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Nonaqueous Cephalosporin Suspension for Parenteral Administration: Cefazolin Sodium

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Abstract □ The flocculation-deflocculation behavior of cefazolin sodium (I) in nonaqueous media and the effect of surfactants as measured by zeta potential, sedimentation, and porosity were studied. A significant difference in zeta potential was observed when the particles were suspended in different nonaqueous media. The addition of surfactant produced a deflocculated state. The surfactant deflocculated the particles by a process of supersaturation and crystallization involving a surfactant-cefazolin complex. The shielding effect of the surfactant on the surface of the particles also apparently affected their electrophoretic properties. Kinetic studies on the stability of the drug as a function of temperature were conducted; it appears that the chemical stability in ethyl oleate at room temperature is adequate for a reasonable shelf life. The efficiency of absorption of the drug from the ethyl oleate suspension was evaluated after intramuscular administration in dogs. The area under the plasma concentration *versus* time curve and urinary recovery indicated that cefazolin was 100% bioavailable from this nonaqueous preparation.

Keyphrases □ Cefazolin sodium—surfactants, stability, bioavailability □ Cephalosporin—nonaqueous suspension, stability, bioavailability

Parenteral pharmaceutical suspensions have been used for a long time (1–4). In general, this dosage form is comprised of a physiologically active agent and a vehicle. The vehicle is typically comprised of a suspending liquid, an emulsifying agent, a surfactant, density- and viscosity-adjusting substances, and preservatives. While water is generally the preferred suspending liquid, some physiologically active agents such as the cephalosporin antibiotics are not chemically stable in water-based parenteral pharmaceutical suspensions. Therefore, to achieve a ready-to-use cephalosporin preparation which can be stored at room temperature, it is desirable to develop a satisfactory suspension utilizing a nonaqueous liquid as the suspending medium. This paper describes the preparation of a unique, simple flocculated ready-to-use cefazolin sodium suspension which is dispersed in a nonaqueous medium. The efficiency of absorption of cefazolin from the nonaqueous system administered intramuscularly in dogs is also reported.

Table I—Flocculation-Deflocculation Behavior of Cefazolin Sodium in Peanut Oil [a]

Conc. of Polysorbate 80, %	Sedimentation Volume, H_u/H_o %[b]	Number of Turns to Redisperse
0	48	268
0.17	87	>3500
0.50	82	∞
3.30	63	∞

[a] In the presence of polysorbate 80. [b] Ultimate settled height (H_u) is based on the sedimentation height on day 33.

EXPERIMENTAL SECTION

The following chemicals were used: sterile micronized cefazolin sodium[1]; polysorbate 80, commercial grade[2]; lecithin[3]; ethyl oleate[4]; peanut oil[5]; cefazolin sodium[6]. For *in vivo* studies, sterile micronized cefazolin sodium and ethyl oleate were prepared under sterile conditions. The sterile cefazolin sodium was aseptically micronized[7] and ethyl oleate was sterilized in a preheated oven at 155°C for 5 h. For *in vitro* studies, the materials were used without further sterilization.

Particle Size Distribution—The determination of particle size distribution was conducted by the sedimentation method using a micromerograph[8]. The mean particle size diameter of the micronized material was 7 μm.

Preparation of Suspensions—For *in vitro* studies, suspensions were prepared using an electric mixer[9] to disperse 12.5 g of micronized cefazolin sodium in 50 mL of peanut oil in the presence of various amounts of polysorbate 80 [*i.e.*, 0, 0.17, 0.50, and 3.3% (w/v), respectively]. Suspensions containing lecithin in ethyl oleate were prepared in the same manner. However, the concentration of lecithin added was 0, 0.2, 0.4, 0.6, 0.8, and 1.0% (w/v). For *in vivo* studies, the suspension was aseptically prepared by dispersing 1 g-equivalent activity of sterile micronized cefazolin sodium in 3.0 mL of sterile ethyl oleate with an electric mixer. The suspension was then passed through a 150-mesh sterile stainless steel screen and mixed until uniform. For *in vivo* control studies, aqueous solutions of cefazolin sodium were prepared for intravenous and intramuscular administration by dissolving the sodium salt[6] in isotonic saline to a final concentration of 100 mg/mL as cefazolin.

Sedimentation Volume—The sedimentation volumes were recorded in terms of the ultimate settled height, H_u, to the original height, H_o, as described in the literature (5, 6):

$$\text{Sedimentation Volume} = H_u/H_o \qquad \text{(Eq. 1)}$$

Redispersibility—The number of revolutions necessary to restore the suspension to homogeneity was recorded by means of a multi-purpose rotator[10] with the modification described by Matthews and Rhodes (7).

Turbidity—The turbidity of the supernatant of the suspensions was measured by an analytical nephelometer[11] after cefazolin sodium suspensions had been kept at 25°C for 1 month. The procedure was carried out as described by the manufacturer (8).

Crystal Growth—Crystal size changes with time were microscopically examined. Cefazolin sodium (I) in the dry state was compared with I in a suspension at initial time, and in a suspension stored for 3 months at 25°C.

Viscosity—The viscosities of ethyl oleate and peanut oil were measured with a viscometer[12].

Porosity—The porosity of a sedimentation bed was determined by the method of Kaneniwa and Takamura (9).

[1] Lilly Research Laboratories, Indianapolis, Ind.
[2] Tween 80; ICI America Inc., Wilmington, Del.
[3] American Lecithin Company, Atlanta, Ga.
[4] Pfaltz and Bauer, Inc., Stamford, Conn.
[5] Sessions Oil Mills, Interprise, Ala.
[6] KEFZOL; Eli Lilly and Company, Indianapolis, Ind.
[7] Sturtvant Mill; Sturtvant Mill Company, Boston, Mass.
[8] Sharples, Pennwalt Corp., Warminster, Pa.
[9] Lightnin' Mixing Equipment Co., Inc., Rochester, N.Y.
[10] Scientific Industries, Inc., Bohemia, N.Y.
[11] Hach Chemical Company, Ames, Iowa.
[12] Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

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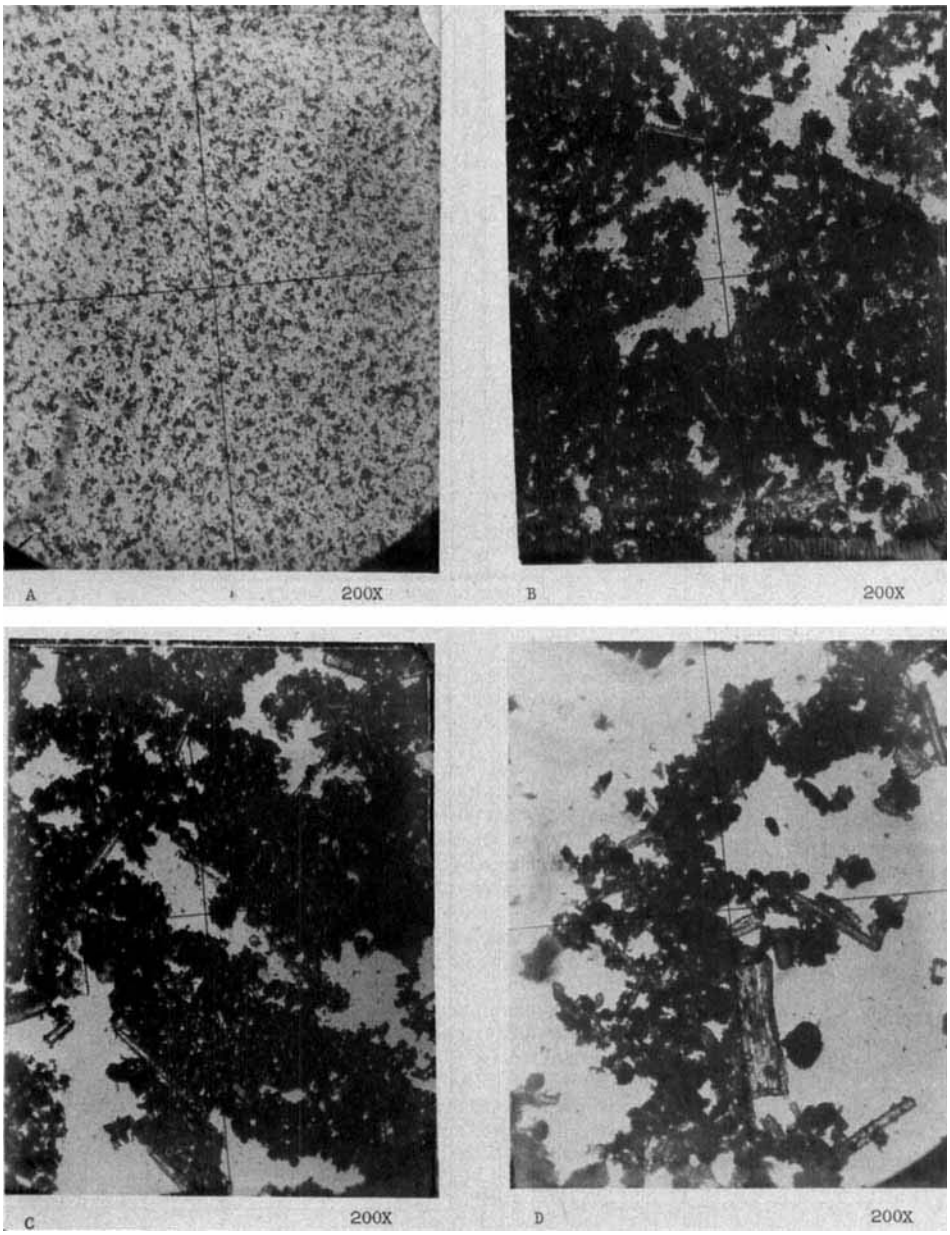

Figure 1—*Microscopic examination of agglomeration and crystal growth of cefazolin sodium suspension in the presence of 0% (A), 0.17% (B), 0.5% (C), and 3.3% (D) polysorbate 80.*

Electrophoretic Mobility—Electrophoretic mobility was measured with a zeta meter[13] equipped with a high voltage power booster[13]. The measurement procedure is described in the literature (10). Zeta potential (ZP) was calculated from the Helmholtz-Smoluchowski equation:

$$ZP = \frac{4\eta}{D} EM \qquad \text{(Eq. 2)}$$

where, at a fixed temperature, η is the viscosity of the suspensing medium in equilibrium, D is the dielectric constant of the suspending medium, EM is the electrophoretic mobility in cm/s per electrostatic volts (esu)/cm, and ZP is the zeta potential of the suspended particle in esu. Since the ZP is always <1 (practical) volt, it is more conveniently expressed in millivolts. Equation 2 then becomes:

$$ZP = 113{,}000 \frac{\eta}{D} EM \qquad \text{(Eq. 3)}$$

where ZP is in the unit of millivolts (mV) and EM is expressed in μm/s per V/cm. The dielectric constants of ethyl oleate and peanut oil have been described (11).

Kinetic Study on Stability of Cefazolin Sodium Suspension—Stability studies were conducted at 57°C, 60°C, 80°C, and 90°C as a function of time. Samples were assayed microbiologically according to CFR§436.105 (*i.e.*, Certified Federation Registration) with *Bacillus subtilis* ATCC 6633 substituted as the assay organism. The kinetic treatment of the data has been previously described (12, 13).

***In Vivo* Bioavailability Evaluation**—Female mongrel dogs (weight 13–20 kg) were fasted overnight with access to water. On the day of the experiment, they were transferred to stocks in which they could stand or sit comfortably for the duration of the test. A retention catheter[14] was inserted into the urinary bladder of each dog for collection of all urine produced during the test period.

Cefazolin sodium (I) was administered as a single 15-mg/kg bolus dose *via* acephalic vein (intravenous) or the biceps femoris muscle (intramuscular). At specified time intervals, samples of urine were collected, chilled in an ice bath, and then frozen until analyzed. At the same time, a blood sample was withdrawn from one cephalic vein into a heparinized evacuated tube[15]. After centrifugation, a plasma sample was removed, chilled, frozen, and analyzed in the following manner. On the day of the assay, the plasma and urine samples were thawed. Urine samples were diluted, as required, in isotonic 0.1 M phosphate buffer (pH 6.0). The antibacterial activity of the diluted samples was assayed in a disk plate agar diffusion test with *B. subtilis* against standard solutions of cefazolin in the same buffer. Plasma samples were diluted with dog serum and assayed in the same way against standard solutions of cefazolin in dog serum.

[13] Zeta-Meter Inc., New York, N.Y.

[14] French No. 14; Foley.

[15] Vacutainer; Becton, Dickinson and Co.

Table II—Chemical Stability of Cefazolin Sodium Suspended in Ethyl Oleate

Lot No.	Age and Storage Condition	Experimental Value[a], % of Initial	Predicted Value, % of Initial
P35294	3 months, 25°C	103.8	99.6
G43061	1 month, 25°C	98.9	99.9
G43060	1 month, 37°C	101.5	99.3
G43046	1.5 months, 40°C	104.1	97.1
G43034	2 weeks, 60°C	90.4	90.8

[a] Mean of three determinations.

RESULTS AND DISCUSSION

During the entire study, cefazolin sodium particles were well suspended in ethyl oleate; a good flocculated system resulted. In contrast, caking was observed in the peanut oil suspensions. A significant difference in zeta potential was observed between cefazolin sodium particles suspended in peanut oil and ethyl oleate (*i.e.*, −2.57 mV ± 0.09 mV *versus* −27.1 mV ± 1.03 mV, respectively). These results are consistent with reported data that the ability to be suspended is correlated with the zeta potential (7, 14).

Effect of a wetting agent on the physical stability of a nonaqueous suspension was evaluated. Surfactants are widely used in pharmaceutical formulations as wetting agents. In the cefazolin sodium suspension in peanut oil, the addition of various amounts of polysorbate 80 produced a deflocculated state. This change was indicated by the difficulty of redispersibility, evidenced by the number of revolutions of the redispersing apparatus required to return the system to a homogenous state (Table I). There was an increase in sedimentation volume by adding 0.17% of polysorbate 80. However, an increase in concentration of polysorbate 80 above this concentration produced a reduction in sedimentation volume. In general, in an aqueous suspension system, a progressive rise in sedimentation volume is accompanied by an increase in the ease of redispersibility (15). In contrast to the conventional aqueous system, no correlation between the redispersibility of the sedimentation bed and the sedimentation volume was found in this nonaqueous system. Considering the interaction of polysorbate 80 with cefazolin sodium, it is possible that cefazolin sodium particles were deflocculated by a process of supersaturating and crystallizing effects produced by the solubilization of the polysorbate 80–cefazolin sodium complex. As shown in Fig. 1, microscopic examination indicated that extensive agglomeration and crystal growth did indeed occur.

An emulsifying agent is commonly included in the formulation of parenteral preparations. Because the agent lowers interfacial tension, the drainage property of a parenteral suspension from the vial can be remarkably improved. In this study, lecithin was chosen as an emulsifying agent and the effect of lecithin on the stability of cefazolin sodium dispersed in ethyl oleate was evaluated. Unlike the peanut oil system, cefazolin sodium in the ethyl oleate suspension in the presence of lecithin produced a deflocculated state accompanied by a decrease in sedimentation volume. The zeta potential of cefazolin sodium decreased with the addition of lecithin and remained essentially constant throughout the concentration range studied (Fig. 2). Furthermore, the effect of increasing lecithin concentration on the zeta potential of cefazolin sodium was paralleled by the effect of lecithin concentration on redispersibility. The data on the porosity of the sedimentation bed correlated well with the data on the sedimentation volume ratio. As shown in Fig. 3, the porosity of the sedimentation bed decreased with the addition of lecithin and the suspension resulted in a deflocculated state which correlates to ability to be suspended.

It is also of interest to compare the appearance of the supernatants of the suspensions relative to their physical stability. As shown in Fig. 4, the turbidity was minimum at 0 and 1.0% of lecithin in the formulation. No correlation was found between the degree of turbidity in the supernatant and the degree of flocculation. These data suggest that the cefazolin sodium particles might be initially well dispersed due to an electrical repulsive force between particles. In the presence of lecithin, however, the contribution of electrical repulsive

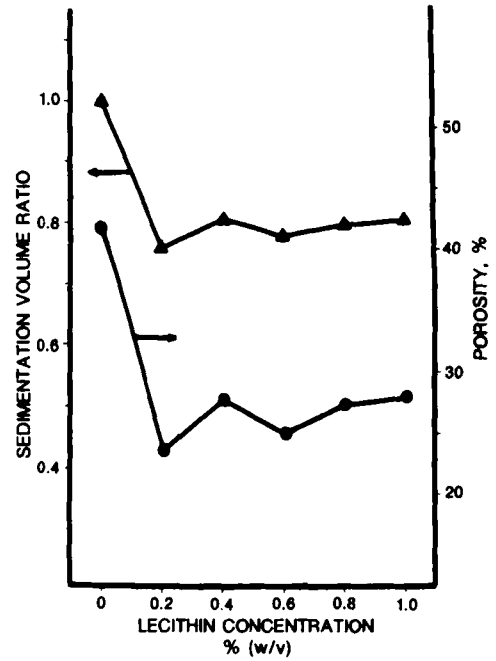

Figure 3—*Influence of lecithin concentrations on sedimentation volume of cefazolin sodium suspensions in ethyl oleate and on porosity of its sedimentation bed.*

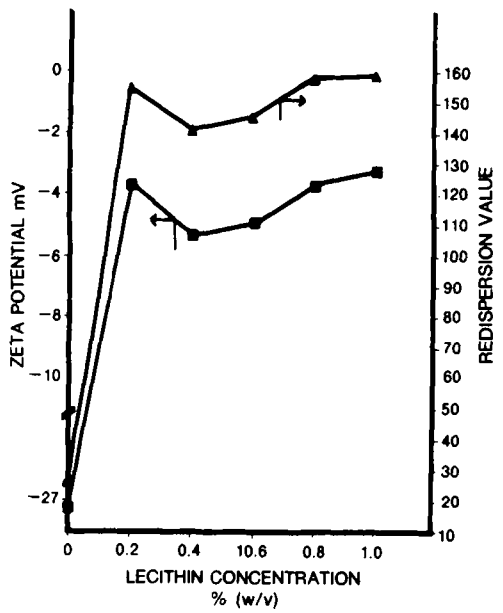

Figure 2—*Influence of lecithin concentrations on zeta potential and redispersibility of cefazolin sodium suspensions in ethyl oleate.*

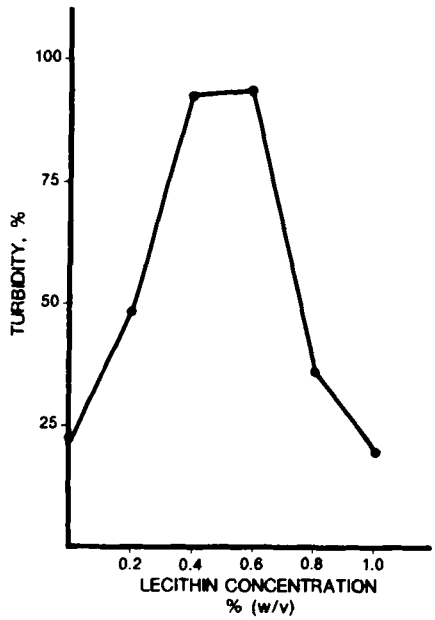

Figure 4—*Influence of lecithin concentrations on turbidities of cefazolin sodium suspensions in ethyl oleate.*

Table III—Comparison of the Performance of Aqueous and Nonaqueous Formulations of Cefazolin in Female Mongrel Dogs

Cefazolin Sodium Formulation	Time of Peak Mean Plasma Concentration, min	Peak Mean Plasma Concentration, μg/mL	Urinary Recovery, % of Dose	AUC[b], μg·min/mL
Solution				
Intravenous	1.0	134 ± 15	89.1 ± 2.4	4609 ± 363
Intramuscular	40	32.4 ± 2.4	79.8 ± 2.4	4319 ± 341
Suspension in Ethyl Oleate				
Intramuscular	40	28.1 ± 4.0	94.1 ± 8.0	4512 ± 256

[a] Cumulative (24-h) recovery of antibacterial activity in urine. [b] Area under the plasma concentration *versus* time curve.

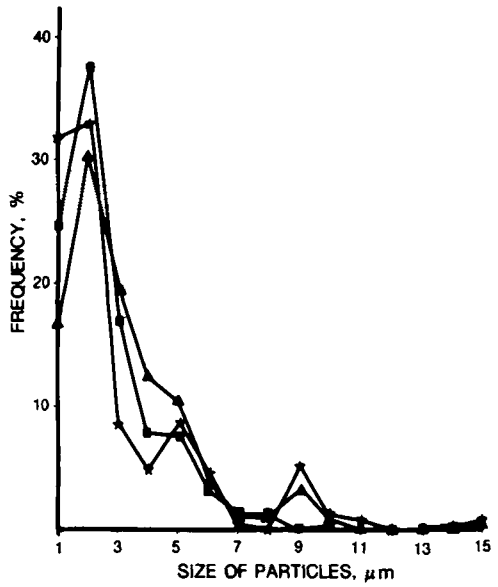

Figure 5—*Particle size frequency distribution curve of cefazolin sodium suspension in ethyl oleate. Key: (■) cefazolin sodium in dry state; (*) cefazolin sodium suspension at initial time; (▲) cefazolin sodium suspension aged 3 months at 25°C.*

force was diminished by a shielding effect on the surface of cefazolin particles as reflected in their electrophoretic properties.

Figure 5 shows the results of the evaluation of crystal growth. Measurement of changing particle size distribution as a function of time under three different storage conditions showed no significant crystal growth.

The potency of cefazolin sodium in ethyl oleate was studied at high temperatures. An Arrhenius plot (with 95% confidence limits) of degradation constants of cefazolin sodium suspension as a function of temperature is shown

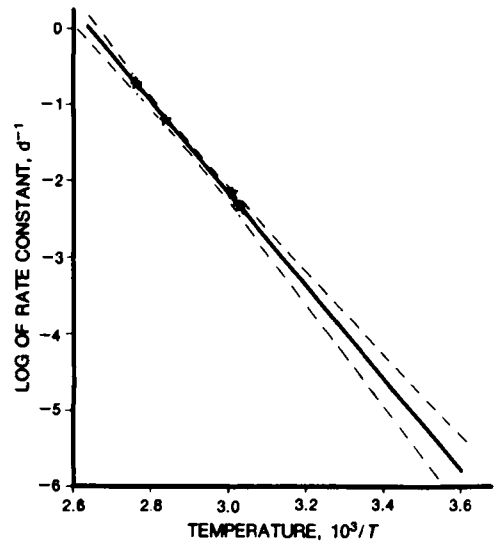

Figure 6—*Arrhenius plot with 95% confidence limits of degradation constants of cefazolin sodium suspension in ethyl oleate:* y = −5.916x + 15.556; r = 0.998.

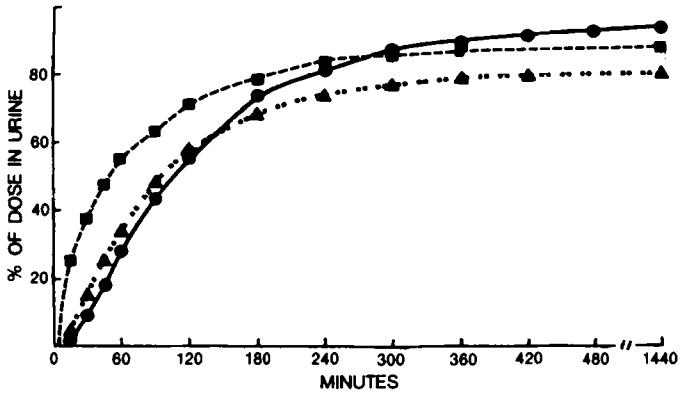

Figure 7—*Mean recovery of antibacterial activity in the urine of female mongrel dogs after administration of 15 mg/kg of cefazolin. Key: (■) aqueous solution administered intravenously; (▲) aqueous solution administered intramuscularly; (●) ethyl oleate suspension administered intramuscularly.*

in Fig. 6 ($y = -5.916x + 15.556$, $r = 0.998$). By extrapolation from the Arrhenius plot, it appears that the chemical stability of cefazolin sodium in ethyl oleate would be adequate for satisfactory shelf life at room temperature. As shown in Table II, the experimental values are well correlated with the predicted values.

In Table III, the performance of one intravenous and two different intramuscular formulations of cefazolin sodium in female mongrel dogs are compared. Based on the results of an analysis of variance, there are no statistically significant differences between the mean values for the area under the plasma concentration *versus* time curves for any of the three treatments ($p > 0.1$). By the same criterion, there are also no differences between the amounts of antibacterial activity recovered in urine by 24 h for the three treatments. The time course of the appearance of antibacterial activity in urine (Fig. 7) suggests that, as expected, cefazolin is more rapidly excreted in the urine following intravenous administration than following intramuscular administration.

The mean peak plasma concentration obtained following intravenous administration of cefazolin was significantly higher than that obtained with the intramuscular preparations, but the mean peak plasma concentrations obtained with the two intramuscular preparations were not different ($p > 0.1$). From these comparisons, it is concluded that in dogs administered 15 mg/kg intramuscularly, the total availability of cefazolin sodium suspended in ethyl oleate is not significantly different from that in aqueous solution. Furthermore, judged by the area under the plasma concentration *versus* time curve and the urinary recovery of antibacterial activity, the bioavailability of cefazolin sodium from this nonaqueous preparation approaches 100%.

In summary, it is known that physical stability is one of the most challenging problems when developing an acceptable suspension. This study demonstrates that by application of fundamental physical parameters, including sedimentation height, sedimentation rate, porosity of sedimentation bed, redispersibility, zeta potential, and turbidity, a good understanding of the physical stability of a nonaqueous cephalosporin suspension can be reached and potential problems relating to physical stability can be identified in the early stages of product development.

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Liquid Chromatographic Assay for Diflorasone Diacetate in Cream and Ointment Formulations

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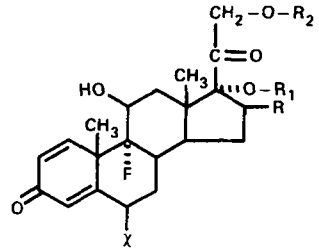
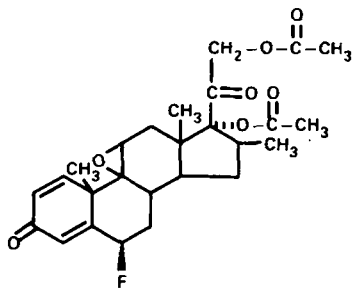
Abstract □ A high-performance liquid chromatographic assay for quantitating diflorasone diacetate (I) in cream and ointment formulations and in bulk form is described. Isoflupredone acetate (II) was used as the internal standard and a 3-μm silica gel column with a mobile phase comprised of water-saturated butyl chloride–water-saturated methylene chloride–tetrahydrofuran–acetic acid (350:125:10:15) led to an efficient separation. The method gave accurate results for four formulations, two creams and two ointments, as well as the bulk drug. The assay has an *RSD* of ~1.8% for the cream formulations, 1.3% for the ointment formulations, and 1.0% for the bulk drug. The method is specific for I and capable of resolving structurally related compounds.

Keyphrases □ Diflorasone diacetate - HPLC, cream, ointment, bulk drug □ HPLC—diflorasone diacetate, cream, ointment, bulk drug

Diflorasone diacetate (I) in topical formulations is indicated for the relief of inflammatory manifestations of acute and chronic corticosteroid-responsive dermatoses (1). It is available as a 0.05% cream- or ointment-based topically applied product[1]. The high-performance liquid chromatographic (HPLC) analysis of similar topical anti-inflammatory steroids has appeared (2). This report describes a new normal-phase HPLC assay that is specific for diflorasone diacetate in two cream and two ointment formulations. The assay is also capable of separating several structurally related compounds: the 9,11-epoxide (III), 6β-fluoro analogue (IV), 6-defluoro analogue (V), the 17-OH,21-OAc analogue (VI), and the 17-OAc,21-OH analogue (VII) of I.



I R=CH_3, R_1=Ac, R_2=Ac, X=α-F
II R=H, R_1=H, R_2=Ac, X=H
IV R=CH_3, R_1=Ac, R_2=Ac, X=β-F
V R=CH_3, R_1=Ac, R_2=Ac, X=H
VI R=CH_3, R_1=H, R_2=Ac, X=α-F
VII R=CH_3, R_1=Ac, R_2=H, X=α-F



III

EXPERIMENTAL SECTION

Materials—Diflorasone diacetate[2] (I), isoflupredone acetate[2] (II), and the related compounds III–VII[2] (3–7) were obtained in pure form and dissolved in water-saturated chloroform for chromatography and spectroscopy. Solvents and reagents were commercial analytical grade. Placebo materials were obtained in-house[2].

Chromatographic Conditions—A modular liquid chromatograph including pump[3], fixed-loop injector[4], fixed-wavelength detector[5] (254 nm), and a minicomputer[6] were used. Commercial 3-μm[7] silica gel columns (10 cm × 4.6 mm i.d.) were used at ambient temperature. The mobile phase, deaerated under vacuum sonication prior to use, consisted of water-saturated butyl

[1] Florone Cream, Florone Ointment; The Upjohn Company, Kalamazoo, Mich.
[2] The Upjohn Company.
[3] Model 110A; Altex Scientific Inc., Berkeley, Calif.
[4] Model AH60 equipped with a 10-μL fixed volume loop; Valco Instruments Co., Houston, Tex.
[5] Model 1203; LDC Corp., Riviera Beach, Fla.
[6] PDP 11-40; Digital Equipment Corp., Marlborough, Mass.
[7] Part #0258-1500; Perkin-Elmer Corp., Norwalk, Conn.

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