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Short Communication

Determination of teicoplanin in plasma using microbore high-performance liquid chromatography and injection-generated gradients

R. B. TAYLOR* and R. G. REID

Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 1FR (U.K.)

and

I. M. GOULD

Aberdeen City Hospital, Urquhart Road, Aberdeen (U.K.)

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ABSTRACT

A reversed-phase isocratic high-performance liquid chromatographic method for the determination of total teicoplanin in plasma is reported. The method developed uses a bracketing injection technique in conjunction with large injection volumes on a 1 mm diameter column to form a limited injection-generated gradient. The chromatography yields adequate resolution among all the major components for individual quantitation and also allows quantitation of total teicoplanin in plasma using ultraviolet detection. Pre-treatment is by solid-phase extraction which uses C_8 Bond Elut cartridges and gives effective clean up from endogenous materials. The method offers a faster and simplified means to determine total teicoplanin in plasma than those previously reported, and has a detection limit of 50 ng/ml.

INTRODUCTION

Teicoplanin is a relatively new glycopeptide antibiotic developed in 1984 and is a complex of closely related molecular species, each consisting of a linear heptapeptide with two interconnected chlorinated hydroxytyrosine units, five substituted phenyl-glycine systems and an acyl-glucosamine unit. Each of the six major components of the complex has differing acyl units (see Fig. 1).

Teicoplanin, because of its complex nature, has proved difficult to assay. A bioassay method [1] and a solid-phase receptor assay [2] were developed but these methods have proved to be rather slow and temperamental [1]. They also suffer the limitation of being unable to distinguish among individual components. Chromatographic methods have been reported using UV detection but are unsatisfactory with regard to resolution of the various components and in terms of

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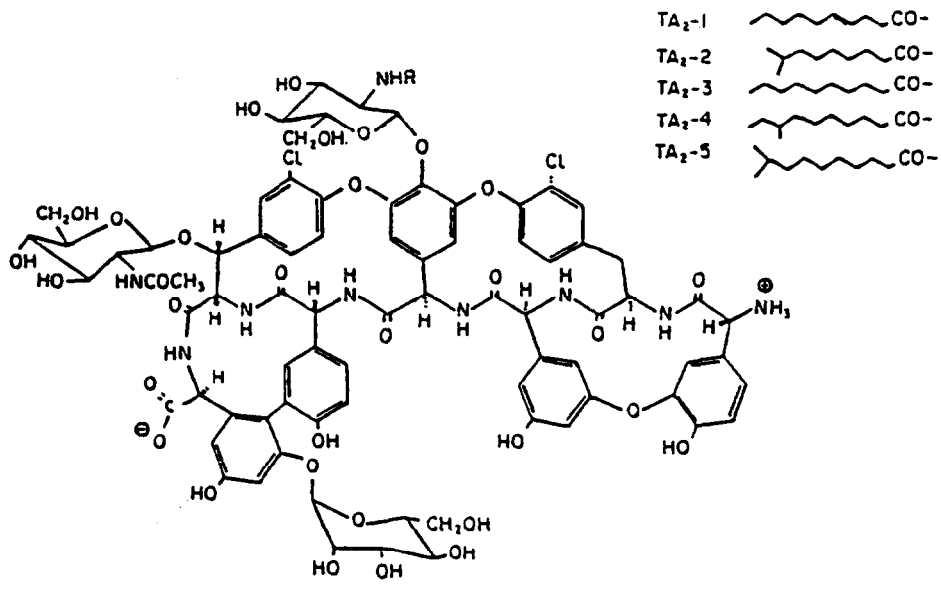

Fig. 1. Structure of the teicoplanin complex.

detection limits [3]. The development of a high-performance liquid chromatographic (HPLC) gradient elution technique [4,5] significantly increased the resolution of the components and allowed other minor constituents of the complex to be observed. A recent method has been reported [6] whereby, following derivatisation of teicoplanin with fluorescamine, the components are separated using an isocratic separation incorporating fluorescence detection.

There are problems associated with these methods. The gradient elution methods have difficulty in achieving adequate sensitivity for biological determinations and also suffer from re-equilibration delays between samples. The fluorescence method, while having a significantly lower detection limit, requires lengthy pretreatment stages and one of the major components of the teicoplanin complex TA_3-1 is not observed. The limitations of current teicoplanin assay methods are demonstrated by a recent report which uses only a single component TA_2-2 as being characteristic of the total antibiotic [7].

It is the purpose of the present work to improve on current assay techniques for teicoplanin and to develop an assay applicable to both routine therapeutic monitoring and also capable of determining all major individual components by using microbore columns in conjunction with a large injection volume to form an injection-generated gradient. Recent work has shown that large volumes can be injected on any columns if the sample has been injected in what has been termed a "non-eluting solvent" [8,9]. This can lead to a decrease in detection limits.

It has been shown recently that by selectively reducing the column diameter significant decreases in detection limits can be achieved [10]. By using a combina-

tion of large injection volumes and narrow-bore columns it has been shown that a fourteen-fold increase in sensitivity can be achieved [11].

EXPERIMENTAL

The chromatographic system used comprised a Varian Assoc. (Walnut Creek, CA, U.S.A.) Model 2510 pump and a Varian Assoc. Model 2550 variable-wavelength detector (210 nm) fitted with an 8 μl flow-cell. The injection was by a Rheodyne (Cotati, CA, U.S.A.) Model 7125 valve, fitted with loops of different volume as required. The detector was directly interfaced with a Jones Chromatography (Hengoed, U.K.) JCL6000 chromatography data system.

Materials to prepare and pack columns were obtained from HETP (Macclesfield, U.K.) as was the Hypersil ODS (5 μm) stationary phase. Bond Elut cartridges (C_8 and C_{18}) used for plasma extractions were obtained from Analytichem (Harbor City, CA, U.S.A.). Methanol and acetonitrile were supplied by Rathburn (Walkerburn, U.K.). Water was specially purified by a Millipore (Harrow, U.K.) Milli-Q system. The sodium dihydrogen orthophosphate, isopropanol and sodium hydroxide were obtained from Fisons (Loughborough, U.K.).

A 100 mm × 1 mm microbore column was prepared by sandwiching a 1.6 mm diameter 2 μm porous stainless-steel gauze between the 1 mm I.D. 1.6 mm O.D. unpolished stainless-steel tubing and the column end. The columns were slurry packed at a constant pressure of 400 bar using isopropanol as slurrying solvent. At optimum conditions of flow-rate and injection volume the measured efficiency was in the range 5000–6000 plates per column.

Chromatography

The extraction method described below, which was required for adequate recovery of the antibiotic from plasma, resulted in a solution of the drug in a solvent comprising acetonitrile–pH 6 buffer (20:80, v/v). This precludes the direct injection of large volumes of the extracted antibiotic solution and the on-column preconcentration approach was modifed to a bracketing technique [12].

A teicoplanin extract obtained from the extraction method described below was injected (50 μl) into a 500-μl loop prefilled with water. A further 200 μl of water were subsequently injected before operating the valve. The resultant chromatogram was identical with that obtained by injecting in 500 μl of water and is shown in Fig. 2A. This bracketing technique did not adversely affect the reproducibility of retention and the precision (relative standard deviation) of retention times for the different individual peaks is approximately 6% ($n=8$).

Extraction method

From preliminary investigations it was found that teicoplanin was adsorbed onto C_8 and C_{18} Bond Elut cartridges but that, after washing with water, methanol or acetonitrile, little teicoplanin was recovered from the cartridge. It was

found, however, that a solvent of acetonitrile–pH 6 buffer (20:80, v/v) eluted the teicoplanin complex and that recovery was greater from C_8 than from C_{18}. The recovery of the extraction procedure for individual components was determined by comparison of peak heights before and after solid-phase extraction, and a value of 98% was obtained for component TA_3-1. This decreased for the longer retained components and only 60% of the TA_2-5 component was recovered.

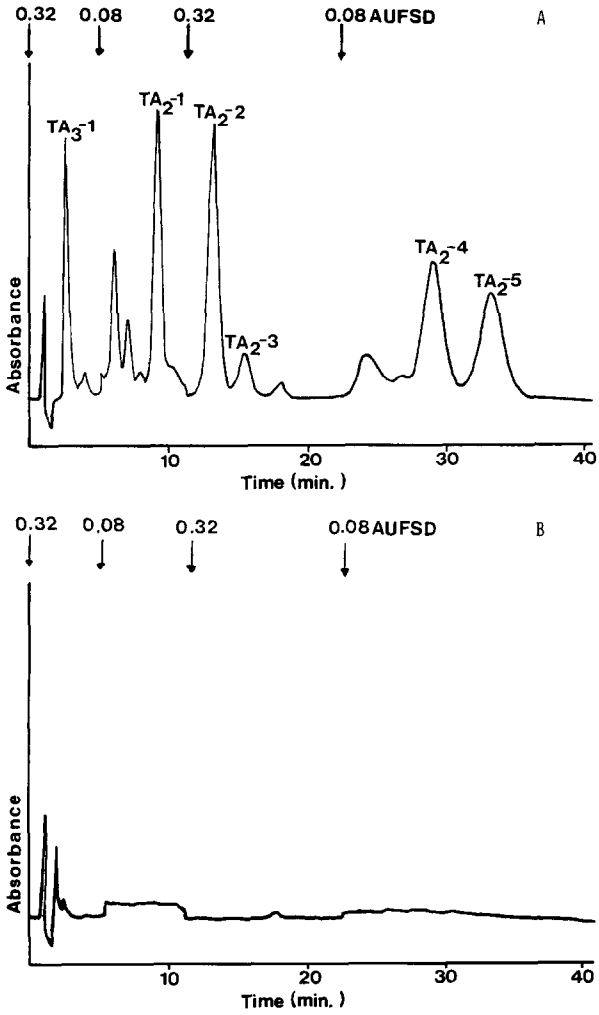

Fig. 2. Representative chromatograms of a teicoplanin plasma standard containing 50 μg/ml (A) and a plasma blank (B). Column, 100 mm × 1 mm, Hypersil ODS, 5 μm; solvent, 20% acetonitrile in 25 m*M* sodium dihydrogen orthophosphate, pH 6.0; flow-rate, 50 μl/min; injection volume, 500 μl; wavelength, 210 nm; sensitivity, 0.32 a.u.f.s.d. for 5 min, reduced to 0.08 a.u.f.s.d. for elution of TA_2-1 and other minor components, returned to 0.32 a.u.f.s.d. for elution of TA_2-2 and TA_2-3, reduced to 0.08 a.u.f.s.d. for the remainder of the chromatogram.

The reproducibility of the extraction and quantitation of total teicoplanin was determined by carrying out eight replicate assays using a single calibration line and a single spiked plasma sample. A relative standard deviation of 3.05% was obtained at a total teicoplanin concentration of 42.4 μg/ml, this being the approximate mean of peak and trough levels found in patient samples.

The effectiveness of the removal of endogenous material is shown in Fig. 2B by the chromatogram of blank plasma, subjected to the above pretreatment.

Quantitation using spiked plasma

Standards were prepared over the range 5–50 μg/ml teicoplaninin in blank plasma since this was the anticipated range to be encountered clinically. These samples were extracted and chromatographed as above and peak heights and areas measured using the JCL6000 data collection system. In order to measure all peaks on scale, changes in the absorbance ranges were required during the chromatography. Higher absorbance ranges were required for components TA_3-1 and TA_2-2. Quantitation of individual compounds was not possible due to lack of pure standard materials but plots of peak height and area for these individual components showed good linearity with their relative concentrations.

Total teicoplanin was established by summing either the peak heights or areas for the six main components, and the regression paramaters for the resulting calibration curves are shown in Table I. From Table I can be seen that good linearity is obtained for both methods of peak quantitation as observed by the excellent r^2 values achieved. The slope of the calibration line for total teicoplanin was found to have a relative standard deviation of 12.4% ($n = 5$) over a period of two months.

The method described is capable of measuring total teicoplanin in plasma with a detection limit of 50 ng/ml taking as a criterion a signal-to-noise ratio of 3 for component TA_2-2.

Quantitation using patient samples

Plasma samples were obtained from patients undergoing treatment with teicoplanin. Dosage was 400 mg intravenously once daily. Samples of plasma were removed prior to dosing to provide trough values and at 45 min post dosing to

TABLE I

RESULTS OBTAINED BY REGRESSING BOTH TOTAL PEAK HEIGHT AND AREA AGAINST CONCENTRATION OF TEICOPLANIN IN PLASMA

	Slope	Intercept	r^2
Peak height	232.7	49	0.9998
Peak area	519.9	357	0.9989

TABLE II

RESULTS OBTAINED FROM PATIENT SAMPLES UNDERGOING TREATMENT WITH TEICOPLANIN

Sample No.	Sample type	Teicoplanin concentration (μg/ml)
1A	Trough	15.4
1B	Peak	68.3
2A	Trough	16.0
2B	Peak	69.2
3A	Trough	16.8
3B	Peak	79.1
4A	Trough	11.1
4B	Peak	48.0

provide peak values. Four such pairs of values were assayed by the above method.

These results show that the method is capable of distinguishing the variation of teicoplanin concentration over the plasma range encountered following recommended therapeutic dosing (Table II).

This method offers a faster and simplified means to determine total teicoplanin in plasma. The detection limits are comparable with those quoted in the fluorescence method [6]. It also allows quantitation of component TA_3-1 which is a fairly major component. Finally the sample pretreatment is much reduced. The method of using 1 mm diameter columns offers several advantages over the conventional gradient methods published [3,4,13]. As demonstrated above sensitivity is increased. Also, a drifting baseline, which is a problem associated with the gradient elution method, is not observed with this injection technique. In addition, because no time is required for column re-equilibration between samples, the bracketing technique requires little more time per analysis than chromatography under standard isocratic conditions.

Teicoplanin has just became available for routine clinical use in the U.K. It is essential that assay facilities are available to ensure adequate therapeutic levels are achieved in seriously ill patients and perhaps also to monitor peak levels to limit toxicity. In addition, further work is needed to investigate the different components separable by HPLC, particularly their respective accumulation at different body sites and in renal failure and the therapeutic and toxic implications of this. Application of this new method should make this task easier.

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