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# DETERMINATION OF STREPTOMYCIN IN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

NAHOKO KUROSAWA, SHOICHI KURIBAYASHI, EIJI OWADA and KEIJI ITO*

*Hokkaido Institute of Pharmaceutical Sciences, 7-1 Katsuraoka-cho, Otaru, Hokkaido 047-02 (Japan)*

and

MASANORI NIOKA, MASAKO ARAKAWA and RYOHEI FUKUDA

*Departments of Pharmacy and Internal Medicine, Otaru Municipal Hospital, 1-2-1 Wakamatsu, Otaru, Hokkaido 047 (Japan)*

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## SUMMARY

A high-performance liquid chromatographic method was developed for monitoring the serum concentration of streptomycin. The method includes clean-up using a Sep-Pak $C_{18}$ cartridge and quantitation using dihydrostreptomycin as an internal standard. Streptomycin and dihydrostreptomycin were separated by reversed-phase ion-pair chromatography on LiChrosorb RP-18 and detected by UV absorption (195 nm). The calibration graph of serum streptomycin concentration was linear over the range 5—50 μg/ml. Streptomycin was added to serum at the level of 20.0 μg/ml and its concentration was determined to be 18.9 μg/ml with a coefficient of variation of 2.07% ($n = 5$). The clinical application of this method was confirmed by comparison with fluorescence polarization immunoassay.

## INTRODUCTION

Although streptomycin (SM) is clinically useful for the therapy of tuberculosis, it has serious side effects such as ototoxicity and nephrotoxicity, which are related to the serum concentration. Therefore, therapeutic drug monitoring and hearing loss tests are essential during streptomycin therapy [1].

Various methods are available for determining aminoglycoside antibiotics in

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serum [2]. The conventional microbiological assay has the disadvantages of being time-consuming, with poor reproducibility and interferences from other co-administered antibiotics. Several methods based on the immunological reaction are rapid, sensitive and accurate, but the commercially available apparatus and reagent kits are expensive.

High-performance liquid chromatography (HPLC) is one of the best methods for monitoring aminoglycoside antibiotics because of its separatory characteristics and relatively low operational costs [2]. HPLC methods involve extraction of aminoglycoside antibiotics by CM-Sephadex or silica gel followed by derivatization to yield fluorigenic [3, 4] or UV-detectable [5] products. Unfortunately, these methods cannot be applied to streptomycin owing to the lack of a primary amino group that is easily derivatized. Whall [6] studied a HPLC method that included the UV detection of streptomycin. Although this method has adequate separation efficiency and sensitivity for the determination of streptomycin products, no application to biological materials was reported.

In this paper, we describe the development of an HPLC method for the determination of serum streptomycin for therapeutic drug monitoring.

## EXPERIMENTAL

### *Apparatus and conditions*

A Shimadzu (Kyoto, Japan) Model LC-3A high-performance liquid chromatograph equipped with a spectrophotometric detector (Shimadzu Model SPD-2A) was employed. The analytical column and the guard column were Hibar LiChrosorb RP-18 (particle size 5 $\mu$m; 25 cm × 4.0 mm I.D.; Cica-Merck, Kanto Chemical, Tokyo, Japan) and Spheri-10 RP-8 (particle size 10 $\mu$m; 3 cm × 4.6 mm I.D.; Brown Labs., Santa Clara, CA, U.S.A.), respectively, and were warmed to 55°C. The column flow-rate was 1.0 ml/min and the detection wavelength was set at 195 nm using 0.08 a.u.f.s.

### *Reagents*

SM sulphate (Lot. No. sss-38808) was obtained from Meiji Seika Kaisha (Tokyo, Japan) and dihydrostreptomycin (DHSM) sulphate (Lot. No. 39c-0161) from Sigma (St. Louis, MO, U.S.A.). The antibiotic concentrations were calculated from their labelled potency. Acetonitrile (HPLC grade, Cica-Merck) was purchased from Kanto Chemical. Sodium 1-hexanesulphonate (Tokyo Kasei Kogyo, Tokyo, Japan) and the other reagents were of analytical-reagent grade. Water was doubly distilled in an all-glass still after passage through an ion-exchange column. A Sep-Pak $C_{18}$ cartridge was purchased from Waters Assoc. (Milford, MA, U.S.A.).

### *Mobile phase preparation*

In about 900 ml of water were dissolved 3.76 g of sodium 1-hexanesulphonate as an ion-pairing reagent and 9.50 g of tribasic sodium phosphate dodecahydrate. The solution was adjusted to pH 3.0 with phosphoric acid and then diluted to 1000 ml with water. Finally, the solution and acetonitrile were mixed (92:8, v/v) to prepare the mobile phase.

*Sample preparation*

The Sep-Pak $C_{18}$ cartridge, connected to a 5-ml syringe as the eluent reservoir, was previously washed with 20 ml methanol and 20 ml water. The buffer for the Sep-Pak $C_{18}$ extraction usually containing sodium 1-hexanesulphonate (50 m*M*) and tribasic sodium phosphate (25 m*M*) was adjusted to pH 2.0 with phosphoric acid. A 2-ml volume of this solution, 0.4 ml of human serum and 100 $\mu$l of aqueous DHSM solution (100 $\mu$g/ml) were poured into the cartridge and 2 ml of water were passed through. After centrifuging at 2300 *g* for 5 min to remove the aqueous solution, the cartridge was eluted with 5 ml of methanol and the eluate was concentrated to approximately 200 $\mu$l under vacuum at 30°C. A 200-$\mu$l volume of the mobile phase was added to the residue, then a 100-$\mu$l aliquot of the solution was injected into the chromatograph.

*Blood sampling*

Blood samples were taken from hospitalized patients receiving intramuscular injections of SM sulphate (0.5 or 1.0 g daily). The sampling times were 1 and 3 h after administration. The serum was obtained by centrifuging the blood samples (2300 *g* for 5 min), immediately after sampling and stored at −20°C until analysis.

*Fluorescence polarization immunoassay*

SM from the same serum was also analysed by fluorescence polarization immunoassay (FPIA) as reported by Schwenzer and Anhalt [7]. A commercially available kit (TDX-streptomycin; Abbott Labs., Irving, TX, U.S.A.) was used.

## RESULTS AND DISCUSSION

We chose DHSM as an internal standard because the similarity of its physicochemical character to that of streptomycin. As far as we could establish, Whall's conditions [6] did not yield an adequate separation of SM and DHSM with our HPLC system. Therefore, the pH of the mobile phase was changed from 6.0 to 3.0 to yield a better separation (Fig. 1). Except for this, we adopted most of the conditions used by Whall [6].

As many of the biological components in serum may interfere with the determination of aminoglycoside antibiotics, clean-up [2] of the serum sample is usually required before injection into the HPLC system. The solvent extraction of SM from biological components is difficult owing to its hydrophilic nature. For some aminoglycoside antibiotics [4], pretreatment using CM-Sephadex gel gave cleaner preparations. However, this procedure is relatively troublesome and was inadequate for removing interfering substances from the serum with our UV detection method. We have already reported ion-pair extraction with a Sep-Pak $C_{18}$ cartridge to separate the hydrophilic compound from the biological components [8]. We tried to use this method for the determination of SM in serum.

For the Sep-Pak $C_{18}$ extraction, the effects of the buffer pH and the concentration of ion-pair reagent in the buffer were examined and the results

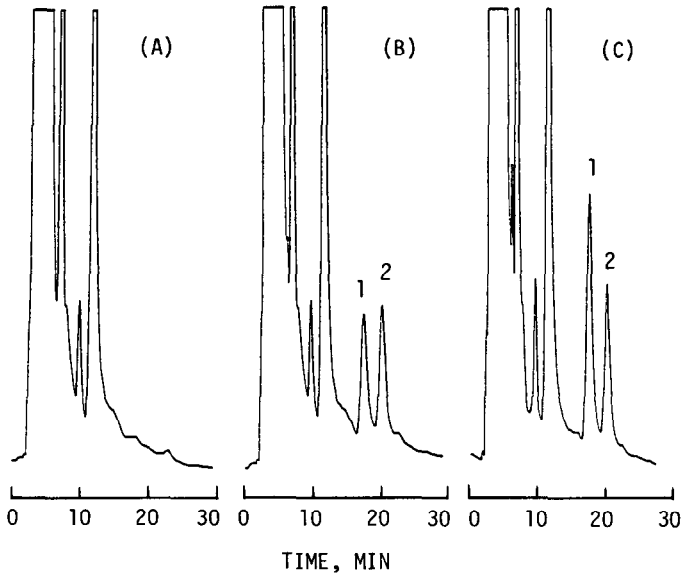


Fig. 1. Chromatograms of (A) serum blank, (B) serum spiked with streptomycin (20 μg/ml) and (C) serum obtained 1 h after intramuscular injection of streptomycin (streptomycin concentration = 32.6 μg/ml). Peaks: 1 = streptomycin; 2 = dihydrostreptomycin (internal standard).

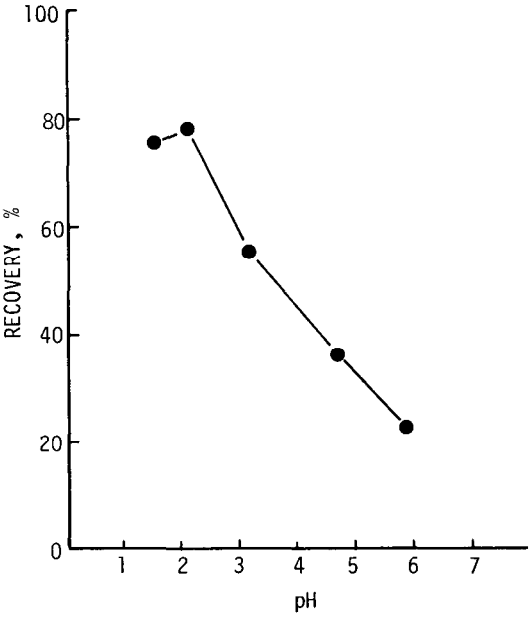


Fig. 2. Effect of buffer pH on extraction of streptomycin from serum with a Sep-Pak $C_{18}$ cartridge (streptomycin concentration = 20 μg/ml, sodium 1-hexanesulphonate concentration = 50 m*M*).

are shown in Figs. 2 and 3. From these results, we adopted pH 2.0 and an ion-pair reagent concentration of 50 m*M* for maximum recovery of SM (about 80%) from serum. After Sep-Pak $C_{18}$ extraction, washing the Sep-Pak $C_{18}$ cartridge with at least 2 ml of water is necessary in order to prevent contamina-

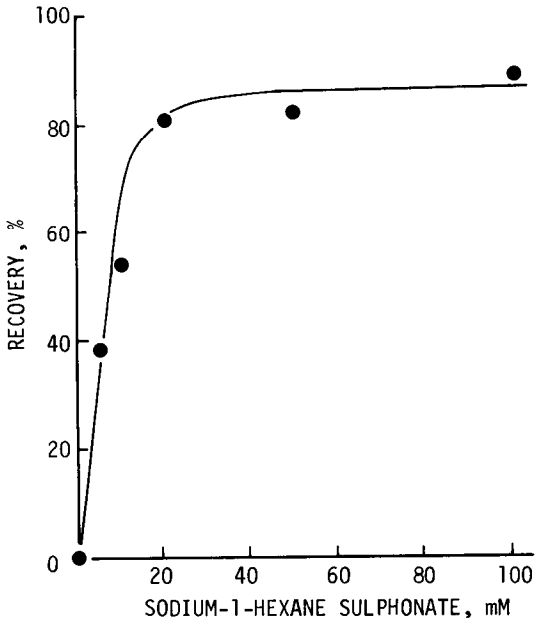


Fig. 3. Effect of sodium 1-hexanesulphonate concentration on extraction of streptomycin from serum with a Sep-Pak $C_{18}$ cartridge (streptomycin concentration = 20 μg/ml, pH = 2.0).

TABLE I

PRECISION AND ACCURACY OF STREPTOMYCIN ASSAY IN DIFFERENT HUMAN SERA ($n$ = 5)

| Added (μg/ml) | Found (μg/ml) | |
|---|---|---|
| | Mean | Coefficient of variation (%) |
| 5.0 | 4.36 | 5.46 |
| 10.0 | 9.14 | 3.45 |
| 20.0 | 18.9 | 2.07 |
| 40.0 | 39.4 | 2.83 |

tion with interfering substances. However, more than 2 ml of water should not be used as the recovery of SM then decreased (65% with 4 ml, 30% with 6 ml of water).

Several solvent systems for the elution of SM from the Sep-Pak $C_{18}$ cartridge were examined. Five millilitres of acetonitrile or diethyl ether did not elute SM. However, the same volume of methanol, a more hydrophilic solvent, successfully eluted 80% of SM, so methanol was chosen for elution. In this procedure, more than 5 ml of methanol did not improve the efficiency. On concentrating the methanol eluates, complete evaporation should be avoided because SM in the dried residue tends to sublime.

Typical chromatograms of sera prepared as above are shown in Fig. 1. The retention times of SM and DHSM were approximately 18 and 20 min, respectively. The calibration graphs of SM in aqueous solution and serum were

linear over the range 5—50 μg/ml and almost passed through the origin ($y$ = $0.0495x$ — 0.0181, $r$ = 0.9998 for aqueous solution; $y$ = $0.0476x$ — 0.0258, $r$ = 0.9997 for serum), and showed fairly good overlapping, which suggested that the serum constituents did not affect the recovery of SM.

The precision and accuracy of the method were determined with sera from five healthy volunteers, to which known amounts of SM had been added. As shown in Table I, good accuracy was obtained, although the relatively low concentrations tended to be slightly underestimated. The variations among the sera were small (less than 6%) at each concentration. The detection limit estimated from a signal-to-noise ratio of 2:1 was about 2 μg/ml in serum. In practical SM therapy, desirable serum concentrations of SM are about 20 μg/ml at the peak and less than 5 μg/ml at the trough in order to avoid ototoxicity and nephrotoxicity [1]. These toxicities have occurred at SM concentrations greater than 40—50 μg/ml [1]. The sensitivity of our method is sufficient to monitor this range.

To confirm the clinical applicability of the method, serum samples were obtained from patients undergoing SM therapy and analysed by the proposed method. The results are shown in Fig. 4. Although wide variations were observed in the concentration of SM among the patients, the data were almost consistent with the results reported by Buggs et al. [9] and Holdiness [10]. Fifty clinial specimens analysed by our HPLC method were compared with the

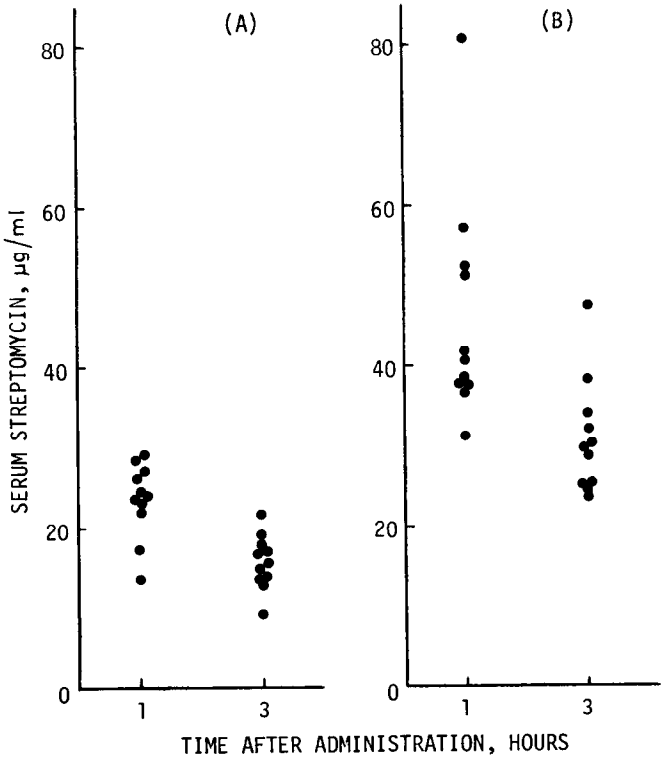


Fig. 4. Serum streptomycin concentrations in patients who had received intramuscular injections of (A) 0.5 g or (B) 1.0 g of streptomycin.

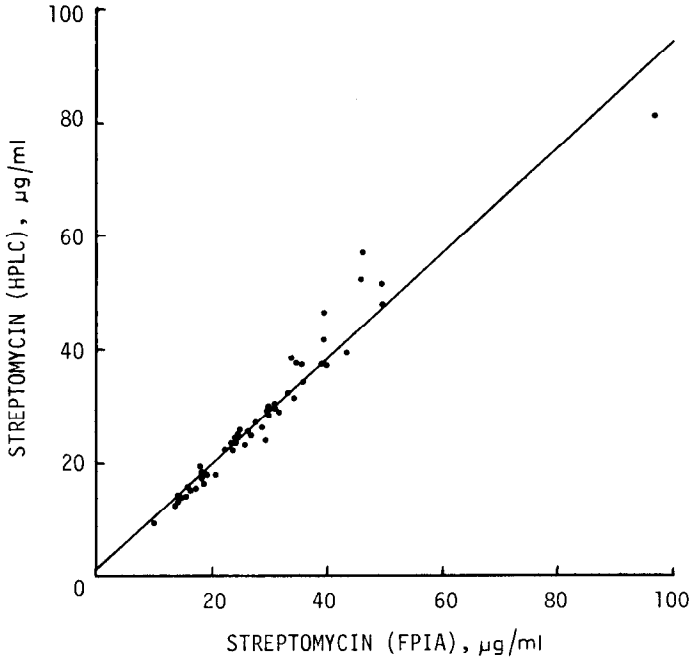


Fig. 5. Correlation between HPLC and FPIA method. The regression equation and correlation coefficient are [HPLC] = 0.931[FPIA] + 1.36 ($n$ = 50) and $r$ = 0.969 ($p < 0.001$), respectively.

results of an FPIA technique [7]. As shown in Fig. 5, a good regression line and a correlation coefficient near unity were obtained.

In conclusion, this relatively simple HPLC procedure is reproducible and sensitive and may be useful for monitoring SM in human serum following intramuscular injection.

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