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Note

Reversed-phase high-pressure liquid chromatography of spiramycin

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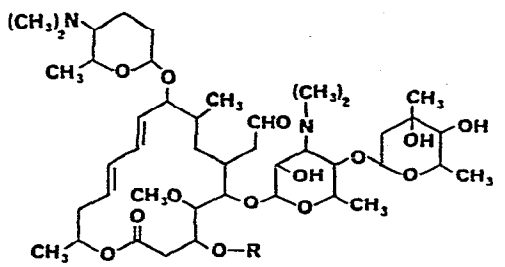
Spiramycin, a macrolide antibiotic, has been isolated from cultures of *Streptomyces ambofaciens*; it consists of three closely related constituents[1]: spiramycin I (63 ± 10%), spiramycin II (24 ± 4%) and spiramycin III (13 ± 5%).



	R
I	—H
II	—COCH$_3$
III	—COCH$_2$CH$_3$

Several methods are available for the determination of spiramycin: microbiological techniques, used directly[2] or after a clean-up procedure by TLC[3], and electrophoresis[4]. Although these methods are very sensitive, they lack selectivity and are thus incapable of differentiating between the three constituents of spiramycin; moreover, they are not suitable for routine analysis. Omura *et al.*[5] have recently examined spiramycin by high-pressure liquid chromatography (HPLC), but they did not separate the three components. This paper describes an HPLC method that provides an efficient analytical separation of the spiramycin components.

EXPERIMENTAL

Chromatographic system

A Varian LC 8500 chromatograph was used, with a variable-wavelength UV detector (operated at 231 nm) and a column (15 cm × 4.7 mm I.D.) of LiChrosorb (particle size 10 μm). The flow-rate of mobile phase was maintained at 100 ml/h. The column pressure was 500 p.s.i.

Chemicals

The reference samples of spiramycin base and spiramycin adipate were a generous gift from SPECIA (Paris, France) and those of spiramycin I, spiramycin

II and spiramycin III from Rhône-Poulenc (Paris). All the solvents were of analytical-reagent grade.

Analytical method

Initially, each of the spiramycins was separately chromatographed to determine its retention time and the order of elution in our chromatographic system. A mixture of the three spiramycins was then prepared to confirm the resolution obtained for the individual peaks. The spiramycins were separated by isocratic elution with concentrated sulphuric acid (1% in water)–acetonitrile (74:26).

RESULTS AND DISCUSSION

Fig. 1A shows a chromatogram of a spiramycin mixture and a suitable internal standard (*p*-nitrophenol). The order of elution is spiramycin I, spiramycin II, *p*-nitrophenol and spiramycin III. Under the experimental conditions indicated,

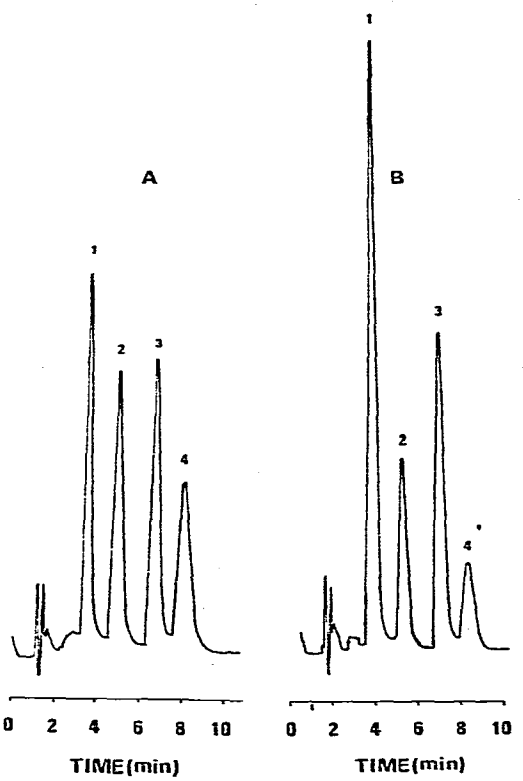


Fig. 1. Chromatogram showing separation of a mixture of spiramycins on a LiChrosorb RP-8 microparticulate column, with isocratic elution. Eluent composition: concentrated sulphuric acid (1% in water)–acetonitrile (76:24); flow-rate, 100 ml/h; detector sensitivity, 0.20 a.u.f.s. A: standard mixture of spiramycin I, spiramycin II and spiramycin III in equal proportions by weight at a concentration of 2 g/l in methanol; B: batch of spiramycin base at a concentration of 2 g/l in methanol. Peaks: 1, spiramycin I; 2, spiramycin II; 3, *p*-nitrophenol (internal standard); 4, spiramycin III.

the retention times are 206, 290, 395 and 470 sec, respectively. Complete separation of the four compounds takes less than 10 min. A typical chromatogram of spiramycin base with its three constituents and the internal standard is shown in Fig. 1B. The quantitative determination of components shows that a batch of spiramycin base contains 58% (w/w) of spiramycin I, 23% (w/w) of spiramycin II and 19% (w/w) of spiramycin III.

The rapid and complete separation of components of the spiramycin mixture makes this procedure practical for quality-control application. The three constituents of spiramycin show quantitative differences in antibacterial activity, so that, as well as quantitative analysis of a batch of spiramycin, the method would also be useful for measuring the period of time during which commercial preparations containing spiramycin as active ingredient may be stored and still remain suitable for use.

We have also ascertained that, in our HPLC system, spiramycin adipate, spiramycin base–adipic acid mixture and spiramycin embonate have the same chromatographic properties and consequently the same retention time as spiramycin base. This method may be applied to the identification or assay of spiramycin in antibiotic formulations, and the use of our internal standard should enable accurate quantitative determinations to be performed.

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We thank SPECIA (Paris) for the generous gift of spiramycin base and spiramycin adipate, and Rhône-Poulenc (Paris) for that of spiramycin I, spiramycin II and spiramycin III.

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