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Development and validation of a liquid chromatographic method for the analysis of capreomycin sulfate and its related substances

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ABSTRACT

A gradient LC method for the analysis of capreomycin sulfate and its related substances was developed. The chromatographic conditions include the use of a Hypersil base deactivated C_{18} (250 mm × 4.6 mm, 5 μm) column maintained at 25 °C, a mobile phase containing acetonitrile, phosphate buffer pH 2.3 and 0.025 M hexanesulfonate at a flow rate of 1.0 mL/min and UV detection performed at 268 nm. Good separation of the four active components of capreomycin and eleven unknown impurities was achieved. A system suitability test to check the quality of the separation is specified. The method shows good repeatability, linearity and robustness.

1. Introduction

Capreomycin is a polypeptide antibiotic, commonly grouped with the aminoglycosides because of its actions on bacterial ribosomes [1]. The four active components of capreomycin (IA, IB, IIA and IIB) have been first isolated from *Streptomyces capreolus* in the 1960s [2]. The components capreomycin IA, IB, IIA and IIB are distributed in the approximate percentages of 25%, 67%, 3% and 6%, respectively [3]. As shown in Fig. 1, the structures of capreomycin I and II differ in R_2, which is β-lysine in capreomycin I and an amine in capreomycin II. The forms A and B of both capreomycins I and II differ in R_1, which is a hydroxyl in form A and hydrogen in form B.

Capreomycin sulfate is the disulfate salt of capreomycin, suitable for parenteral use [4,5] or aerosol administration (liposome formulations) [6,7]. It is a second-line antituberculosis drug used for the treatment of multidrug-resistant tuberculosis (MDR-TB). Capreomycin sulfate is used in combination with other antituberculosis drugs when resistance to the first-line drugs such as isoniazid, ethambutol and p-aminosalicylic acid, is developed. A sensitive CE method has been reported for the simultaneous determination of capreomycin, ofloxacin and pasiniazide in urine [8]. For the determination of the capreomycin content, a normal-phase liquid chromatographic (NP-LC) method has been described in the British Pharmacopoeia (BP) [4] and the United States Pharmacopeia (USP) [5]. UV spectrophotometry and reversed-phase liquid chromatography (RP-LC) have been proposed as valid alternative methods for capreomycin assay in liposome formulations [7], whereas for the related substance analysis of capreomycin, thin layer chromatography (TLC) has been applied [9]. It is important to note that the presence of impurities causes a difference in the safety and pharmacokinetic profiles of the drug. Their removal decreases the toxicity without affecting the efficacy of capreomycin [9]. So far, no LC methods have been reported for the determination of related substances of capreomycin sulfate. Therefore, the aim of the present work was to develop and validate a LC method for the separation of capreomycin sulfate from its related substances. Since capreomycin is frequently used in developing countries with limited capabilities, the method should not be unnecessarily complicated.

2. Experimental

2.1. Reagents and samples

Sodium 1-hexanesulfonate (HSA), phosphoric acid and potassium phosphate were purchased from Acros Organics (Geel, Belgium), sodium hydroxide from J.T. Baker (Deventer, Holland), hydrochloric acid from Chem-Lab (Zedelgem, Belgium) and hydrogen peroxide from Merck (Darmstadt, Germany). LC gradient grade acetonitrile (ACN) was purchased from Fisher Scientific (Leicester, UK). Capreomycin sulfate samples were obtained from Macleods Pharmaceuticals Limited (Daman, India) and the WHO (World Health Organization, Geneva, Switzerland). A concentration of 2 mg/mL was chosen for method development so as to detect small amounts of impurities as much as possible. The sample solution is

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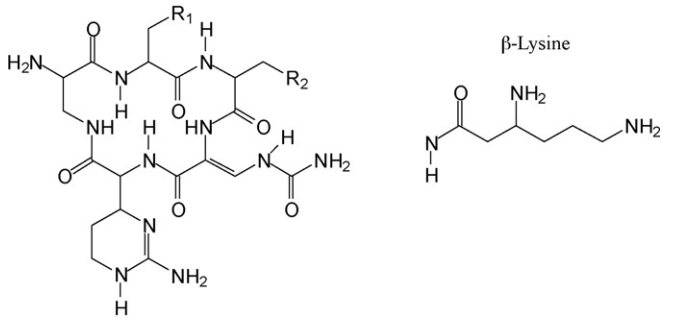

Component	R_1	R_2
Capreomycin IA	OH	β-Lysine
Capreomycin IB	H	β-Lysine
Capreomycin IIA	OH	NH_2
Capreomycin IIB	H	NH_2

Fig. 1. Chemical structure of the components of capreomycin.

stable for 14 days in the refrigerator or for 48 h at room temperature. The relative content of capreomycin IA, IB, IIA and IIB (25%, 67%, 3% and 6%) [3] corresponding to the peak areas was used to identify the four main components. The buffers and sample solutions were prepared with ultrapure Milli-Q water (Millipore, Milford, MA, USA).

2.2. LC instrumentation and chromatographic conditions

The LC LaChrom Elite apparatus (Merck Hitachi, Darmstadt, Germany) consisted of an L-2130 pump, an L-2200 autosampler (20 μL injection volume), an L-2450 diode array detector (DAD) set at 268 nm and EZChrom Elite 4.0 software for data acquisition. In the final method, a Hypersil BDS C18 column (250 mm × 4.6 mm I.D.), 5 μm (Thermo Electron Corporation, Waltham, USA), maintained at 25 °C in a water bath by means of a Julabo ED thermostat (Julabo, Seelbach, Germany), was used. The mobile phases consisted of ACN-phosphate buffer pH 2.3 with 0.025 M HSA, (A) (5:95, v/v) and (B) (15:85, v/v). The phosphate buffer pH 2.3 containing 0.025 M HSA was prepared by dissolving 54.4 g of KH_2PO_4 in about 1.5 L Milli-Q water and the buffer pH was adjusted to 2.3 using 1 M phosphoric acid. To this solution, 9.4 g of HSA was added and the volume was made up to 2 L. The mobile phases were degassed by sparging helium. Gradient elution was performed at a flow rate of 1.0 mL/min (see Table 1). pH measurements were performed on a Metrohm 691 pH meter (Herisau, Switzerland). To ensure consistent results, it was calibrated before each measurement with reference buffer solutions as prescribed in [10].

2.3. Forced degradation studies

The drug was subjected to forced degradation under acidic, basic and neutral conditions by heating in a boiling water bath in 0.1 M HCl, 0.1 M NaOH and water, respectively. Oxidative stress studies were carried out at room temperature for 2 h in 3% H_2O_2. For thermal stress, the drug was kept in an oven at 100 °C for 24 h. In all conditions, the drug concentration was 2 mg/mL.

Table 1
Gradient elution programme applied.

Time (min)	Mobile phase A (%, v/v)	Mobile phase B (%, v/v)	Comments
0–25	55–52	45–48	Linear gradient
25–40	52	48	Isocratic
40–60	30	70	Isocratic
60–70	55	45	Re-equilibration

2.4. Experimental design

A robustness study was performed by means of an experimental design and multivariate analysis using Modde 5.0 software (Umetrics AB, Umeå, Sweden). A central composite face centered (CCF) design was applied. The CCF design permits the response surface to be modeled by fitting a second order polynomial model. In particular, the CCF consists of points of a two level full factorial design (2^k), which were augmented with ($2k+n$) star points to enable this model to estimate the response curvature plot. The star points are located at the center and both extreme levels of the experimental domain. For a complete central composite design, the number of runs is equal to 2^k+2k+n, where k is the number of parameters and n is the number of centre points. In this study, five chromatographic parameters (buffer pH, concentration of HSA, acetonitrile content in mobile phase B and percentage of mobile phase B at start and end of linear gradient) were investigated. With this number of parameters and three centre points, a complete central composite design would result in a number of runs equal to 45. In order to reduce the number, a central composite design which includes points of a two level half fractional factorial design was chosen, with a number of runs equal to $2^{k-1}+2k+n=29$. These experiments were performed in duplicate and the average retention times and peak widths were used to calculate the resolution between peaks [10]. For an experimental design with five factors, the model including linear, quadratic and cross terms can be expressed as

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{25}x_2x_5 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{45}x_4x_5 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{55}x_5^2 + E$$

where b are the regression coefficients and E is the overall experimental error. The square term of each variable describes the nonlinear effect on the response and the cross term of two different variables the effect of their interaction.

3. Results and discussion

3.1. Method development

Taking into account the better selectivity of the RP-LC method proposed for capreomycin assay [7] compared to NP-LC [4,5], it was chosen as the starting point for the present LC method development. The RP-LC method used a Phenomenex C18 (150 mm × 2 mm I.D., 3 μm) column maintained at 25 °C, UV detection at 268 nm and an isocratic mobile phase consisting of a mixture of ACN–0.2 M KH_2PO_4 buffer pH 2.3 with 0.3% heptafluorobutyric acid (10:90, v/v) at a flow rate of 0.2 mL/min. For the purpose of the universality of the LC method, which can be used in countries all over the world, a more common Hypersil BDS C18 (250 mm × 4.6 mm I.D., 5 μm) column was used to replace the Phenomenex C18 column (150 mm × 2 mm I.D., 3 μm). Accordingly, a flow rate of 1 mL/min was used with a run time of 25 min. Unfortunately, capreomycin II A was co-eluted with an unknown impurity peak and capreomycin IIB was poorly separated from capreomycin IA.

Further method development was performed by changing other chromatographic conditions such as the type and concentration of ion-pairing agents, buffer pH and column temperature. Some sulfonate ion-pairing agents with different alkyl chain lengths