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Short Communication

Spectrophotometric determination of fusidic acid and sodium fusidate in dosage forms*

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Introduction

Fusidic acid (FA) and its sodium salt (FS) are antibacterial agents with unusual steroid structures, chemically represented as: (3α,4α,8α,9β,11α,13α,14β,16β,17Z)-16-Acetyloxy-3,11-dihydroxy-29-nordammara-17(20),24-dien-21-oic acid (Fig. 1). The 16β-desacetyl derivative is a degradation product of FA, which lacks antistaphylococcal activity. The published methods for the quantitative analysis of FA and SF in dosage forms are based mainly on microbiological procedures [1, 2], which though specific, require considerable expenditure of time and specialized skills. Various instrumental methods have been used for the quantification of FA and SF in pharmaceutical formulations and biological fluids including: colorimetry [3, 4], derivative UV-spectrophotometry [5], potentiometric titration and LC [6–8] procedures.

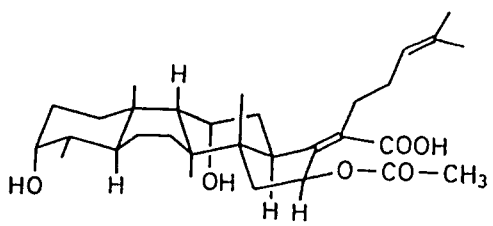

Figure 1
The stereochemical structure of fusidic acid (FA).

The colorimetric methods are based on the reactions of acetic anhydride–conc.sulphuric acid (4:1, v/v) [3] and phosphomolybdic acid in acid media [4]. Unfortunately, the colorimetric methods are not sufficiently selective, because of interference from impurities such as 3-ketofusidic acid, and 16β-desacetylfusidic acid.

A general method for determination of derivatives of organic acids (esters, amides, cyclic imides, lactones, etc.) as complexes with iron(III)-ion, after their quantitative conversion into the corresponding hydroxamic acids in alkaline pH has been described [9]. The stability of the red-violet coloured complexes depends mainly on the iron(III)-ion and hydroxylamine concentrations and pH value of the final reaction mixture [10, 11]. The reaction conditions, favouring quantitative formation of acetylhydroxamic acid from 16β-acetyloxy moiety of FA make possible a specific determination of this antibacterial agent. Under these conditions, the impurities such as 16β-desacetylfusidic acid would not react with hydroxylamine. The main aim of this work, therefore, was to investigate the actylhydroxamic acid-iron(III), [AcHA-Fe(III)], complex formation and to propose a new colorimetric method for the determination of FA and SF in dosage forms.

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Experimental

Apparatus

A Specord M40, (Carl-Zeiss, Jena, Germany) spectrophotometer equipped with 10 mm glass cells was used.

Reagents and solvents

FA (Lot 6552), and SF (Lot 6057, Upjohn Co., Kalamazoo, MI, USA) were used as working standards. $Fe(ClO_4)_3$, $NH_2OH.HCl$, cyclohexane, CH_3COOH (Fluka), NaOH (Zorka, Yugoslavia), anhyd.Na_2SO_4, $CHCl_3$, $HClO_4$, MeOH and EtOH (all manufactured by Merck, Germany) were used. All solvents and reagents were of analytical grade of purity.

Dosage forms

Stanicid® film coated tablets (250 mg) and Stanicid® oral suspension (50 mg ml^{-1}) were obtained from Hemofarm–Upjohn (Yu-USA).

Solutions

Reagent A. Equal volumes of 12.5% methanolic $NH_2OH.HCl$ and 12.5% methanolic NaOH solution were mixed and filtered. The prepared solution was stable for 4 h.

Reagent B. (Stock soltuion); Accurately weighed 5 g of $Fe(ClO_4)_3$ was dissolved in the mixture of 10 ml $HClO_4$ and 10 ml H_2O. The prepared solution was diluted with EtOH up to 100 ml with cooling.

Reagent B_1. 1.2 ml of $HClO_4$ was added to 4 ml of the reagent B with cooling. The prepared solution was diluted to 100 ml with EtOH.

Standard solutions

Standard stock solution A. Accurately weighed 26.7 mg of FA was transferred in a 10 ml-calibrated flask and dissolved in 5 ml of methanol. The prepared solution was diluted up to 10 ml with ethanol.

Standard stock solution B. Accurately weighed 26.0 mg of SF was transferred to a 10 ml-calibrated flask and dissolved in 5 ml of MeOH. The solution was diluted up to 10 ml with EtOH.

Standard solution 1. Accurately weighed 20.0 mg of FA was transferred to a 10-ml calibrated flask and dissolved in 5 ml of MeOH. The solution was diluted up to 10 ml with EtOH.

Standard solution 2. Accurately weighed 50.0 mg of SF was transferred to a 5 ml-calibrated flask and dissolved in MeOH.

Sample solution (I). An appropriate volume of suspension containing 50.0 mg of FA was transferred to a separating funnel. The FA was extracted with 4 × 5 ml of HCl_3. The extracts were passed through a layer containing 1.0 g of anhyd.Na_2SO_4, collected and transferred to a 25-calibrated flask and diluted with $CHCl_2$. Of this extract, 2.0 ml was evaporated at 40°C and diluted up to 10 ml with EtOH.

Sample solution (II). A quantity of powdered film coated tablets containing 100 mg of SF was transferred to a 10 ml-calibrated flask and dissolved in 5 ml of MeOH. The prepared mixture was sonicated during 20 min, diluted to 10 ml with EtOH and filtered through Schleicher–Schüll filter paper.

General procedure

Equal volumes of each standard solutions 1 and 2 (0.5 ml) were transferred to 5 ml-volumetric flasks. After adding 0.3 ml of reagent A to each the volumetric flasks were stopped and heated for 40 min at 70°C. On cooling, the solutions were diluted to volume with reagent B_1. The absorbances were measured at 524 nm against the blank, prepared as described above but without standard solutions. The same procedure was carried out for sample solution (I) to determine of FA from the investigated dosage form.

Calibration curves. Nine samples containing 0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8 and 1.0 ml of the standard stock solution A was transferred to 5 ml-calibrated flasks. After adding to each sample 0.3 ml of reagent A, the reaction mixtures were stopped and heated for 40 min at 70°C. After cooling, the reaction mixtures were diluted up to the mark with reagent B_1. Absorbances were measured at 524 nm against blank. The same procedure was repeated for the standard stock solution B.

Chromatography

One hundred microlitres standard solution 1 and 100 µl sample solution II was applied to TLC plates (200 × 200 mm, precoated with

silica gel $60F_{254}$, Merck). The plates were developed in a solvent system consisting of $CHCl_3$–CH_3COOH–cyclohexane–MeOH (160:20:20:5, v/v/v/v). After development, the spots were detected under UV light (254 nm) and areas coresponding to the SF were marked. The silica gel was quantitatively scraped off and eluted with 2 ml EtOH. The eluents were transferred to 5-ml calibrated flasks and further procedure of colour development was performed as described above.

Results and Discussion

The kinetics and mechanism of hydroxyaminolysis of simple imides and esters have been studied previously [10, 11]. The stability of the violet coloured complexes depends mainly on the iron(III)-ion concentration and pH value of the final reaction mixture [12]. The desacetyl analogue of FA does not react with Fe(III)-ion to form a coloured complex, therefore, FA can be simply determined in the presence of 16β-desacetyl derivative without prior separation. According to the literature [9] the quantitative formation of an AcHA-Fe(III) complex (Fig. 2) from esters was carried out by heating the reaction mixture during 5–10 min at 70°C. The quantitative formation of AcHA-Fe(III) complex needed considerably more time (40 min), presumably due to the stereochemical hindrance in the molecule of FA. The absorption maximum was carried out by heating the reaction mixture at 70°C during 40 min (Fig. 3).

The graph of absorbance vs the concentration of FA was linear in the range from 53.6 to 428.0 μg ml^{-1}. Beer's law for FA was given by the equation: $y = 0.013 + 1113.7x$; $r = 0.9998$ and molar absorptivity 1.1×10^3 l mol^{-1} cm^{-1}. The intercept $a = 0.013$ statistically was insignificant. The detection limit for FA was 52.0 μg ml^{-1}.

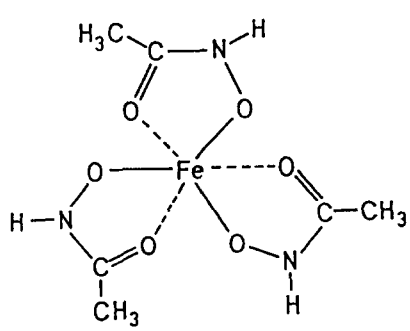

Figure 2
Structure of AcHA-Fe(III) complex.

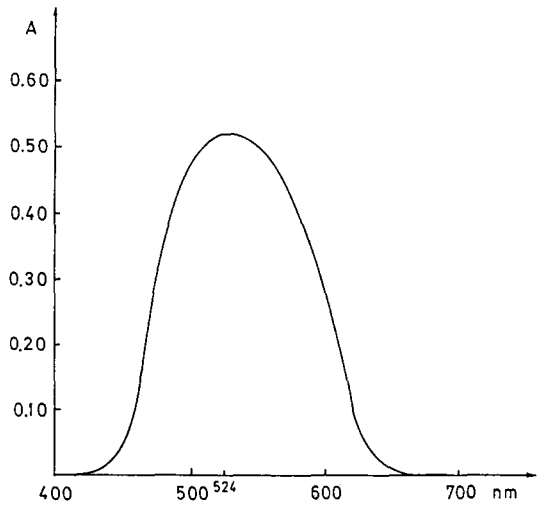

Figure 3
Absorption spectrum of AcHA-Fe(III) complex; 4.08×10^{-4} M of fusidic acid.

The graph of absorbance vs the concentration of SF was linear in the range from 52.1 to 416.4 μg ml^{-1}. Beer's law was given by the equation: $y = 0.0023 + 1045.6\ x$; $r = 0.9990$ and molar absorptivity 1.05×10^3 l mol^{-1} cm^{-1}. The intercept $a = 0.0023$ statistically was insignificant, the limit of detection of SF was 50.0 μg ml^{-1}.

The precision of the proposed method was checked at three different concentrations of FA and SF. The obtained results with statistical parameters are presented in Table 1.

The accuracy of the method was proved by direct determination of SF from the laboratory-made tablets (250 mg) spiked with a determined quantity of SF; recovery was 98.50%, ($n = 5$); RSD = 1.61% (Table 2).

The proposed procedure was applied to the direct determination without prior TLC separation of FA in the investigated dosage form. The commercially available film coated tablets consisted esters of cellulose which takes part in the reaction of hydroxyamonolysis and complex formation with Fe(III)-ion. The assay of SF in film coated tablets was preceded by TLC separation, due to the interference of cellulose esters on the intensity of developed colour.

The analytical results obtained for the mentioned dosage forms of FA and SF are listed in Table 2. The recoveries ranged from 100.41 to 105.39% and RSD from 1.51% to 2.75%, respectively.

Table 1
The precision of the spectrophotometric determination of FA and SF

Sample	Taken (mg)	X (n = 6)	RSD (%)
FA	160.8	157.8	1.66
FA	268.0	264.3	1.36
FA	375.2	368.1	1.98
SF	208.5	205.4	1.50
SF	260.2	258.0	0.84
SF	312.4	306.5	1.89

Table 2
The determination of FA and SF in dosage forms

Dosage form	Taken	X (mg)	(n = 5)	RSD (%)
Stan. 1*	50.0	49.25		1.61
Stan®. 2†	50.0	51.12		1.92
Stan®. 3‡	50.0	50.70		1.50

*Laboratory made tablets (250 mg) of SF.
†Film coated tablets (250 mg).
‡Oral suspension (50 mg ml^{-1}).

The obtained results and statistical parameters for the determination of FA and SF demonstrate that the proposed procedure was suitable for application in a routine control of sodium fusidate in dosage forms.

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