the former and 5.041 mg of the latter in 500 ml of 0.75% CH_3OH in methylene chloride. The stock solutions were diluted with this same solvent to prepare solutions of 5, 10, 25, 50, and 75 ng/10 μl. Ten-microliter aliquots of these solutions were injected as external standards for establishing the HPLC parameters. The computed UV peak areas, as well as peak heights, were plotted *versus* concentration, and the UV detector was shown to respond linearly and equivalently to both diastereomers.

Quantitation and Calculations—Free and total carprofen concentrations were determined in urine and feces of subjects given [^{14}C]carprofen by the radiometric method (6). Free carprofen in blood was quantitated by HPLC (9). UV detection and computer integration of the areas under the (*S*,*S*) and (*R*,*S*) diastereomer peaks were used to determine the (*S*)-(+):(*R*)-(−) ratios, which were applied to the carprofen concentrations to determine the concentrations of the individual carprofen enantiomers.

[10] Whatman Partisil 10 adsorbent.
[11] Whatman HC Pellosil packing (38 μm silica gel).
[12] Model ALC/GPC 204, Waters Associates.
[13] Model 440, Waters Associates.
[14] Model 6000A, Waters Associates.
[15] Schoeffel model FS-970.
[16] Corning No. 0-52 filter.
[17] All-glass filter apparatus, 47 mm, Millipore Corp.
[18] Hewlett-Packard model 7132A.

RESULTS AND DISCUSSION

Characteristics of Stereospecific Carprofen Assay—Typical chromatograms for a reagent blank, standard carprofen, and spiked human urine are shown in Fig. 1. To minimize the presence of UV-absorbing impurities and to obtain adequate HPLC separation of the diastereomers, it was necessary to modify the procedures described in the original radio-TLC method (6). Extraction of the reaction product into hexane from an alkaline aqueous phase (instead of ether at pH 5) removed certain acidic and more polar by-products and provided a product sufficiently pure for direct HPLC analysis. The concentration of early eluting UV-absorbing impurities that interfered with the (*R*)-(−)-carprofen diastereomeric amide peak could be reduced satisfactorily by reacting carprofen with half the amount of each reagent used previously (6).

[^{14}C]Carprofen was used to determine the overall formation of the diastereomeric amides. The radioactivity determined in the reaction extract indicated that the reaction yield was 62.3 ± 2.9% (*SD*), equivalent to the yield found with the original procedure (6).

The overall percent recovery of carprofen from urine after extraction, preparative TLC, a second extraction, derivatization, and final extraction was 39.3 ± 2.3% (*SD*) with a detection limit of 0.6 μg/ml when 10 ml was extracted. The feces assay had a recovery of 29.2 ± 3.75% with a detection limit of 0.6 μg/ml of homogenate when a 10-ml aliquot of homogenate was extracted. The blood assay had a recovery of 41.5 ± 8.3% with a limit of detection of 0.27 μg/ml when a 5-ml aliquot was extracted.

Table I presents the statistical evaluation of the (*S*)-(+):(*R*)-(−) values found for carprofen reacted over a range of concentrations. The results show that the method is reproducible. Therefore, modification of the derivatization and extraction procedure did not affect overall yield and reproducibility.

To check the accuracy and precision of the method, biological samples spiked with carprofen were analyzed. The following mean (*S*)-(+):(*R*)-(−) ratios (±*SD*) were found at the indicated concentrations of carprofen: 0.27 μg/ml in blood, 1.03 ± 0.05 ($n = 4$); 0.83 μg/ml in blood, 0.98 ± 0.04 ($n = 9$); 1.0 μg/ml in urine, 0.99 ± 0.02 ($n = 7$); 2 μg/ml in urine, 0.99 ± 0.01 ($n = 3$); and 22 μg/ml of fecal homogenate, 1.02 ± 0.03 ($n = 3$). In addition, blood and urine were spiked with synthetic mixtures of carprofen enantiomers with varying (*S*)-(+):(*R*)-(−) ratios. The coefficients of variation of the ratios found were ≤2.7% (Table II). These results indicate that the method is accurate and precise.

Fluorescence detection was used for determining the (*S*)-(+):(*R*)-(−) values in a limited number of samples. Carprofen standards of 10 and 20 μg were reacted, and the diastereomers were quantitated by computer-integration of the peak areas or by measurement of peak heights. The (*S*)-(+):(*R*)-(−) values had a mean of 0.987 ± 0.012 (± *SD*) ($n = 22$). Fluorescence detection shows promise as an alternative detection mode, especially when UV contaminants are present.

Application to Biological Specimens—To compare the radio-TLC and HPLC methods, urine samples from a subject who had ingested 50 mg of [^{14}C]carprofen (0.5 μCi/mg) were analyzed by both methods. The (*S*)-(+):(*R*)-(−) ratios obtained by the two methods were nearly identical (Table III).

Blood specimens were obtained from three human subjects who were

Table IV—Determination of (*S*)-(+):(*R*)-(−) Values in Human Blood following a Single 100-mg Oral Dose of Carprofen [a]

Subject	Hours after Administration							
	0.5	1.0	2.0	4.0	6.0	8.0	12.0	16.0
III/14	1.23	1.40	1.16	1.45	1.46	1.52	1.41	1.40
V/41	1.19	1.22	1.15	1.51	1.35	1.77	1.65	1.92
V/42	NA[b]	1.08	1.22	1.35	1.31	1.38	1.40	1.36

[a] Ratios for Subjects III/14 and V/41 were determined by the HPLC method, and the ratios for Subject V/42 were determined using a fluorometric TLC procedure. For the latter subject, carprofen was extracted by the standard procedure from 9–11 ml aliquots of blood containing 0.2–1.8 μg of carprofen. After cleanup of carprofen by extraction with sodium hydroxide and by TLC, the diastereomeric derivatives were formed, separated by TLC, and extracted from the silica gel bands with 2.5 ml of ethanol–1% acetic acid. Comparison of the fluorescence intensities measured at 370 nm (emitting wavelength) after activating at 295 nm resulted in the fluorescence (*S*)-(+):(*R*)-(−) ratios for racemic carprofen of 0.986 ± 0.002. [b] Not analyzed.

Table V—Urinary and Fecal Excretion of Carprofen and Its Individual Enantiomers in Three Human Subjects Following Single 50-mg Doses of [^{14}C] Carprofen

Excreta	Excretion Period, hr	Carprofen and Enantiomer Excretion in Percent of Dose														
		Subject I					Subject II					Subject III				
		Total Carbon 14	Total[a] (T) or Free (F) Carprofen	(*S*)-(+):(*R*)-(−)	(*S*)-(+)	(*R*)-(−)	Total Carbon 14	Total[a] (T) or Free (F) Carprofen	(*S*)-(+):(*R*)-(−)	(*S*)-(+)	(*R*)-(−)	Total Carbon 14	Total[a] (T) or Free (F) Carprofen	(*S*)-(+):(*R*)-(−)	(*S*)-(+)	(*R*)-(−)
Urine	0–24	57.6	53.5 T	1.2[b]	29.2	24.3	62.4	53.4 T	1.3[b]	30.2	23.2	44.8	39.7	1.3[b]	22.4	17.3
	24–48	9.6	8.3 T	1.0	4.2	4.2	5.7	4.6 T	1.0	2.3	2.3	7.7	6.3	1.1	3.3	3.0
	48–72	4.2	3.3 T	0.7	1.4	1.9	2.4	2.0 T	NA[c]	NA	NA	2.5	2.0	1.0	1.0	1.0
	0–72	71.4	65.1 T		34.8	30.4	70.5	60.0 T		32.5[d]	25.5[d]	55.0	48.0		26.7	21.3
Feces	0–24	ND[e]	ND	NA	NA	NA	3.0	2.0 F	NA	NA	NA	1.0	NA	NA	NA	NA
	24–48	3.6	2.0 F	0.4	0.6	1.4	1.0	0.3 F	0.3	0.1	0.2	19.6	10.0	0.4	1.2	2.9
	48–72	3.1	2.0 F	0.4	0.6	1.4	4.4	2.0 F	0.3	0.5	1.5	8.5	3.0	0.5	0.7	1.6
	72–96	5.1	3.0 F	NA	NA	NA	4.3	2.0 F	0.3	0.5	1.5	3.5	1.0	0.5	0.4	1.1
	0–96[d]	11.8	7.0 F		1.2	2.8	12.7	6.3 F		1.1	3.2	32.6	14.0		2.3	5.6
Total (urine and Feces)[d]		83.2	72.1 F		36.0	33.2	83.2	66.3 F		33.6	28.7	87.6	62.0		29.0	26.9

[a] Free carprofen plus ester glucuronide. [b] The (*S*)(+):(*R*)-(−) values were determined for the excretion periods 0–2, 2–4, 4–6, 6–9, 9–12, and 12–24. The mean (*S*-)(+):(*R*)-(−) values for 0–24 hr are presented. The standard deviation was ±0.1 in each case. [c] Not analyzed. [d] These sums for carprofen and its enantiomers are minimum values because not all samples over the time period were analyzed. However, the samples not analyzed contained little radioactivity so their absence should not significantly affect the total excretion values. [e] Not detected.

each administered a single oral dose of 100 mg of carprofen[19]. Intact carprofen had been shown by Rubio *et al.* (10) to be the predominant component in human plasma during the first 12 hr after single-dose administration. Data on the enantiomeric ratios in the blood are presented in Table IV. The (*S*)-(+) enantiomer exceeded the (*R*)-(−) enantiomer at all time points, which is consistent with previous results (7). In addition, there was a small increase in the (*S*)-(+):(*R*)-(−) ratios with respect to time. The mean (± *SD*) of the ratios in the 4–16-hr blood samples was 1.48± 0.17, which was significantly higher ($p < 0.001$, Student *t* test) than the mean (± *SD*) of 1.21 ± 0.09 found for the early blood samples (0.5–2 hr). The relatively constant (*S*)-(+):(*R*)-(−) ratios in the 4–16-hr blood samples from the three human subjects resulted in parallel fall-off curves for the two enantiomers (Fig. 2). This finding suggests that assays for total carprofen would reflect the concentration of the pharmacologically more active (*S*)-(+) enantiomer, at least during this time period.

Previously a larger increase in (*S*)-(+):(*R*)-(−) ratios was seen in the blood of two rats given carprofen intravenously (6). The 1-hr blood samples had ratios of 1.14 and 1.21, while the 6-hr blood samples had ratios of 2.09 and 2.39. In these rats, the (*R*)-(−) enantiomer was eliminated from the blood at a rate approximately twice that of the (*S*)-(+) isomer. Therefore, the stereoselective disposition of carprofen is more pronounced in rats than in humans.

Rubio *et al.* (10) reported that three human subjects administered single oral 50-mg doses of [^{14}C]carprofen excreted (0–120 hr) a mean (± *SD*) of 2.8 ± 0.5% of the dose in the urine as free carprofen and 63.1 ± 10.8% as carprofen ester glucuronide. In the feces, a mean of 7% of the dose was recovered as directly extractable intact carprofen. Similar results were obtained by Ray *et al.* (12) after administration of single 100-mg doses of [^{14}C]carprofen to three human subjects.

Urine and fecal samples from the Rubio *et al.* study (10) were analyzed by the stereospecific assay (Table V). In all urine samples collected between 0 and 24 hr, the (*S*)-(+) enantiomer exceeded the (*R*)-(−) enantiomer; after 24 hr, urinary (*S*)-(+):(*R*)-(−) values were close to unity with the exception of the 48–72-hr urine of Subject I where the ratio was 0.7. The (*R*)-(−) enantiomer of free carprofen predominated in all fecal samples between 24 and 96 hr. These data demonstrate the stereoselective excretion of carprofen by humans.

Other α-methylarylacetic acids that are structurally related to carprofen undergo stereoselective inversion. Cicloprofen undergoes (*R*)-(−) to (*S*)-(+) inversion in rats (2, 3), monkeys (2), and dogs (4), and both ibuprofen (1) and benoxaprofen (5) undergo (*R*)-(−) to (*S*)-(+) inversion in humans. In each case, inversion was unequivocally demonstrated by the administration of the (*R*)-(−) enantiomer. Only the racemic mixture of carprofen has been given to humans. The amount of the (*S*)-(+) enantiomer of carprofen in the total urinary and fecal excretion exceeded the amount of the (*R*)-(−) enantiomer by only 2.1–4.9% of the dose (Table V). The data did not show any significant amount of stereoselective inversion of carprofen. However, since excreted carprofen accounted for only 62–72% of the administered dose, stereoselective inversion cannot be excluded as a possibility.

[19] Three normal volunteers, each receiving a single 100-mg dose of carprofen, were part of a study conducted by Dr. James D. Moore, University of Montana Foundation, Deer Lodge, Mont.

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Hydration and Percutaneous Absorption II: Influence of Hydration on Water and Alkanol Permeation through Swiss Mouse Skin; Comparison with Hairless Mouse

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Abstract □ *In vitro* permeation studies with biological membranes often involve long, aqueous maceration of the tissue. The present investigation examined the possible effects of hydration on barrier integrity of Swiss mouse skin, using water, methanol, ethanol, and butanol as permeants and a previously developed procedure involving multiple, sequential permeation runs on each piece of skin. The permeation rate of water increased almost linearly up to 30 hr of hydration and then tended to level off. Transport rates of methanol and ethanol increased asymptotically and then plateaued at ~15 hr. These results contrast with earlier findings on hairless mouse skin where the permeabilities of these three compounds were unaffected by aqueous immersion. The permeation rate of butanol also increased during the first 15 hr of hydration but gradually declined over the next 25 hr. This result again contrasts with the hairless mouse species in which butanol permeability doubled in 10 hr and then plateaued. The species differences in the hydration profiles appear related to the vastly dissimilar pellages and, in the Swiss mouse, may indicate greater involvement of the transfollicular pathway.

Keyphrases □ Hydration—effect on water and alkanol permeation through Swiss mouse skin, comparison with hairless mouse □ Absorption, percutaneous—effect of hydration on water and alkanol permeation through Swiss mouse skin, comparison with hairless mouse □ Permeability—effect of hydration on water and alkanol absorption through Swiss mouse skin, comparison with hairless mouse

A previous study (1) on the influence of long aqueous immersion (hydration) on the permeability properties of hairless mouse skin used a method involving sequenced, *in vitro* diffusional experiments on each skin membrane. Hydration-related alterations of the permeation behaviors of a series of compounds were systematically investigated, and the observed effects were related to the physicochemical properties of the permeants and mass transport mechanism. The permeabilities of water, methanol, and ethanol were not affected by immersion of the skin in normal saline. The permeabilities of butanol and hexanol doubled and reached asymptotes in 10 hr of hydration. The permeation rate of heptanol only increased by ~50%, while octanol showed an initial 50% increase followed by a 25% decline to assume an invariant rate with a net 25% hydration-induced alteration.

In view of the experimental and possible mechanistic significance of the hydration-induced permeability alterations, the present study compared the influence of hydration on the skin permeability of the Swiss mouse, to that of the hairless mouse. The results obtained proved the skins of the two species to be exceedingly different in their chemical barrier properties, both in terms of absolute rates of permeation of the low molecular weight alcohols and in their hydration sensitivities.

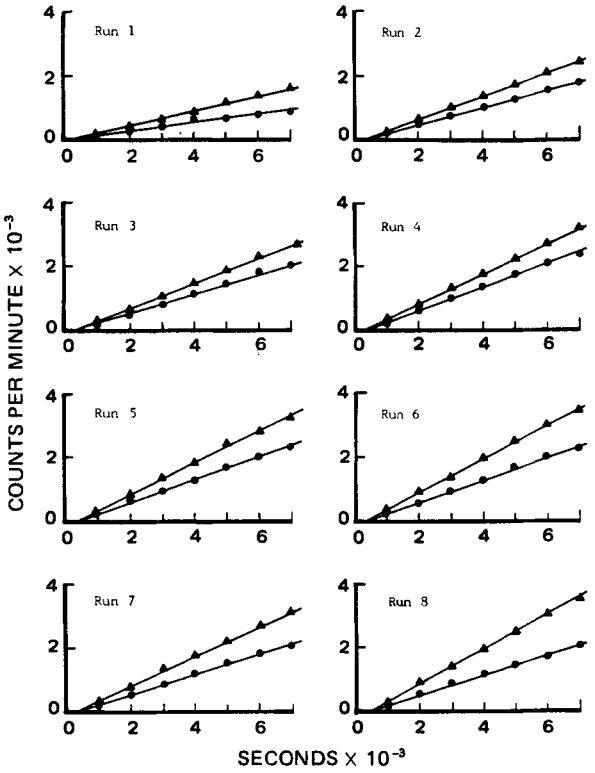

Figure 1—*Series of receiver concentration* versus *time profiles for eight sequential permeation runs on a single skin. This figure is one set of data from the methanol (▲)–butanol (●) series detailed in the text. Key: Run 1, t_0 = 0 hr; Run 2, t_0 = 5 hr; Run 3, t_0 = 10 hr; Run 4, t_0 = 15 hr; Run 5, t_0 = 20 hr; Run 6, t_0 = 25 hr; Run 7, t_0 = 30 hr; and Run 8, t_0 = 43 hr.*

EXPERIMENTAL

Chemicals—[^{3}H]Water[1], [^{3}H]methanol[1], [^{14}C]ethanol[2], and [^{14}C]-butanol[2] were used as received. The radiochemicals were diluted into 0.9%

[1] New England Nuclear, Boston, MA 02218.
[2] International Chemical and Nuclear Corp., Irvine, CA 92715.

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