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High-performance liquid chromatographic method for the simultaneous assay of a new synthetic penem molecule and its salt-forming agent in injectable formulations

MARINA FARINA*, GIORGIO FINETTI and VIRGINIO BUSNELLI
Galenical Research and Development, Farmitalia Carlo Erba, Via Carlo Imbonati 24, 20159 Milan (Italy)

SUMMARY

A rapid, stability-indicating, reversed-phase high-performance liquid chromatographic method for the direct and simultaneous determination of a new β-lactam molecule and its salt-forming agent in finished dosage forms was studied for the development of injectable formulations.

INTRODUCTION

threo-*trans*-(*R*)-6-Hydroxyethyl-2-carbamoyloxymethyl-2-penem-3-carboxylic acid, laboratory code FCE 22101 (Fig. 1), is a synthetic β-lactam antibacterial agent[1] belonging to the penem class, with a broad antibacterial spectrum which includes both aerobic and obligate anaerobic species[2–4]. FCE 22101 is designed for parenteral administration in the treatment of infections of the urogenital and upper respiratory tracts.

The injectable formulations of this active drug substance and of all the similar synthetic β-lactam molecules on the market need the presence of a salt-forming agent to allow a rapid reconstitution of the pharmaceutical preparation.

On the basis of its good stability and compatibility with the drug, L-arginine was chosen as the salt-forming agent for FCE 22101; these two components are present in the finished dosage form in the molar ratio 1:1.

The determinations of similar active drug substances and of their salt-forming agents were previously carried out by different time-consuming methods[5]. This study

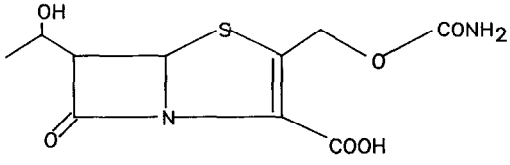

Fig. 1. Structural formula of FCE 22101.

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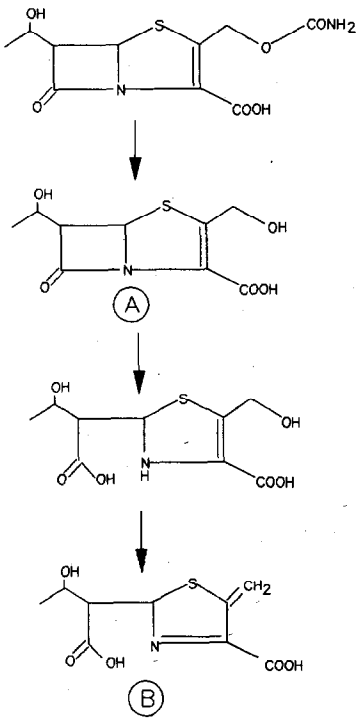
was aimed at setting up a rapid and stability-indicating high-performance liquid chromatographic (HPLC) assay method allowing the simultaneous determination of FCE 22101 and L-arginine.

EXPERIMENTAL

The trials were carried out with a Spectra-Physics Model SP8770 liquid chromatograph equipped with a Whatman PartiSphere C_{18} column (110 × 4.6 mm I.D., average particle size 5 μm), a Rheodyne 7125 injection valve, a Knauer variable-wavelength UV detector and a Spectra-Physics Model SP4270 integrating recorder. The chromatographic column was kept at 25 ± 1°C by means of a water jacket. High-precision glassware was used for all the sampling operations.

The analytical wavelength was set at 206 ± 1 nm, which gave the best results for



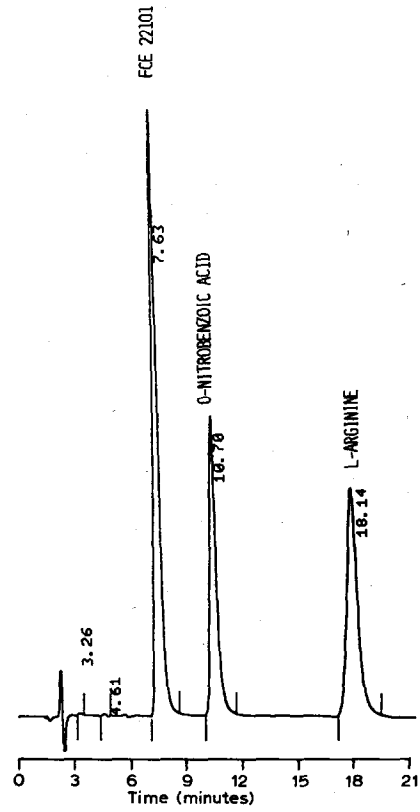


Fig. 2. Degradation pathway of FCE 22101, showing the formation of its more probable degradation products (A and B).

Fig. 3. Typical chromatogram of FCE 22102, L-arginine and *o*-nitrobenzoic acid (internal standard) obtained with a Whatman PartiSphere C_{18} column (110 × 4.6 mm I.D.; average particle size 5 μm) using as the mobile phase phosphate buffer (pH 2.5)–acetonitrile (92.5:7.5, v/v) containing 0.01 *M* heptanesulphonic acid. Analytical wavelength: 206 ± 1 nm.

the statisfactory simultaneous detection of the components of the pharmaceutical preparations and of the most probable degradation products, indicated as products A and B[6], which arise from hydrolytic cleavage of the FCE 22101 molecule (Fig. 2).

FCE 22101 was supplied by Carlo Erba and L-arginine by C.F.M. (USP grade). Acetonitrile was of HPLC grade. All other reagents were analytical-reagent grade.

Phosphate buffer (pH 2.5) was prepared by dissolving 2.722 g of potassium dihydrogenphosphate in distilled water, adjusting the pH to 2.5 with phosphoric acid and diluting to 1000 ml with distilled water.

The mobile phase was phosphate buffer (pH 2.5)–acetonitrile (92.5:7.5, v/v), containing 0.01 *M* sodium heptanesulphonate. The mobile phase flow-rate was 0.7 ml/min and the chart speed 0.5 cm/min.

The integrator attenuation was set at 32 for FCE 22101 and *o*-nitrobenzoic acid and then switched to 8 for L-arginine, during the same run, after about 13–14 min of analysis. For all the analyses a solution containing about 0.1 mg/ml of FCE 22101, about 0.06 mg/ml of L-arginine and 0.2 mg/ml of *o*-nitrobenzoic acid (internal standard) was prepared using as the solvent the HPLC mobile phase. In this way the molar ratio between FCE 22101 and L-arginine was identical with that present in the finished dosage form submitted to HPLC analysis.

Under these conditions, FCE 22101, *o*-nitrobenzoic acid and L-arginine showed retention times of about 8, 10 and 19 min, respectively, and were efficiently separated from the most common related substances present (Fig. 3).

RESULTS AND DISCUSSION

In order to effect the simultaneous elutions of the two component peaks under isocratic conditions, the mobile phase composition was studied and optimized.

Phosphate buffer was chosen as the aqueous component of the mobile phase; the pH for optimum resolution of the compounds under analysis was 2.5. A satisfactory separation was obtained with a mobile phase consisting of a phosphate buffer (pH 2.5)–acetonitrile (92.5:7.5, v/v).

In order to obtain an optimum column retention capacity for L-arginine, an ion-pairing agent was added to the mobile phase, otherwise the retention time of L-arginine would have been very close to the solvent front (Fig. 4). The ion-pairing agents tested were sodium alkanesulphonates salt with a carbon chain length from C_5 to C_8 (Fig. 5). On the basis of the results obtained, sodium heptanesulphonate salt was chosen as it allowed a good separation of L-arginine from the solvent front together with a suitable retention time for FCE 22101. The optimum concentration of this ion-pairing agent was determined from a plot of log k' *versus* sodium heptanesulphonate concentration for the determination of L-arginine (Fig. 6). This plot shows that an increase in ion-pairing agent concentration was followed by a significant increase in the retention time of L-arginine. The best compromise between a good resolution of the L-arginine peak and a practicable analysis time was obtained when sodium heptanesulphonate was added to the mobile phase at a concentration of 0.01 *M*.

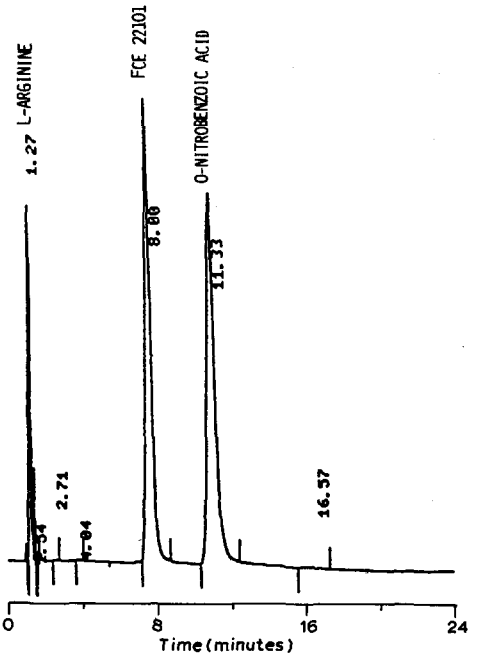


Fig. 4. Typical chromatogram obtained injecting FCE 22102, L-arginine and *o*-nitrobenzoic acid (internal standard) in the absence of the ion-pairing agent.

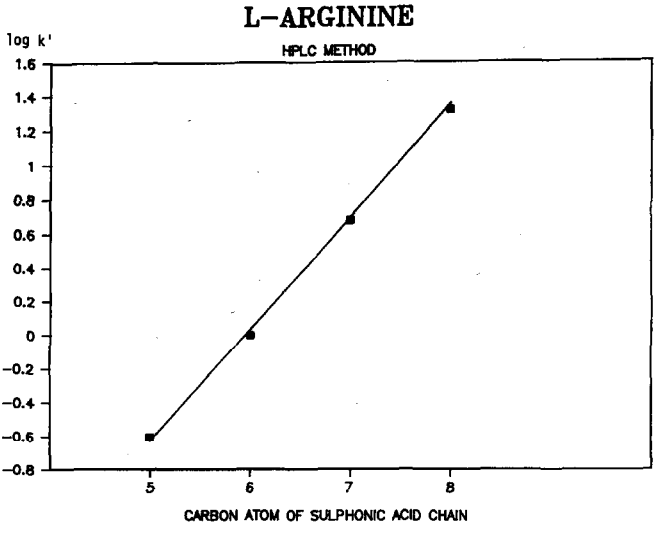


Fig. 5. Choice of the optimum ion-pairing agent: sodium alkanesulphonates with a carbon chain length from C_5 to C_8, at 0.01 *M* concentration.

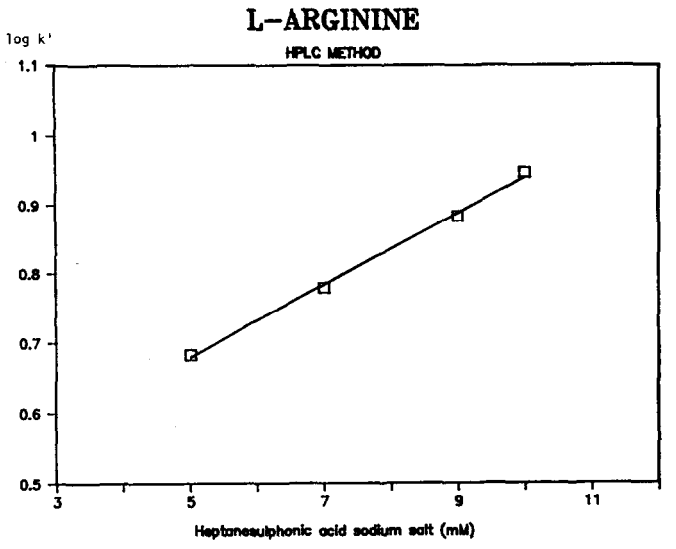


Fig. 6. Determination of the ideal concentration of the ion-pairing agent in the mobile phase.

CONCLUSIONS

The proposed method allowed the simultaneous determination of the active drug substance (FCE 22101), its salt-forming agent L-arginine and the prevalent related substances and degradation products, permitting very detailed stability trials on pharmaceutical preparations during their development to be carried out (Fig. 7),

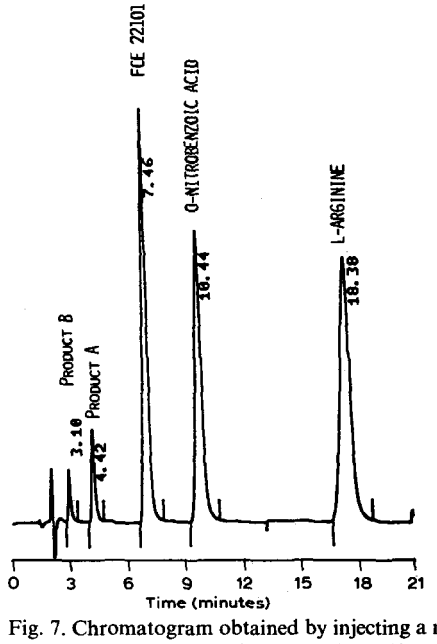


Fig. 7. Chromatogram obtained by injecting a mixture of FCE 22101, L-arginine, *o*-nitrobenzoic acid and the most probable degradation products (A and B) under the optimized analytical conditions.