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Journal of Chromatography B, 730 (1999) 55–59

JOURNAL OF CHROMATOGRAPHY B

Simple and sensitive high-performance liquid chromatographic method for the determination of an everninomycin, SCH 27899, in rat plasma

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Received 19 January 1999; received in revised form 6 April 1999; accepted 13 April 1999

Abstract

A simple and sensitive high-performance liquid chromatographic (HPLC) method was developed for the determination of SCH 27899, an everninomycin antibiotic, in rat plasma. The method involved plasma protein precipation with acetonitrile, followed by reversed-phase HPLC analysis using a polymeric column and a mobile phase containing acetonitrile and ammonium phosphate, pH 7.8. The linear relationship between detector response and concentration was demonstrated with a correlation coefficient of larger than 0.996 at concentrations ranging from 0.2 to 100 μg/ml. The results showed that the HPLC method was accurate (bias ≤6%) and precise (coefficient of variation, C.V.≤6%). The limit of quantitation was 0.2 μg/ml with a C.V. of 2.6% and bias of 5%. SCH 27899 was stable in rat plasma at −20°C for at least 40 days. The HPLC method has been utilized for the determination of SCH 27899 in plasma samples from rats following single intravenous administration (3 mg/kg).

Keywords: Everninomycin

1. Introduction

SCH 27899, (Fig. 1), is a novel oligosaccharide compound of the everninomycin class. It exhibits greater antibacterial activity against *Streptococcus* spp. and *Streptococcus pneumoniae* than clinafloxacin, teicoplanin and vancomycin [1]. SCH 27899 also shows excellent activity (MIC_{90}, 0.25 μg/ml) against the fluoroquinolone-resistant strains and all gram-positive strains resistant to vancomycin (MICs≤4 μg/ml) [1]. These in vitro data indicate that SCH 27899 could be useful against emerging gram-positive strains resistant to other contemporary antimicrobial agents.

Vancomycin has emerged as the dominant alternative therapy for serious infections caused by oxacillin-resistant staphylococci and any gram-positive infection observed in a patient with intolerance to β-lactams or macrolides. Furthermore, enterococci that are resistant to penicillins are often treated with vancomycin [2–7]. However this choice has been recently comprized by the rapidly increasing development of vancomycin-resistance among enterococci [8]. Therefore, it is important that new compounds, such as SCH 27899, be developed.

This report describes a high-performance liquid chromatographic (HPLC) method for the determi-

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0378-4347/99/$ – see front matter
PII: S0378-4347(99)00204-2

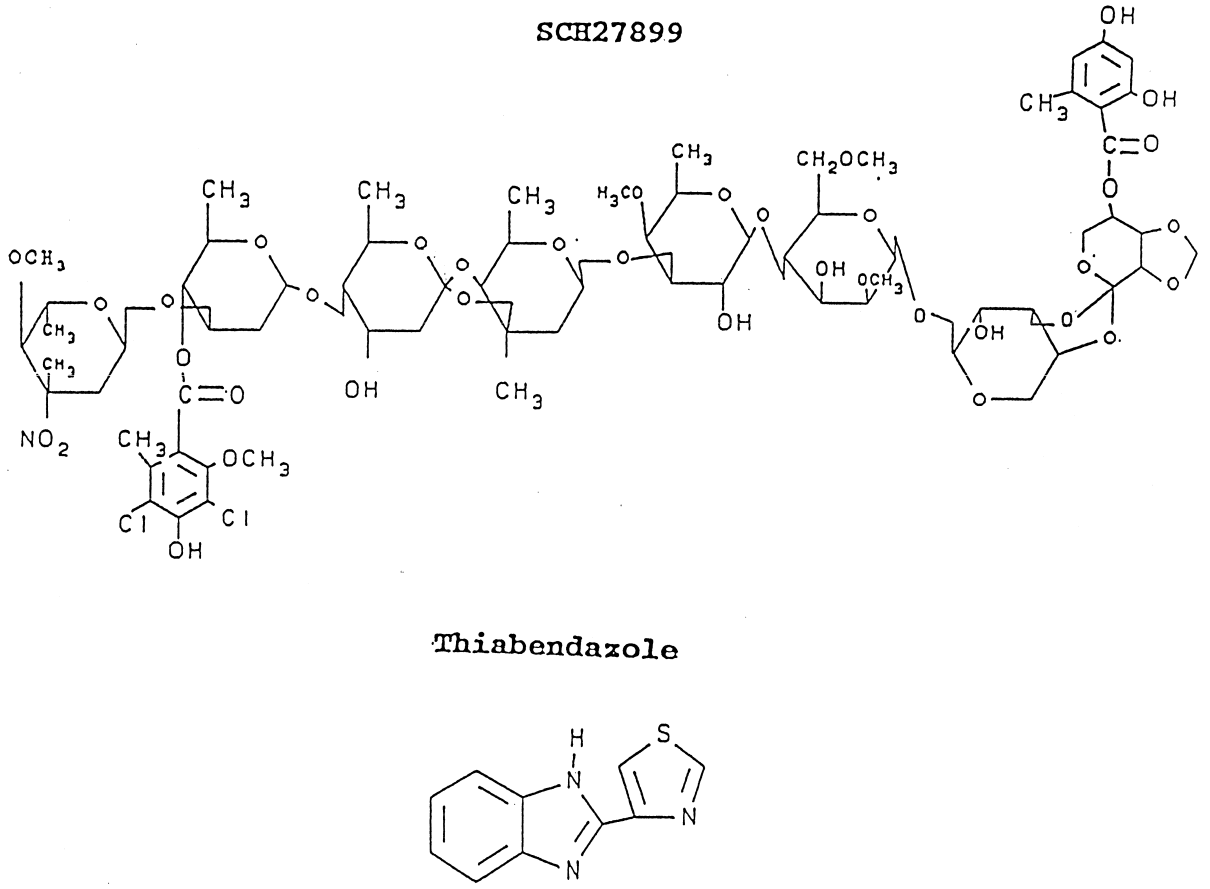

Fig. 1. Chemical structures of SCH 27899 and internal standard (thiabendazole).

nation of SCH 27899 in rat plasma. This assay was then validated in rat plasma and used in pharmacokinetic study in rats.

2. Experimental

2.1. Chemicals

SCH 27899 was supplied by Schering-Plough (Kenilworth, NJ, USA). Thiabendazole (internal standard) was obtained from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade), ammonium hydroxide (29%, ACS grade), ammonium phosphate (HPLC grade), methanol (HPLC grade), orthophosphoric acid (85%, HPLC grade) and water (HPLC grade) were obtained from Fisher Scientific (Pittsburg, PA, USA).

2.2. Drug administration and plasma sample collection

Male Charles River rats (weighing 121–182 g) received an intravenous bolus dose (3 mg/kg) of SCH 27899 as an intravenous solution of SCH 27899: NMG (*N*-methylglucmino): HpβCD (hydroxypropyl-β-cyclodextrin) at a molar ratio of 1:3:5. Blood samples were obtained at 5, 15, 30 and 45 min and also at 1, 2, and 3 h post-dose into heparinized tubes by cardiac puncture following anesthesia with ketamine. Plasma samples were obtained following centrifugation at 4°C and stored at −20°C.

2.3. Sample preparation

To 200-μl aliquot of rat plasma were added 50 μl of internal standard (0.2 μg per ml of methanol), 50 μl of 50% acetonitrile–water and 1000 μl of acetonitrile. The mixture was vortex-mixed for 20 min and then centrifuged (3000 rpm) for 10 min. The organic layer was transferred to another tube and evaporated to dryness at 50°C. The residue was reconstituted in 400 μl of the HPLC mobile phase (described in Section 2.4.), and a 100-μl aliquot of the mixture was injected onto the HPLC.

2.4. Chromatography

The HPLC system consisted of a Tosoh Model TSK-6011 pump (Novex, San Diego, CA, USA), a Model AS8020 autosampler and a Model TSK-6041 tunable ultraviolet absorbance detector set a wavelength of 300 nm. Separation was accomplished on a Hamilton 75 A poly(styrene-divinyl benzene), PRP-1 column (5 μm, 150×4.1 mm, Hamilton Reno, NV, USA). The absorbance detector output was monitored with a Model 3392-A integrator (Hewlett-Packard, Roseville, CA, USA). The mobile phase consisted of acetonitrile, 0.2 *M* ammonium phosphate, and water (50:5:45, v/v/v). It was then adjusted to pH 7.8 with concentrated ammonium hydroxide and was delivered at 0.55 ml/min. All separations were carried out at ambient temperature.

2.5. Method evaluation

The precision (%C.V.) and accuracy (%bias) were calculated from the back-calculated concentrations of nine standard curves prepared in plasma. The lowest limit of quantitation was established as the lowest concentration in the standard curve where the %C.V. (nine replicates) and bias from the back-calculated concentrations were ≤20%. The specificity of the assay was established by the lack of interference peaks at the retention time of the internal standard and SCH 27899. The recovery of internal standard and SCH 27899 was determined using a standard curve set up in a mixture of water–acetonitrile (50:50, v/v). Stability of SCH 27899 (0.4, 40 and 80 μg/ml) was determined for 2 h at 23°C, at the end of three freeze–thaw cycles and 40 days after storage at −20°C.

3. Results

A typical chromatogram for SCH 27899 and internal standard (thiabendazole) extracted from rat plasma is shown in Fig. 2. The retention time was 7.4 min for SCH 27899 and 5.0 min for thiaben-

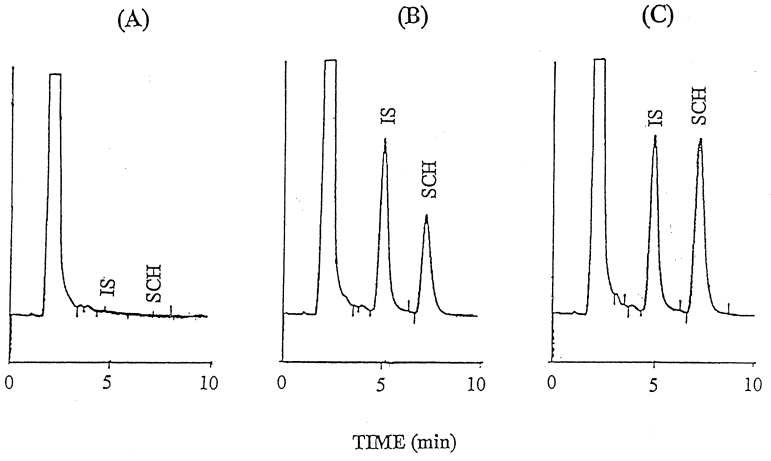

Fig. 2. Typical chromatograms of SCH 27899 (SCH) and internal standard (I.S.) in: (A) blank rat plasma; (B) blank rat plasma spiked with SCH 27899 (5 μg/ml) and internal standard (0.5 μg/ml); and (C) blank rat plasma spiked with SCH 27899 (10 μg/ml) and internal standard (0.5 μg/ml).

Table 1
Precision and accuracy of SCH 27899 analysis

	Concentration added (μg/ml)	Concentration found (mean±SD)[a]	C.V. (%)	Bias (%)
Intra-day	0.4	0.40±0.031	7.8	0
	40	39.5±2.11	5.3	−1.3
	85	81.0±3.41	4.2	−4.7
Inter-day	0.4	0.42±0.031	7.4	5.0
	40	40.6±2.63	6.5	1.5
	85	83.0±5.09	6.1	−2.4

[a] $n=3$ for intra-day analysis and $n=9$ for inter-day analysis.

dazole. The standard curve was obtained by plotting the ratio of peak height of SCH 27899 to that of internal standard against the concentration (0.2–100 μg/ml) of SCH 27899 added ($y=0.10863x+0.00216$). There was a linear relationship between the peak-height ratio and the plasma concentration, with a correlation coefficient of 0.9996. The limit of quantitation was 0.2 μg/ml, with a small coefficient of variation (2.6%). There were no interfering peaks in blank plasma at the retention time of either SCH 27899 or its internal standard, indicating that the HPLC analysis for SCH 27899 was selective.

Intra- and inter-day precision and accuracy of the method was validated at 0.4, 40 and 85 μg/ml. The results (Table 1) demonstrated that HPLC method was accurate (bias≤5.0%) and reproducible (C.V.≤7.8%).

The recovery of SCH 27899 (0.4, 40 or 85 μg/ml) and internal standard (2 μg/ml) was determined to be more than 90% with C.V. ranging from 11 to 14% (Table 2). SCH 27899 was found to be stable at 23°C for 2 h, −20°C for 40 days and after three freeze–thaw cycles (Table 3).

The method was used to analyze plasma samples from rats which received an intravenous dose of 3 mg of SCH 27899/kg. The mean plasma concentration–time curve for SCH 27899 is shown in Fig. 3. The mean plasma concentration of SCH 27899 was 16.4 μg/ml at 5 min and decreased rapidly thereafter. At 3 h, the plasma concentration was 0.41 μg/ml in one rat and below the limit of quantitation in two other rats.

Table 2
Recovery of SCH 27899 and thiabendazole

Compound	Concentration added (μg/ml)	Recovery (mean±SD, $n=9$) (%)	C.V. (%)
SCH 27899	0.4	99.1±10.6	10.7
	40	94.2±11.5	12.2
Thiabendazole	85	93.4±12.6	13.5
	2	97.8±6.56	6.7

4. Discussion

SCH 27899 is an oligosaccharide compound of the everninomycin class [9,10]. It contains a phenol side chain and a dihydroxy benzoate and has a $pK_a \geq 9.0$. We have previously developed a reversed-phase ion-pair chromatographic method for SCH 27899 using tetramethylammonium hydroxide as the ion pairing

Table 3
Stability of SCH 27899 in rat plasma

Time and condition of storage	Concentration added (μg/ml)	Concentration found (Mean±SD, $n=3$)	C.V. (%)
Day 0	0.4	0.40±0.031	7.8
	40	39.5±2.11	5.3
	85	81.0±3.41	4.2
23°C, 2 h	0.4	0.42±0.023	5.5
	40	39.1±0.200	0.5
	85	86.7±4.69	5.4
−20°C day 40	0.4	0.41±0.021	5.1
	40	40.0±1.33	3.3
	85	85.5±3.01	3.5
End of three freeze–thaw cycles	0.4	0.38±0.010	2.6
	40	39.6±1.28	3.2
	85	80.0±1.23	1.5

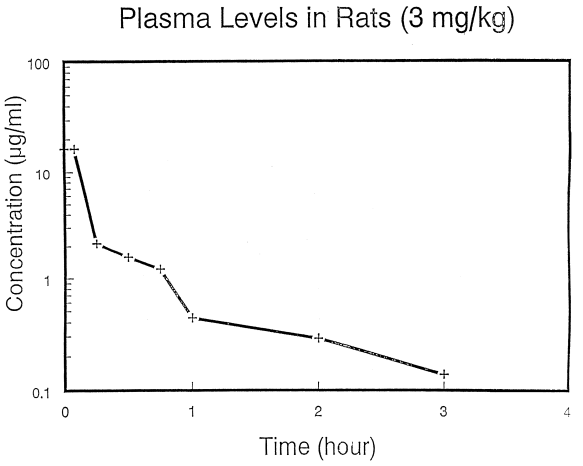

Fig. 3. Plasma concentration–time profile of SCH 27899 in rats following intravenous administration (3 mg/kg); *y*-axis represents the concentration (μg/ml); *x*-axis represents time (h).

reagent. However the method required extensive and tedious plasma sample preparation prior to injection into the HPLC system.

The HPLC method reported in this paper represents a breakthrough for the analysis of SCH 27899 in biological fluids. Instead of reversed-phase ion-pair chromatography, a simple reversed-phase HPLC method was developed using a mixture of acetonitrile (50%), 0.2 *M* ammonium phosphate, pH 7.8 (5%) and water (45%) as the mobile phase. This was achieved by using a polymeric HPLC column [poly-(styrene-divinyl benzene)] with an unique stability to pH ranging from pH 1 to 13. This property contrasts with the silica-based columns used in most reversed-phase HPLC which are intolerable to mobile phase with pH≥7.6 and/or a high percentage of aqueous buffer. The present HPLC method which involves protein precipitation with 1 ml of acetonitrile, followed by HPLC analysis is simple and rapid.

The results demonstrate that the HPLC method is a simple, rapid and sensitive assay technique for determination of SCH 27899 in rat plasma. The utility of this new reproducible and accurate HPLC method was demonstrated in the evaluation of the pharmacokinetics of the drug in rats following an intravenous dose of 3 mg/kg. Based on this HPLC assay in rat plasma, assays in mouse, cynomolgus monkey and human plasma were validated and successfully utilized in the pharmacokinetic evaluation of SCH 27899.

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