(40:1) as the eluting solvent. The compound was further purified by crystallization from acetone.

TLC—Silica gel 60 F_{254}-precoated aluminum sheets were used with chloroform–methanol (10:1), giving R_f 0.62 for I and R_f 0.8 for II. The spots were visualized by spraying with the following mixture: anisaldehyde (21 ml), phosphomolybdic acid, 10% in methanol (5 ml), acetic acid (45 ml), sulfuric acid (22.5 ml), and methanol (430 ml). The plates were heated to 120–125° and development of color was observed.

Isolation of Solasodiene—Solasodine (400 mg) was dissolved in 100 ml of ethanol, and 24 ml of concentrated hydrochloric acid was then added. The resulting solution was refluxed for 3 hr, cooled, and neutralized with aqueous ammonia, and the methanol was evaporated. The residue was extracted with ether, and the combined extracts were washed with saturated aqueous sodium chloride and then dried over magnesium sulfate. After evaporation of the ether, the mixture was separated by silica gel column chromatography, using chloroform–methanol (40:0.5) as the eluting solvent. Crude solasodiene (25%) was obtained. Crystallization from acetone gave pure solasodiene, mp 173°.

General Procedure for Hydrolysis—Solasodine (0.005 g) was dissolved in methanol, ethanol, 1-propanol, or 1-butanol, and then concentrated hydrochloric acid was added to give the required acid concentration (0.5, 1, and 2 *N* for each alcohol used). The total volume of each solution was 10 ml. The solution was refluxed for 3 hr and neutralized with aqueous ammonia. After evaporation to dryness in a vacuum, a second portion of aqueous ammonia was added, making the reaction mixture alkaline. This mixture was then extracted with ether, and the combined extracts were washed with saturated sodium chloride, dried over magnesium sulfate, and evaporated to dryness. The residue was then diluted with methanol to the required volume for UV spectroscopic reading.

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Analysis of Cefazolin in Serum or Urine

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Abstract ☐ A method was developed to determine cefazolin in serum or urine. The drug is extracted from serum or urine with ethyl acetate, separated by TLC, and determined by fluorescence quenching densitometry. The method was developed to study the pharmacokinetics of the compound in humans.

Keyphrases ☐ Cefazolin—TLC-densitometric analysis in biological fluids ☐ TLC-densitometry—analysis, cefazolin in biological fluids ☐ Antibacterials—cefazolin, TLC-densitometric analysis in biological fluids

To carry out pharmacokinetic studies on cefazolin, 7-[1-(1*H*)-tetrazolylacetamido]-3-[2-(5-methyl-1,3,4-thiadiazolyl)thiomethyl]ceph-3-em-4-carboxylic acid (1), an assay was developed to determine antibiotic concentrations in serum and urine. The method provides a convenient alternative to other published procedures (1, 2).

EXPERIMENTAL

Reagents—Ether[1], ethyl acetate[1], chloroform[1], acetic acid[2], 1 *M* HCl, and 0.2 *M* HCl were used.

TLC-Densitometry—Precoated TLC plates[3] (20 × 20 cm) were developed in glass tanks. Solutions were applied to the thin-layer plates using an automatic plate spotter[4]. Plates were scanned using a densitometer[5]. The settings on the instrument were: mode, log −; span, 980; level, e5; damping, 2; rotating-spot diameter, 8 mm; scan speed setting, 3; and chart speed setting, 5.

Analysis of Samples—A sample of serum or urine (1.0 ml diluted 0–500-fold) was pipetted into a 10-ml stoppered tube. The sample pH was adjusted to 2 by adding 1 *M* HCl (250 μl) to serum and 0.2 *M* HCl (250 μl) to urine. The sample was extracted with ethyl acetate (2 × 3 ml), and the combined extracts were evaporated under nitrogen at 37°. The residue in the tube was dissolved in 50 μl of methanol and transferred quantitatively, using a 100-μl syringe, to the origin of the thin-layer plate. The tube was rinsed with methanol (50 μl), and the rinsings also were applied to the respective spot.

The plate was developed for approximately 15 cm in ether, dried at air temperature, and redeveloped again for 15 cm in chloroform–methanol–acetic acid (60:40:5). The drug traveled with an R_f of approximately 0.3, and the drug spot was measured on the densitometer. The area of the peak on the chart recorder was calculated for each spot as the product of peak height and width at half peak height.

Standard Curve—A standard calibration curve was prepared daily for each batch of samples. Aliquots of serum or urine (1.0 ml diluted 0–500-fold) spiked with a range of drug concentrations were processed according to the described method, and peak area was plotted against drug concentration. The standard curve was linear over the 1–8-μg/ml range.

Accuracy and Precision—Control serum samples were spiked with unknown quantities of I. The spiked concentrations and concentrations found (in parentheses) were 6.8 (6.8), 1.4 (1.6), 4.1 (3.8), 8.2 (8.2), 2.7 (2.9), and 5.4 (5.5) μg/ml. Control urine samples spiked with unknown quantities of I also were processed, and the results were 4.2 (4.4), 6.3 (6.2), 2.1 (2.3), 7.4 (7.7), and 3.2 (3.2) μg/ml. Six replicate assays were carried out on serum spiked with I at 4.0 μg/ml. The mean value was 4.1 ± 0.1 (*SD*) μg/ml.

DISCUSSION

The described method gives an accurate means of determining the cefazolin concentration in serum and urine and has been used to study its pharmacokinetics in humans. The homogeneity of the spots corresponding to I in extracts of the serum or urine of subjects given I was established by two-dimensional TLC in chloroform–methanol–acetic acid (60:40:5, R_f 0.3) and 2-propanol–water–acetic acid (28:8:1, R_f 0.5). No metabolites were detected in either system. Data from three subjects

[1] Analar.
[2] General-purpose reagent.
[3] Kieselgel $F60_{254}$, E. Merck, Darmstadt, Germany.
[4] TLC Multi-spotter, Analytical Instrument Specialities, Libertyville, Ill.
[5] TLD 100, Vitatron, Dieren, Holland.

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Table I—Serum Concentrations (Micrograms per Milliliter) and Urinary Excretion of Cefazolin in Three Subjects Given a Single Intramuscular Dose (8 mg/kg) of Drug

Subject	Hours after Dose[a]								Excretion in 0–24-hr Urine, % of Dose
	0.5	1	1.5	2	4	5.5	7	8	
1	25	30	30	28	14	7.7	3.5	1.8	45
2	39	42	31	29	13	6.4	3.5	1.8	41
3	21	35	32	28	16	11	6.0	5.7	65

[a] No cefazolin was detected at zero hour.

given a single intramuscular dose (8 mg/kg) of cefazolin are shown in Table I.

The procedure is specific in subjects known to be taking only cefazolin but has not been checked to determine whether related compounds may interfere.

The method may have general applicability to other cephalosporins that can be extracted from serum and/or urine and that contain a structural moiety absorbing in the UV spectrum.

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GLC Assay of Fenclorac in Human Plasma

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Received May 11, 1977, from the *Research Division, William H. Rorer, Inc., Fort Washington, PA 19034.* Accepted for publication November 10, 1977. *Present address: Department of Drug Metabolism, Bristol Laboratories, Syracuse, NY 13201.

Abstract □ A simple, sensitive GLC assay for fenclorac is described. Plasma proteins were precipitated with methanol, and the methanolic extract was refluxed with hydrochloric acid to form the methyl esters of fenclorac and the internal standard. The esters were purified by partitioning into benzene. Aliquots of 1 μl of the concentrated benzene phase were injected into the gas chromatograph and quantitated by a ^{63}Ni-electron-capture detector. Recovery of fenclorac from plasma averaged 82 ± 1.6%.

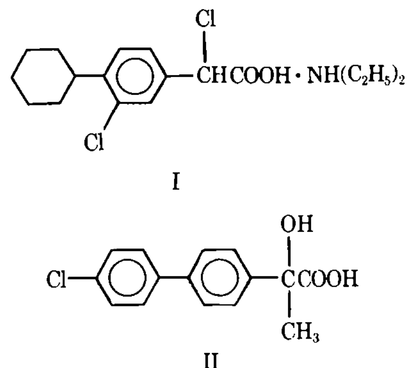
Keyphrases □ Fenclorac—GLC analysis in plasma □ GLC—analysis, fenclorac in plasma □ Anti-inflammatory agents—fenclorac, GLC analysis in plasma

Fenclorac (DL-α,3-dichloro-4-cyclohexylphenylacetic acid) is a potent nonsteroidal anti-inflammatory agent with significant analgesic and antipyretic activities (1–3). In humans and animals, the compound is rapidly absorbed, extensively metabolized, and quantitatively eliminated within 24–48 hr (4, 5). Fenclorac is the primary circulating drug species (>85%) with small amounts of the α-hydroxy metabolite (3-chloro-4-cyclohexylphenylglycolic acid), which has about 0.3 times the anti-inflammatory potency of fenclorac.

A spectrophotometric method (6) was used to quantitate fenclorac in biological samples. However, this method is not specific for the parent drug, and the sensitivity is approximately 1 μg/ml. This report describes a simple, sensitive, specific GLC assay of fenclorac in plasma.

EXPERIMENTAL

Materials—The chemicals and reagents used were: fenclorac diethylammonium salt (I), DL-4′-chloro-α-methyl-4-biphenylglycolic acid (internal standard) (II), GC–spectrophotometric quality benzene and ethyl acetate, and reagent grade methanol and hydrochloric acid.



Instrumentation—*GLC*—GLC analysis was performed on a gas chromatograph[1] equipped with a ^{63}Ni-electron-capture detector and fitted with a 0.9-m × 2-mm coiled glass column packed with 3% silicone[2] [methyl–cyanopropyl (50:50)] on 80–100-mesh Gas Chrom Q. The column was conditioned at 220° for 18 hr. The methyl esters of fenclorac and the internal standard were formed by refluxing in hydrochloric acid–methanol. Purification and concentration of the resultant product are described later.

The methyl esters of fenclorac and the internal standard were eluted isothermally from the column under the following temperature conditions: column, 220°; injection port, 240°; manifold, 240°; and detector, 300°. Argon (95%)–methane (5%) gas flow was maintained as follows: column, 40 ml/min; and auxiliary, 60 ml/min. Samples (0.5–1.0 μl) were injected manually or with an automatic injector[3].

GLC–Mass Spectrometry—Mass spectra of standards and extracts of plasma were obtained using a GLC–mass spectrometer[4]. A 0.9-m × 2-mm coiled glass column packed with 5% OV-17 on Chromosorb W(HP) was used. The ester fractions were eluted isothermally at 225° with a

[1] Perkin-Elmer model 90013.
[2] Applied Science Laboratories.
[3] Hewlett-Packard model 7076.
[4] Hewlett-Packard model 5930-A.

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