Determination of Sodium Fusidate and Fusidic Acid In Dosage Forms by High-Performance Liquid Chromatography and a Microbiological Method

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Received November 2, 1981, from the *Department of Pharmaceutics, College of Pharmacy, King Saud University of Riyadh, Riyadh, Saudi Arabia.* Accepted for publication January 21, 1982.

Abstract □ A new High-performance liquid chromatographic (HPLC) method for the assay of sodium fusidate (I) or fusidic acid in dosage forms was developed and compared to a microbiological assay. A linear relationship was obtained between absolute peak area and amount of I (r = 0.99+) in the 50–1000-μg/ml range. In the microbiological assay, *Staphylococcus aureus* (NCTC 6571) was the test organism, using an agar diffusion technique. With five test levels of the standard, potencies were interpolated from standard curve using a log transformation straight-line method with least-squares fitting (r = 0.99+). Both methods were applied to assay I (or fusidic acid) in tablets, a suspension, and an ointment. Excellent agreement was observed between results of the two methods.

Keyphrases □ Fusidate sodium—determination in dosage forms, high-performance liquid chromatography, microbiological method, tablets, suspension, ointment □ Fusidic acid—determination in dosage forms, sodium fusidate, high-performance liquid chromatography, microbiological method, tablets, suspension, ointment □ High-performance liquid chromatography—determination of fusidate sodium and fusidic acid in dosage forms, microbiological method, tablets, suspension, ointment

Fusidic acid is an antibiotic produced by the growth of certain strains of *Fusidium coccineum* (K. Tubaki). Its structure is related to helvolic acid and to cephalosporins. The structure also includes the cyclopentanoperhydrophenanthrene ring system, thus, it belongs to the steroid group of substances. Fusidic acid, or its sodium salt, shows a very high antistaphylococcal activity (1, 2). Available methods for the quantitative determination of fusidic acid are based mainly on microbiological procedures (3, 4). A colorimetric assay has been reported (5) which involved modification of an earlier sulfuric acid color test for steroids and measurement in the visible range of the spectrum (365–595 nm). Microbiological procedures are, by nature, time consuming and call for specialized technique, while the colorimetric assay involves the added steps of producing the color.

The present report describes a simple, sensitive, and specific procedure for the quantitative determination of fusidic acid and its sodium salt using high-performance liquid chromatography (HPLC). This new procedure was compared to a microbiological assay and was applied to the determination of fusidic acid or its sodium salt in tablets, a suspension, and an ointment.

EXPERIMENTAL

Reagents—Sodium fusidate, BP[1], was purified by crystallization from absolute ethanol before use. All other chemicals were USP or analytical reagent grade, and were used as obtained. All solvents were spectroscopic or chromatographic grade; water used in the mobile phase was double-distilled in an all-glass still. Drug products containing sodium fusidate or fusidic acid were purchased from commercial sources.

[1] Leo Pharmaceutical Products, Ballerup, Switzerland, lot no. 22176.

Apparatus—The liquid chromatograph[2] used was equipped with an automatic sample injector[3], a variable wavelength detector[4] set at 254 nm, a dual-pen recorder[5], and a data system[6]. The column was stainless steel 30 cm × 4-mm i.d. packed with microparticulate silica (5 μm) bonded with octadecylsilane[7]. Experiments were conducted at ambient temperature (25°).

Mobile Phase—The mobile phase consisted of a 3:1 mixture of

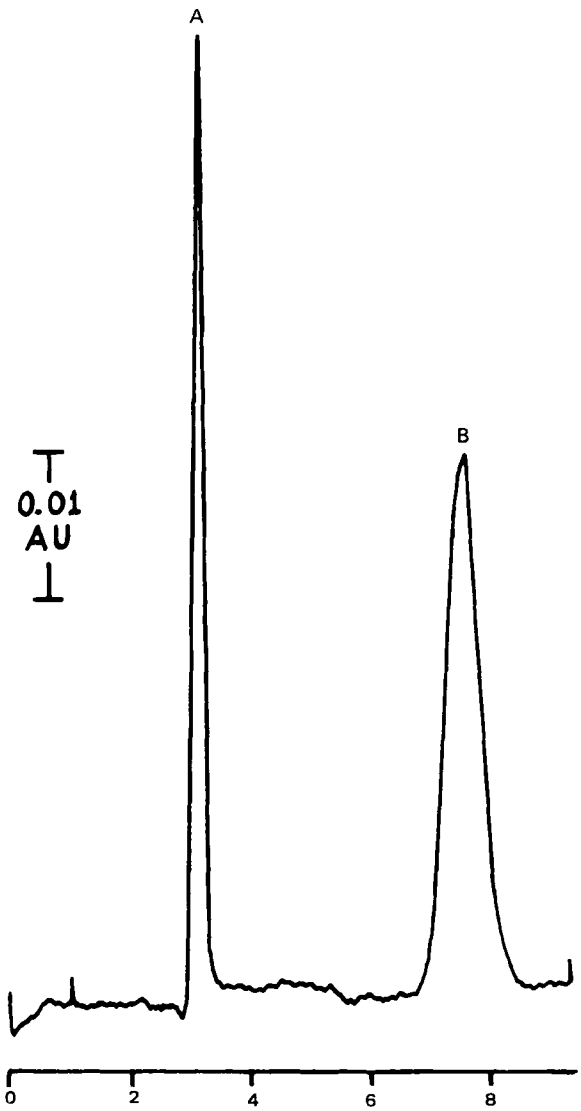

Figure 1—*Chromatogram of extract of sodium fusidate tablets. Key: (A) unassigned peak; (b) sodium fusidate.*

[2] Model 5021, Varian Instrument Group, Palo Alto, Calif.
[3] Model 8055, Varian Instrument Group.
[4] Model UV-50 Varian Instrument Group.
[5] Model 9176, Varian Instrument Group.
[6] Model CDS 111L, Varian Instrument Group.
[7] Micropak MCH, Varian Instrument Group.

0022-3549/82/1100-1297$01.00/0

Table I—Percent of Label Sodium Fusidate or Fusidic Acid Found in Dosage Forms

Dosage Form	Run Number	HPLC Method	Microbiological Method
Tablets	1	96.70	98.80
	2	93.22	95.60
	3	89.90	92.10
	4	95.07	94.50
	5	95.70	91.86
	6	87.90	86.81
	Mean ± *SD*	93.08 ± 3.49	93.28 ± 4.06
Suspension	1	100.47	98.71
	2	108.13	108.66
	3	102.13	104.57
	4	103.81	97.21
	5	105.84	100.00
	6	100.52	99.86
	Mean ± *SD*	103.48 ± 3.06	101.50 ± 4.29
Ointment	1	94.40	94.45
	2	101.60	100.00
	3	99.52	99.36
	4	101.93	100.00
	5	103.80	100.00
	6	99.97	94.50
	Mean ± *SD*	100.20 ± 3.23	98.05 ± 2.78
Overall	Mean ± *SD*	97.42 ± 8.77	97.61 ± 4.96

methanol[8] and 0.01 *M* KH_2PO_4 (pH ~6.6, not adjusted), at a flow rate of 2 ml/min.

Calibration Curve—Using the mobile phase as the solvent, nine different solutions of fusidate sodium were prepared to cover the 50–1000-μg/ml range. Duplicate injections of 10 μl were made, and the peak area was plotted against concentration. The line of best fit was calculated by linear regression, and was used to determine concentrations of the test solutions. The standard solutions were interspersed with solutions obtained from the extraction of dosage forms (see below), and all were run on the same working day.

Extraction Procedure—*Tablets*[9]—Six tablets were individually crushed in glass stoppered flasks, and shaken with 100 ml of methanol for 30 min. The extract was filtered through a membrane filter[10]. A 10-ml aliquot was diluted with 65 ml of methanol and 25 ml of 0.01 *M* KH_2PO_4 for HPLC assay. A 1-ml aliquot was used for microbiological assay as described below.

Suspension[11]—After shaking the bottle thoroughly, six 5-ml aliquots were pipeted using a wide-mouthed pipet, individually placed in 100-ml volumetric flasks, and treated with 0.5 ml of 1 N NaOH solution. Methanol was then added to volume, and the solution was filtered through a membrane filter[10].

Ointment[12]—Six 1-g aliquots were accurately weighed in small beakers and individually extracted as follows: Five milliliters of benzene was added, the mixture was transferred to a separatory funnel, and extracted with three 10-ml portions of 0.01 N NaOH. The combined aqueous extracts were filtered through a membrane filter[13]. A 10-ml aliquot was diluted with the mobile phase to a concentration of ~200 μg/ml for HPLC assay. A 1-ml aliquot was used for microbiological assay as described below.

Microbiological Assay—Aliquots obtained from extraction of tablets or suspension were evaporated at 50° and redissolved in 10 ml of saline. Solutions were transferred to 100-ml volumetric flasks, and saline was added to volume. Aliquots obtained from extraction of ointment were placed in 50-ml volumetric flasks, and saline was added to volume. An agar plate diffusion technique was employed using *Staphylococcus aureus* (NCTC 6571) as the test organism. A 1:10 dilution of an 18-hr culture in antibiotic assay broth[14] was added to melted and cooled (50°) antibiotic assay agar medium[14], mixed thoroughly, and poured into replica plate petri dishes. After the agar had solidified, wells (4-mm i.d.) were punched out, and a drop of a standard or test dilution was applied. Standard dilutions contained 10, 5, 2.5, 1.25, or 0.625 μg/ml of fusidate sodium in saline. Test solutions were diluted similarly. The zone of inhibition diameter was measured after an 18-hr incubation at 37°. Potencies were interpolated from a standard curve using a log transformation straight-line method with least-squares fitting.

RESULTS AND DISCUSSION

Inasmuch as fusidic acid is structurally related to steroid hormones, HPLC conditions that previously (6, 7) have been employed for steroid hormones were tried before the conditions described herein were selected. Under the experimental conditions described, sodium fusidate showed a single peak with a retention time of 7.4 min. A plot of peak area *versus* concentration produced a linear relationship (r for the least-squares line = 0.999, y-intercept = −294851, and slope = 22456); thus, no internal standard was needed. Figure 1 shows a representative chromatogram of the solution obtained from the extraction of tablets. It can be seen that the chromatogram shows an additional peak prior to the fusidate sodium peak. A similar peak appeared in the chromatogram of the solution obtained from the suspension. These additional peaks may be caused by formula additives or decomposition products; however, no definite assignment can be made at present. The absence of any interference with the sodium fusidate peak is an indication of the specificity of this procedure.

Table I gives the results of assaying commercial dosage forms by HPLC and by microbiological procedure. Good agreement is seen between results of the two methods. When six tablets were assayed by both methods, the mean (±*SD*) percent of labeled amount was 93.08 ± 3.49 by HPLC, and 93.28 ± 4.06 by microbiological procedure. The mean (±*SD*) percent of labeled amount obtained in the assay of the suspension was 103 ± 3.06 by HPLC and 101 ± 4.29 by microbiological method. HPLC assay of the ointment gave a mean (±*SD*) percent of labeled amount of 100.20 ± 3.23 compared to 98.05 ± 2.78 obtained by the microbiological method.

Microbiological procedures require considerable expenditure of time and effort and call for specialized skills. The HPLC procedure described is simple, sensitive, specific, and can be applied in routine quality control.

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[8] BDH Chemicals, Ltd., Poole, England.

[9] Fucidin Leo, fusidate sodium, 250 mg/tablet, Leo Pharmaceutical Products, Ballerup, Denmark, Lot No. A85K.

[10] Type FH, 0.5 μm, Millipore Corp., Bedford, Mass.

[11] Fucidin Leo, fusidic acid, 50 mg/ml, Leo Pharmaceutical Products, Lot. No. B08AA.

[12] Fucidin Leo, fusidate sodium, 20 mg/g, Leo Pharmaceutical Products, Lot No. L06B.

[13] Type AH, 0.45 μm, Millipore Corp.

[14] Oxoid Ltd., London, England.