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Note

Isotachophoretic assay of aminoglycosides and lincomycins in pharmaceuticals

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Aminoglycoside antibiotics are used in the treatment of severe infections of men and animals[1]. They are indicated when antibiotics with inferior toxic potential are contraindicated and when the organisms are susceptible to the aminoglycosides concerned. Lincomycin and clindamycin, which are pyranosides, are indicated, for example, for the therapy of infections induced by peniciilin-, oxacillin- and cephalosporin-resistant staphylococci[2].

The quantitation of the active substances of tobramycin sulphate, sisomicin sulphate, clindamycin hydrochloride, lincomycin hydrochloride as well as spectinomycin dihydrochloride by chromatographic techniques is still laborious and time-consuming[3–5]. Microbiological techniques[6] also do not meet the requirements of a rapid, precise and economical method for the quantitation of the aminoglycosides and lincomycins discussed herein. However, the present results show that analytical isotachophoresis can be successfully used for the determination of these active substances. This technique does not require more than 10 min for a complete assay.

MATERIALS AND METHODS

The pharmaceuticals were obtained from commercial sources. Spectinomycin dihydrochloride, clindamycin hydrochloride and lincomycin hydrochloride served as reference substances, *e.g.*, for the construction of the calibration graphs (Fig. 1). The structural formulae of these compounds as well as those of the antibiotics sisomicin and tobramycin are listed in Table I. All reagents were prepared with double distilled water. They were purchased as analytical grade chemicals. Hydroxypropylmethylcellulose (HPMC 15000) was obtained from Dow Chemical (Stade-Brunshausen, G.F.R.), 4-amino-butyric acid from Serva (Heidelberg, G.F.R.), glycylglycine and potassium acetate from E. Merck (Darmstadt, G.F.R.) and β-alanine from Sigma (München, G.F.R.).

For isotachophoresis, a number of suitable aqueous electrolyte systems is available. A system of 0.020 mol/l potassium acetate plus 0.3% HPMC 15000 (to avoid electroendosmosis[7,8]), pH 4.95, proved to be excellent for the qualitative and quantitative determination of the aminoglycosides and lincomycins. A mixture of 20 mmol/l 4-aminobutyric acid/acetic acid, pH 4.72, or 0.020 mol/l glycylglycine or 0.020 mol/l β-alanine, was selected as terminator. HPMC 15000 was purified by means of dialysis[9].

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Determinations were performed with the "Tachophor" (LKB, Bromma, Sweden), Type 2127, at a constant current of 200 μA and a constant temperature of 5°C. The length of the capillary was 23 cm. The measurement range of the recorder (LKB 2210) was 100 mV and the chart speed was 6 cm/min. Aqueous solutions of the antibiotics were injected with a 10-μl Hamilton microsyringe, the volumes injected being 3–5 μl. During the separation (cationic) by a discontinuous electrolyte, the cations migrate according to their net mobilities between the leading and terminating electrolyte[10]. The compounds discussed were investigated in the form of their sulphates or hydrochlorides, which are their common application forms. As these antibiotics show very little UV-absorption, they are identified by their differing electrical conductivities. The concentration of the standard solutions was chosen such that the concentration of the active substances based on the corresponding salts amounted to 1 mg/ml.

RESULTS AND DISCUSSION

The results confirm the possibility of using analytical isotachophoresis for the determination of aminoglycoside antibiotics and lincomycins, under the conditions mentioned. While, on the one hand, the lack of distinct UV absorption renders difficult the identification by means of high-performance liquid chromatography and no conditions are known under which the unaltered molecules can be exactly determined by gas chromatography, the conductivity detector proves to be excellent for the determination of these compounds.

The isotachopherograms show that the separation of the active substances in the pharmaceuticals is practicable without the application of other techniques of analysis, such as thin-layer, ion-exchange or column chromatography. However, the simultaneous determination of not only lincomycin hydrochloride and clindamycin hydrochloride but also of tobramycin sulphate and sisomicin sulphate implies some problems. The mobility of the cations depends on charge, viscosity, molecular size and shape, solvation, dielectric constant and temperature. The mixtures concerned contain weak organic bases differing only slightly in size (Table I) and in pK value

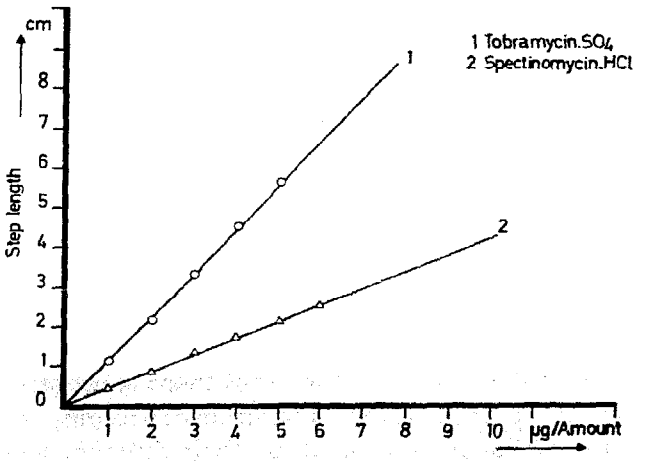
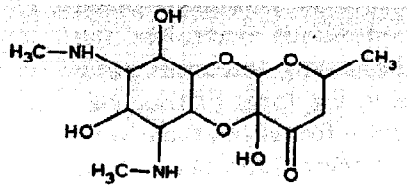
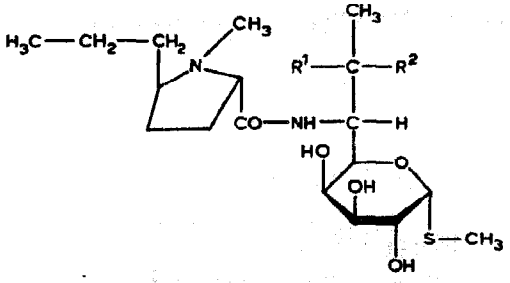
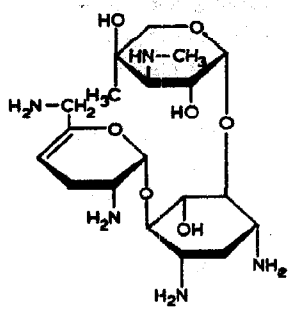
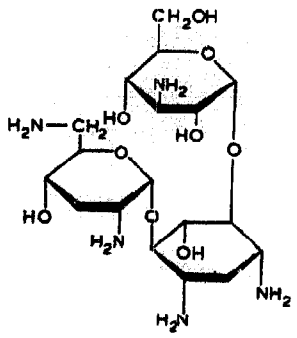


Fig. 1. Calibration graphs for quantitation of tobramycin sulphate and spectinomycin dihydrochloride.

TABLE I

ANTIBIOTICS STUDIED

Structural formulae (base)		*Antibiotics*	*Anion*
		Spectinomycin	Cl^-
	Lincomycin : R^1 = OH, R^2 = H Clindamycin : R^1 = H, R^2 = Cl	Lincomycin Clindamycin	Cl^- Cl^-
		Sisomicin	SO_4^{2-}
		Tobramycin	SO_4^{2-}

and, consequently, also in their effective mobilities. Nevertheless, since pharmaceuticals generally contain only one of these active compounds there is no practical difficulty. The isotachopherogram in Fig. 2 illustrates a separation of tobramycin/sisomicin, spectinomycin and lincomycin/clindamycin based on their different molecular structures[11].

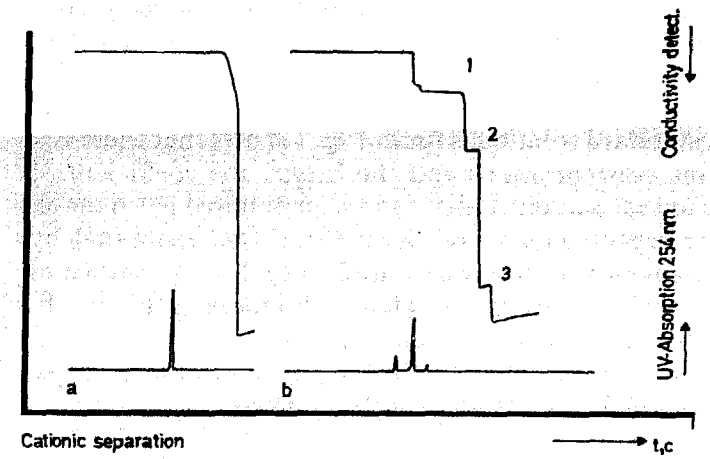


Fig. 2. Isotachophoretic separation of aminoglycosides and lincomycins (b) (1 = tobramycin sulphate; 2 = spectinomycin dihydrochloride; 3 = clindamycin hydrochloride) and UV and conductivity diagram of electrolyte system (a).

If the compounds discussed herein have to be determined during an in-process control, this is likely to be accomplished by means of spacer-ions[12]. Although the salts of the antibiotics do not show any UV signals at 254 nm, the limits of the zones, in the case of spectinomycin and lincomycin/clindamycin, are still recognizable as

TABLE II

RESULTS OF THE QUANTITATION OF ACTIVE SUBSTANCES IN SOME PHARMACEUTICALS

Cps = Capsule; tbl = tablet.

Pharmaceutical	*Active substance*	*Quantity declared*	*Quantity found*	*Content related to quantity declared (%)*
A	Clindamycin-hydrochloride	85.2 mg/cps.	90.3 mg/cps.	106.0
B	Lincomycin-hydrochloride	567.8 mg/cps.	620.0 mg/cps.	109.2
C	Lincomycin-hydrochloride	567.8 mg/cps.	555.3 mg/cps.	97.8
D	Lincomycin-hydrochloride	681.3 mg/2 ml	624.2 mg/2 ml	91.6
E	Lincomycin-hydrochloride	113.4 mg/1 ml	123.0 mg/1 ml	108.5
F	Lincomycin-hydrochloride	226.8 mg/tbl.	240 mg/tbl.	105.8
G	Spectinomycin dihydrochloride	3 g/package	3.01 g/package	100.3
H	Spectinomycin dihydrochloride	3.0 g/package	3.07 g/package	102.3

there are always so-called spacers because of traces of natural UV-absorbing contaminant. These impurities are present even in analytical grade agents, such as glycyclglycine, β-alanine and 4-aminobutyric acid (Fig. 2).

For the quantitation of the antibiotics, a direct comparison of the length of the zones of a sample and those of a standard solution is used. Fig. 1 shows that there is a direct proportionality between the concentrations and the length, the coefficient of correlation being $r = 1.000$. The lowest amount which can be quantitated is 1.6 nmol, under the conditions given. The reproducibility of the method was examined by replicate analyses and the coefficient of variation was found to be 2%. As shown in Table II, the pharmaceuticals contain a surplus of active substances —this is obviously to ensure a sufficient antibiotic content until the expiry date.

We hope that this publication will stimulate further research into more simple and precise assays of pharmaceuticals by means of isotachophoresis.

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