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Short communication

Monitoring of lithium levels in human serum after therapy with lithium preparations by capillary isotachophoresis

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Abstract

An isotachophoretic method for the evaluation of the level of lithium salts in serum samples was optimized. Use of operating systems containing polyethylene glycol permitted the separation of cationically migrating components from Li (i.e., Na, K and Ca). The pretreatment of serum samples involves only appropriate dilution with demineralized water depending on the concentration of the major components such as sodium. The lithium levels were studied both in model samples and serum from patients treated with lithium preparations.

1. Introduction

Lithium salts are administered to patients suffering from affective psychotic diseases as a prophylactic to prevent recidivism, in the therapy of mania, thyrotoxicosis and granulocytophenia [1].

Therapy with lithium salts leads to side-effects and the possibility of intoxication, and therefore the permanent monitoring of lithium levels in body fluids is very important. Recent analytical methods have been mainly directed to the monitoring of lithium in blood plasma and serum.

The mechanism of lithium action along with the pathogenesis of manic-depressive diseases is not entirely understood. Lithium is believed to take part in sustaining the function of the nerve membranes and depressing the liberation of norepinephrine in neurons, and it probably influences the metabolism of norepinephrine and serotonin in the brain.

Lithium therapy requires the maintenance of a constant lithium concentration in the serum (0.6–1.2 mmol/l) [2]. The determination of lithium in biological materials is important first as a check on the correctness of a given therapy, and second as a physiological check. A main criterion in the selection of a method is the accuracy of determination and the amounts of biological material required. In toxicological analyses for lithium there are widely used optical [3–6] and spectral methods [7–12]. More recently the use of ion-selective electrodes has increased [4,13].

The use of capillary isotachophoresis (ITP) seems to be a very promising method, because it offers a truly selective determination of Li without disturbances from the matrix or background, which means that ITP is not influenced by the matrix composition, in contrast to atomic absorption spectrometry or flame photometry. A dis-

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advantage of photometric methods is the long analysis time (about 20 min).

2. Experimental

2.1. Materials and chemicals

The leading electrolyte was 10 mmol/l ammonia solution with acetic acid as a counter ion, adjusted to pH 5.4, 0.1% of *m*-hydroxyethylcellulose as an additive and water–polyethylene glycol (70:30, v/v) as a solvent. The terminating electrolyte was 5 mmol/l tetraethylammonium. Polyethylene glycol with molecular mass in the range 200–300 (Loba Chemie, Vienna, Austria) was purified by demineralization on an Amberlite MB-1 mixed-bed ion exchanger in a polyethylene vessel [14].

The chemicals used for the preparation of both electrolyte solutions were obtained from Serva (Heidelberg, Germany), Loba Chemie and Lachema (Brno, Czech Republic). The solutions used for the construction of calibration graphs were prepared by appropriate dilution of a stock solution of LiCl ($c_{Li} = 1$ g/l). For the calibration measurements of Li in serum the one healthy human subject was used. The samples for measurements were prepared as follows: to 0.9 ml of serum diluted with demineralized water (1:50) were added 100 μl of an external standard containing 0.125–0.208 μg of Li ($C_{Li} = 0.125$–0.208 μg/ml). The LiCl used was of analytical-reagent grade and was dried to constant mass at 105°C and kept in a desiccator.

The samples of serum obtained from psychotic patients treated with lithium preparations were treated only by appropriate dilution with freshly demineralized water (1:50; in the case of lower dosages of Li to a patient, 1:20 dilution was necessary) before the ITP analysis. The samples were stored at −30°C prior to their analysis.

2.2. Apparatus

A CS isotachophoretic analyser (VVZ PJT, Spišská Nová Ves, Slovakia) was used in the column-coupling configuration of the separation unit. The lengths of the zones from the conductivity detectors were measured electronically.

2.3. Procedure

The serum samples after dilution as described above by means of dosing device were injected into the interface of the leading and terminating electrolytes in 35-μl amounts. The determination of lithium was evaluated from the response of the analytical column detector at a driving current of 45 μA. Completion of analysis required 20 min. The concentration of lithium in serum was determined by the calibration graph method. The parameters of the calibration graphs were determined by linear regression of the zone lengths measured as a function of Li concentrations in the calibration solutions.

3. Results and discussion

The conditions for the isotachophoretic separation and determination of lithium in serum from patients under the lithium preparations therapy were studied and optimized.

An operating system containing polyethylene glycol [14,15] permits the separation of cationically migrating components from Li^+, i.e., Na^+, K^+ and Ca^{2+}. Its suitability may be judged from considering possible interferences and the concentration ratios of other major components (Na^+, K^+). The serum for the lithium level study presents a very complicated matrix. Figs. 1 and 2 show that in the mobility range of interest the ions of other metals or organic bases do not migrate. The absence of interfering species indicates the high selectivity of system used. Pretreatment of the serum sample involved only appropriate dilution with demineralized water (1:50) or in the case of lower dosages of Li to a patient, 1:20 dilution. With this approach, only the concentration of sodium as a major component needed to be taken into account.

Table 1 summarizes the parameters of the regression equations obtained by evaluation of the calibration relationships $y = a + bx$ covering the concentration range 0.018–0.030 mmol/l. In

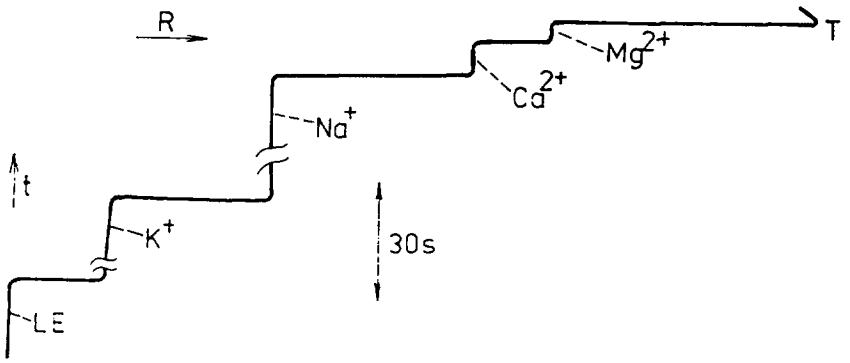

Fig. 1. Isotachopherogram from the analysis of a human serum sample. The injected sample (35 μl) was diluted 1:50 with demineralized water. The driving currents were 250 and 45 μA in the preseparation and analytical columns, respectively. *R* and *t* = increasing resistance and time, respectively.

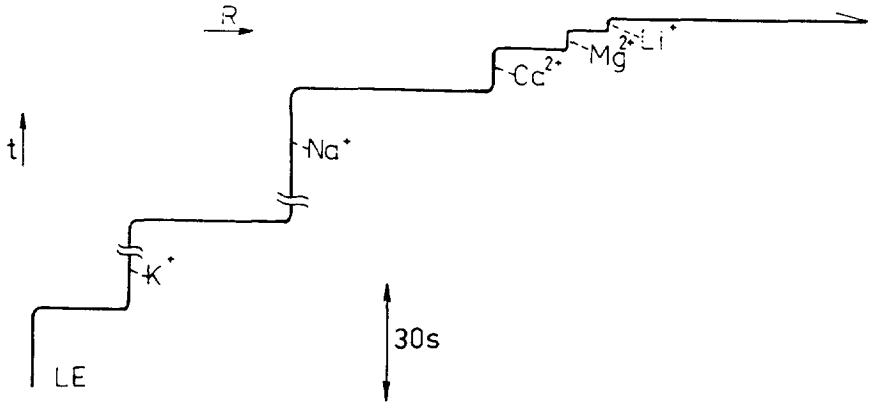

Fig. 2. Isotachopherogram from the analysis of a human serum sample after lithium preparations treatment and dilution 1:50. Volume injected, 35 μl; driving currents, 250 and 45 μA in the preseparation and analytical columns, respectively. The Li concentration in the sample was 1.10 mmol/l.

Table 1
Regression equations and correlation coefficients for Li in the concentration range 0.018–0.030 mmol/l

Ion Li^+	Regression equation[a]	Correlation coefficient
Standard LiCl	$y = 0.573 + 86.982x$	0.999
Standard LiCl added to serum	$y = -0.150 + 105.172x$	0.999

No. of data points = 15.
[a] x = Concentration (mmol/l); y = zone length (mm).

this concentration range the calibration line for the conductivity detector used is linear. The values of the correlation coefficients show the good linearity of the calibration relationships. The precision of these determinations is characterized by the values of the relative standard deviations, which were 0.8 and 0.4% for the determination of 0.018 and 0.030 mmol/l concentrations for the lithium standard in water and 1.2 and 0.6% for the determination of 0.018 and 0.030 mmol/l concentrations for the Li standard added to the serum. The detection limit obtained under the conditions of analysis for practical samples is 0.015 mmol/l, at which the detector gives an unambiguous quantitatively evaluable response.

The lithium levels were investigated both in model samples and real serum samples obtained from 37 patients under lithium therapy. The lithium concentrations in serum as measured were in the range 0.50–1.25 mmol/l. The reproducibility of the determination was R.S.D. = 0.65% and 0.40% ($n = 10$) for the concentrations found for two different samples of serum with average Li concentrations of 0.57 and 1.25 mmol/l, respectively. The ITP method for the separation and determination of lithium in serum demonstrated in all cases that it is sufficiently specific, sensitive and suitable for clinical practice.

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