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Note

Liquid chromatographic determination of fusidic acid in serum

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Fusidic acid (Fucidin®), (17*Z*)-16β,3α,11α-dihydroxyfusida-17(20),24-dien-21-oic acid (Ia, Fig. 1), is an important antibiotic, produced by the growth of certain strains of *Fusidium coccineum* [1], that is particularly useful for the treatment of staphyloccocal infections. Recently it has been reported [2] that fusidic acid inhibits the human immunodeficiency virus (HIV) in vitro.

Although various preparations of fusidic acid have been available since 1962, studies of its pharmacokinetics have appeared only sporadically [3–6]. The assay methods used were mostly microbiological [3,6–8]. A straight-phase high-performance liquid chromatographic (HPLC) method involving extraction of fusidic acid from plasma with methylene chloride has been published [9].

To carry out pharmacokinetic studies on new formulations in both human volunteers and patients, a simple, specific and sensitive assay method with no interference from metabolites [10] or other antibiotics used concomitantly with fusidic acid was needed. A method based on the reversed-phase HPLC used at Leo Pharmaceutical Products for fusidic acid, its salts and formulations is presented here.

EXPERIMENTAL

Chemicals and reagents

Diethanolamine fusidate and the internal standard, 24,25-dihydrofusidic acid (II, Fig. 1) were obtained from Leo Pharmaceutical Products (Copenhagen, Denmark). Acetonitrile used was HPLC grade, all other solvents and chemicals were of analytical grade.

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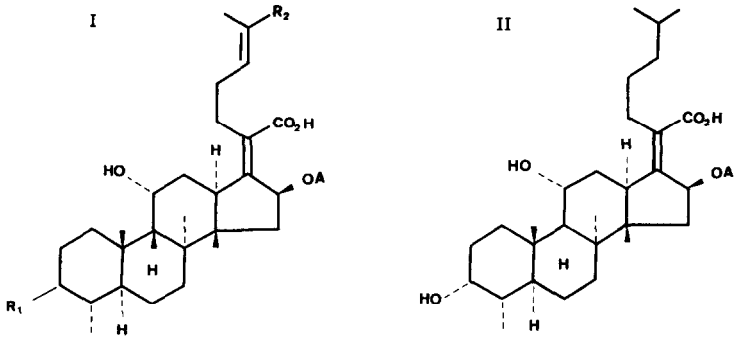

a R_1 = H, α-OH, R_2 = CH_3
b R_1 = O, R_2 = CH_3
c R_1 = H, α-OH, R_2 = COOH

Fig. 1. Structures of fusidic acid (Ia), 3-ketofusidic acid (Ib), 27-carboxyfusidic acid (Ic) and internal standard (II).

Instruments and conditions

Instrumentation consisted of an LDC Model 711-57 solvent delivery system, an LDC SpectroMonitor III variable-wavelength detector (Laboratory Data Control, Riviera Beach, FL, U.S.A.) and a WISP Model 710 A autoinjector (Waters Assoc., Milford, MA, U.S.A.). Peak responses were measured using a data module as recording integrator (Waters Assoc.).

The column was stainless-steel, 250 mm × 4 mm I.D., packed with 7-μm LiChrosorb RP 18 (Merck, Darmstadt, F.R.G.) and protected by a guard column (Waters Guard-PAK™ precolumn module with a C_{18} cartridge, Waters Assoc.).

The eluent was acetonitrile–methanol–0.01 *M* phosphoric acid (50:20:30, v/v/v), delivered at a flow-rate of 3 ml/min and monitored at 235 nm. The column temperature was ambient (ca. 24°C).

Standard solutions

Standard solutions of diethanolamine fusidate in acetonitrile containing 125 and 25 μg/ml, corresponding to ca. 100 and 20 μg/ml fusidic acid, were prepared. The internal standard solution was prepared by dissolving 2 mg in 100 ml of acetonitrile.

Sample preparation

To 100 μl of serum in a tapered centrifuge tube were added 100 μl of internal standard solution and 100 μl of acetonitrile. The tubes were capped and mixed by vortexing, and then centrifuged for 10 min at 2200 *g*. A 75-μl volume of the clear supernatant was injected.

The consentrations of fusidic acid in the samples were determined from the peak-height ratios relative to the internal standard and the corresponding standard determinations, in which 100 μl of standard solution and 100 μl of internal standard solution were added to 100 μl of blank serum. The low standard solution

concentration was used when the sample concentrations were in the range 1–50 μg/ml. The high concentration was used for the 51–250 μg/ml range.

RESULTS AND DISCUSSION

Typical chromatograms obtained from a serum standard and from human volunteer samples are shown in Fig. 2. Good separation from serum constituents and from the metabolites 3-ketofusidic acid (Ib, Fig. 1), 27-carboxyfusidic acid (Ic, Fig. 1) and fusidic acid-21-glucuronide was achieved. The capacity factors of fusidic acid and metabolites are shown in Table I. It appears from the table and from the chromatograms that the method is also suitable for measuring contents of 3-ketofusidic acid, whereas other metabolites do not separate from endogenous serum constituents.

Other antibiotic drugs that may be concomitantly administered with fusidic acid were tested for potential interference in the assay. No interference was observed for the following antibiotics: amikacin, ceftazidime, ciprofloxacin, cloxacillin, colistin, dicloxacillin, erythromycin, flucloxacillin, gentamicin, piperacillin, rifampicin, thienamycin and vancomycin.

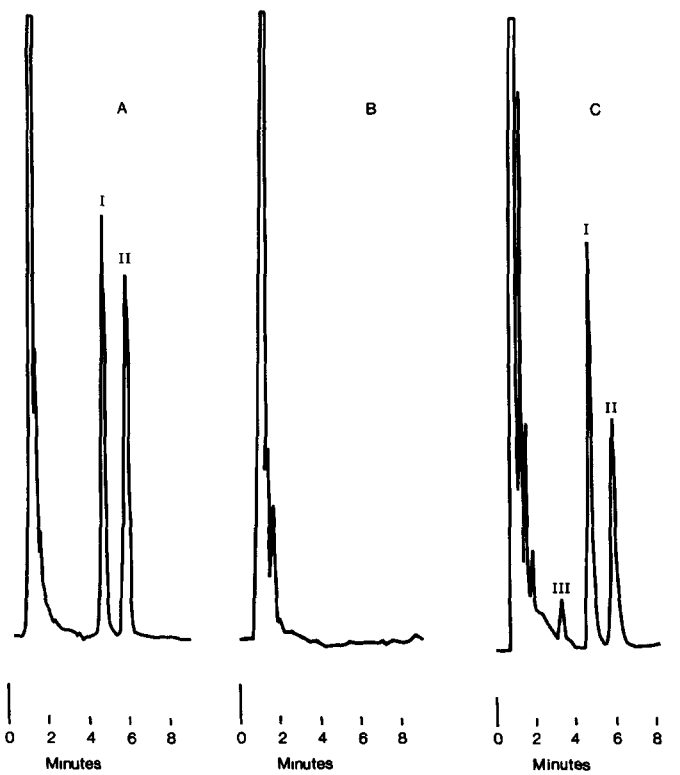

Fig. 2. Chromatograms of fusidic acid in serum. (A) Serum standard containing 20 μg/ml fusidic acid. (B) Serum blank. (C) Human volunteer serum 4 h after a 500-mg dose of sodium fusidate tablets. Peaks: I = fusidic acid; II = internal standard; III = 3-ketofusidic acid.

TABLE I

CAPACITY FACTORS OF FUSIDIC ACID AND METABOLITES

Compound	k'
Fusidic acid	4.7
3-Ketofusidic acid	3.0
27-Carboxyfusidic acid	1.6
Fusidic acid-21-glucuronide	1.2

TABLE II

SUMMARY OF ACCURACY AND PRECISION DATA ($n=15$)

Concentration added (μg/ml)	Concentration found (μg/ml)	Relative standard deviation (%)	Relative error (%)
4.9	4.6	8.2	−6.2
24.6	25.3	3.5	+2.5
49.3	50.2	1.8	+1.8
98.6	100.1	2.3	+1.6
246.4	245.9	1.6	−0.2

Sample recovery

The recovery of I and II from serum was determined by comparison of peak heights from spiked serum prepared by the procedure to solutions of the analytes in acetonitrile–water (1:2). The recovery was essentially 100%.

Precision and accuracy

The precision and accuracy of the method were investigated by spiking blank serum with fucidic acid at five different concentrations. Five replicates from each pool were assayed on three different days. The concentrations ranged from 5 to 250 μg/ml. The results are shown in Table II. Relative standard deviations ranged from 8.2 to 1.6%. The accuracy of the determinations is expressed in terms of percent relative error. Good accuracy was obtained, with values ranging from −6.2 to 2.5%.

Linearity and sensitivity

The relationship between the concentrations of I and its peak-height ratios relative to the internal standard II was linear over a wide concentration range: 1 μg/ml to at least 300 μg/ml. The detection limit for fusidic acid in serum was ca. 1 μg/ml, based on a signal-to-noise ratio of 3.

Stability

The stability of fusidic acid in serum was determined by spiking a known amount of fusidic acid to human serum. Aliquots of the serum pool were stored in plastic sample vials at 37, 4 and −20°C, and samples were analysed at proper intervals.

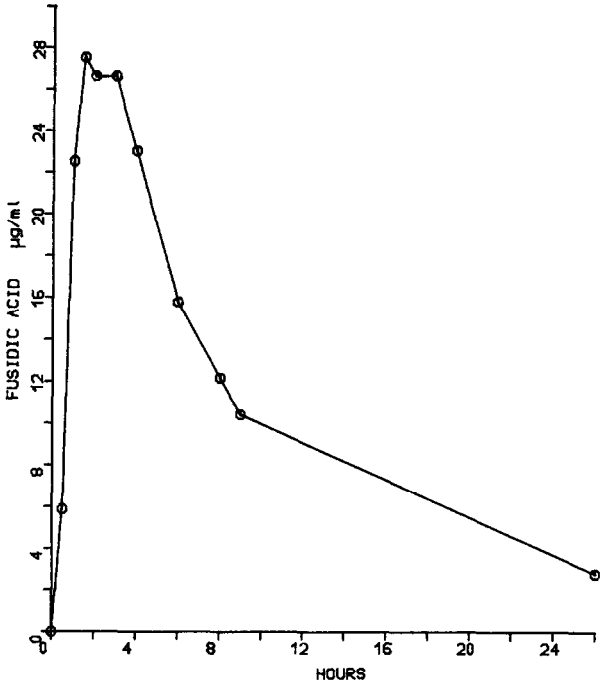

Fig. 3. Fusidic acid serum concentration–time curve following a 500-mg dose of sodium fusidate tablets. Values reflect a mean of twelve human volunteers.

Fusidic acid was observed to be stable at 37 °C for a week, at 4 °C for a month and at −20 °C for at least six months.

The stabilities of standard solutions and of internal standard solutions were determined by storing both solutions at 4 °C and at room temperature and analysing them against freshly prepared solutions. No deterioration was observed in either of the solutions within a month.

Prepared standards were injected periodically over 15 h. The peak heights were measured and compared with those obtained initially. No significant change was observed over that period of time, thus the method is suited to automatic samplers.

Application of the method in pharmacokinetic studies

The method has been used for pharmacokinetic studies involving intravenous as well as oral administration of sodium fusidate in single-dose studies and in repeated-dose studies. The serum profile after a single dose of 500 mg given as two tablets is presented in Fig. 3. In all studies the serum concentrations were within the linear range of the method, and the sensitivity was sufficiently high.

CONCLUSIONS

A precise, accurate and selective liquid chromatographic method has been developed for the selective determination of fusidic acid in human serum. No en-

dogenous serum components or metabolites interfere, and neither do antibiotics commonly used together with fusidic acid.

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