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Note

Determination of rubidium, sodium, calcium and thiamine in a pharmaceutical preparation by capillary isotachophoresis

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Rubidium and sodium iodide in association with calcium and vitamins such as thiamine and vitamin C are used in Italy in the treatment of cataracts, iritis etc.[1]. Several methods have been used to determine alkali and alkaline-earth metals and thiamine, including atomic emission spectrometry, ion-selective electrodes, high-performance liquid chromatography and isotachophoresis[2–6]. The method recommended by the Italian Pharmacopoeia for sodium and calcium determination is titrimetry[7].

The composition of the pharmaceutical formulation is particularly appropriate for capillary isotachophoretic analysis, and this method was therefore applied to the determination of rubidium, sodium, calcium and thiamine in eye-drop samples.

As rubidium has a relatively high mobility, which is similar to that of the common leading cations used, we considered the possibility of modifying its effective mobility. For the separation of alkaline-earth metals by capillary isotachophoresis, complex-forming equilibria [*e.g.*, with SO_4^{2-}, α-hydroxyisobutyric acid (HIBA) and 1,2-cyclohexanediamine-N,N,N′,N′-tetraacetate (CyDTA)] have been used[8–10]. In order to modify the mobility of alkali metal ions, generally inclusion complex formation with non-ionic substances (*e.g.*, 18-crown-6) is used[11–13].

In this work, leading electrolyte containing 30 m*M* 18-crown-6 was used for the rapid determination of rubidium, sodium, calcium and thiamine in an eye-drop formulation.

EXPERIMENTAL

Apparatus

The analysis was carried out by using a LKB (Bromma, Sweden) 2127 Tachophor apparatus equipped with a conductivity detector. Also used were a PTFE capillary tube (240 mm × 0.5 mm I.D.) and a laboratory-made conductivity detector cell[14]. The resistance and its derivative were recorded with an LKB 2210 line recorder at a chart speed of 50 mm/min. The current used was 200 μA, changed to 25 μA during detection.

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Chemicals

Ammonia solution and acetic acid were purchased from Carlo Erba (Milan, Italy), 2-pyridinecarboxylic acid (2-picolinic acid), 18-crown-6, Triton X-100 from Fluka (Buchs, Switzerland) and thiamine hydrochloride from Sigma (St. Louis, MO, U.S.A.).

The sample composition was 0.800 g of rubidium iodide, 0.500 g of calcium formate, 1.200 g of sodium iodide, 0.225 g of sodium ascorbate and 0.250 g of thiamine hydrochloride in 100 ml of solution; boric acid, sodium tetraborate and lactose were also present as excipients. The sample and the standards analysed were kindly supplied by Dipartimento di Studi Farmaceutici, Università la Sapienza (Rome, Italy).

Electrolytes

A 10 m*M* solution of ammonium picolinate (pH 5.4) containing 30 m*M* 18-crown-6 and 0.4% (w/v) Triton X-100 was used as the leading electrolyte and 5 m*M* acetic acid as the terminating electrolyte.

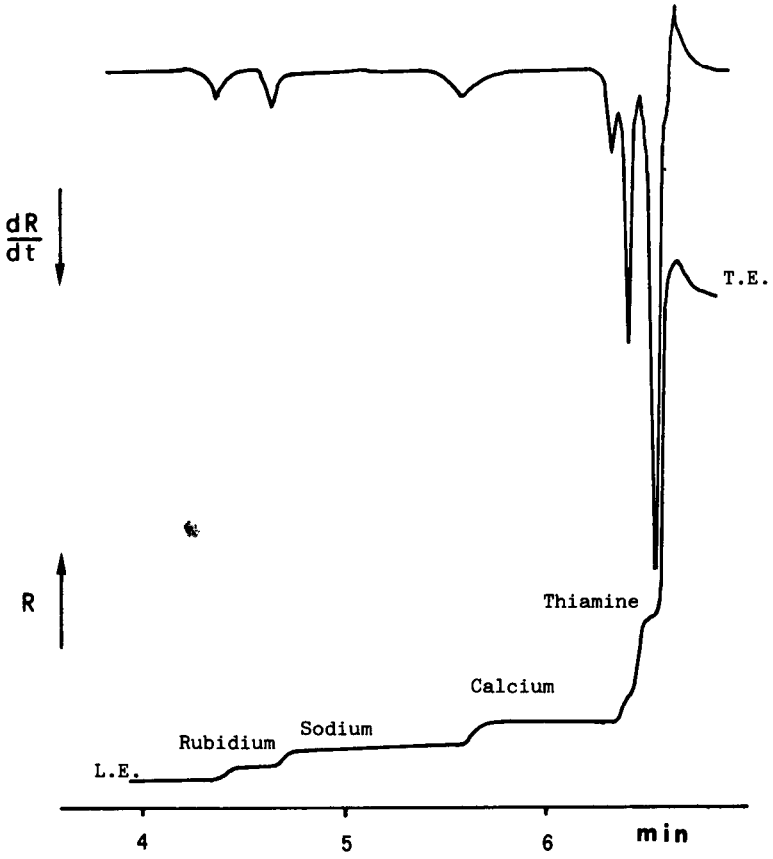

Fig. 1. Isotachopherogram for the separation of rubidium, sodium, calcium and thiamine in an eye-drop sample diluted 100-fold; 8 μl injected. Leading electrolyte: 10 m*M* ammonium picolinate (pH 5.4) containing 30 m*M* 18-crown-6 and 0.4% (w/v) Triton X-100. Terminating electrolyte: 5 m*M* acetic acid.

RESULTS AND DISCUSSION

In order to find optimal conditions for the separation of rubidium, sodium, calcium and thiamine, several electrolyte systems were tested. H^+ was used as a terminator, ammonium was the leading ion and, to ensure very low mobility for H^+, picolinate anion was the leading counter anion[15]. With this electrolyte system it was possible to obtain good resolution between sodium, calcium and thiamine. Rubidium gave a mixed zone with ammonium.

In isotachophoresis, if mixed zones are obtained it is necessary to alter the original electrolyte system in order to change the effective mobilities, usually by appropriate selection of the pH of the leading electrolyte and the use of complex formation[16]. In order to reduce the mobility of rubidium, inclusion complexation was used and 18-crown-6 was added to the leading electrolyte.

Different concentrations of the inclusion complexing agent were added to the leading electrolyte and the relative step heights were observed. The effective mobilities of calcium and thiamine were not reduced, and rubidium was separated from ammonium at a concentration of 18-crown-6 of 25 m*M*.

The optimum concentration of 18-crown-6 was found to be 30 m*M*. A standard solution containing rubidium, sodium, calcium and thiamine at concentrations similar to those in the diluted sample was used for the calibration graphs.

Fig. 1 shows an isotachopherogram of a sample of the pharmaceutical preparation analysed. The linearity of the calibration graphs was tested in the ranges $(7.2–38) \cdot 10^{-10}$, $(8–80) \cdot 10^{-10}$, $(3.8–38) \cdot 10^{-10}$, and $(2–10) \cdot 10^{-10}$ mole for rubidium, sodium, calcium and thiamine, respectively. The correlation coefficients and the relative standard deviations (eight determinations) were 0.9979 and 1.3%, 0.9989 and 0.7%, 0.9984 and 0.8%, and 0.9927 and 2.1% for rubidium, sodium, calcium and thiamine, respectively.

Complete resolution of a standard mixture is possible if the sodium and calcium amounts are not greater than $8 \cdot 10^{-9}$ and $3.8 \cdot 10^{-9}$ mole, respectively.

The results obtained show that capillary isotachophoresis is readily applicable to the determination of inorganic and organic ions in pharmaceutical formulations (no preliminary operations are required), rapid (less than 7 min) and sensitive, with good reproducibility. The method can be used for routine drug monitoring.

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