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Direct analysis of bromide in human serum by capillary electrophoresis ☆

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Abstract

The purpose of the present work was the development and validation of a simple, rapid and reliable method for direct bromide quantification in serum based on capillary electrophoresis (CE). The analysis was carried out with an automated capillary electropherograph. Analytical conditions were as follows. Capillary: uncoated fused silica, effective length 50 cm, internal diameter 50 μm; voltage: 20 kV in reverse polarity mode; temperature: 25 °C; running buffer: 90 mmol/L sodium tetraborate decahydrate and 10 mmol/L NaCl, pH 9.24; detection: direct UV absorption at 200 nm; sample treatment: dilution of serum 1:10 with the internal standard solution (2 mmol/L thiocyanate). Under the described conditions, bromide ions and internal standard were baseline separated in 7 min. No interferences from other serum components were observed. The analytical sensitivity was characterized by a LOD: 0.05 mmol/L and a LOQ of 0.1 mmol/L. Excellent linearity was verified in the range from 2.5 to 60 mmol/L [$y = 0.0746x - 0.0372$; $R^2 = 0.9995$ (x = bromide concentration; y = bromide peak area/internal standard (I.S.) peak area)]. Quantitative imprecision in intra-day ($n = 7$) and day-to-day ($n = 7$) experiments was always within R.S.D. values <2%. Recovery was quantitative throughout the range of linearity of the method. Clinical cases of infants undergoing potassium bromide therapy for refractory epilepsy were analyzed with results in agreement with literature data. On the basis of these considerations, capillary electrophoresis can be proposed as the method of choice for bromide analysis in serum samples, especially for therapeutic drug monitoring purposes.

Keywords: Serum bromide analysis; Capillary electrophoresis; Potassium bromide therapy; Epilepsy

1. Introduction

Bromide was the first drug found to be effective for the treatment of epilepsy, and for this application was largely used in the second half of the 19th century and in the first years of the 20th century [1–3]. With the introduction of Phenobarbital (1912) and, later, of phenytoin and other safer and more effective antiepileptics, the therapeutic use of bromide was almost dismissed. However, many physicians still consider inorganic bromide salts as one of the most effective treatment for refractory epilepsies, particularly in early childhood [3].

However, relevant adverse, potentially life threatening, effects have been reported including drowsiness, restlessness, delirium and dementia, cutaneous effects (acneiform rashes and granulomatous lesions) and gastrointestinal effects (tongue sore, loss of appetite, nausea and emaciation), and are reported as dose dependent. The therapeutic blood bromide concentration is reported to be approximately 10–30 mmol/L [3], above which intoxication symptoms may occur. Because of this narrow therapeutic window, strict monitoring of serum bromide concentrations during treatment is recommended [3]. For this purpose, different analytical techniques have been used, including colorimetry [3], coulometry [3], potentiometry [4], ion selective electrodes [5], energy dispersive X-ray spectrometry [6], cyclic voltammetry [7] and ion-chromatography [8,9]. Among them, the colorimetric methods and ion selective electrodes are the most common only used in clinical chemistry laboratories. However, they may suffer from specific and non-specific interferences. In particular, colorimetric methods require a preliminary sample deproteinization. In addition, non-separative methods may be affected by concentrations of other serum anions, such as chloride and iodine.

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In the past decade capillary electrophoresis (CE) has been introduced in many fields of analytical chemistry and clinical chemistry, showing a wide analytical spectrum (from inorganic ions to large biopolymers), easy and low cost operation, high productivity and versatility, good sensitivity, accuracy, precision and excellent robustness [10]. In particular, CE, in the capillary ion analysis (CIA) mode, has shown a great potential for the separation and determination of organic and inorganic anions in different matrices [10–12]. However, so far, only few applications of CIA to serum anion analysis have been reported [13,14] and, to the best of our knowledge, no one has been published on serum bromide determination.

Based on previous experience in the development of a CIA method for nitrite and nitrate analysis in which bromide was analyzed as the internal standard [15], in the present work a simple, rapid and reliable method for direct bromide quantification in serum based on capillary electrophoresis is described, together with its application to the monitoring of serum bromide in pediatric samples from subjects undergoing anti-epileptic therapy with oral potassium bromide.

2. Materials and methods

2.1. Serum samples

Serum samples (≥30 μL) used for development and evaluation of the method were collected from 3 pediatric patients (<1 year old) under oral potassium bromide treatment for refractory epilepsy at doses ranging between 150 and 675 mg/day. Blank sera were from the authors of the present study. Serum was obtained from blood (sampling of 100–200 μL) after clothing at room temperature and centrifugation at 10,000 rpm in a benchtop Microfuge Lite Centrifuge (Beckman, Fullerton, CA, USA). Usually sera were analyzed within few hours after blood collection; if not possible, samples were stored at −20 °C.

2.2. Chemicals

Salts used for buffer preparation were of analytical reagent grade. Stock solutions of sodium chloride, sodium tetraborate decahydrate (both Sigma–Aldrich, MO, USA) and potassium bromide (Carlo Erba, Milan, Italy) were prepared in sterile deionized water at concentration of 0.1 M and stored at 4 °C for a maximum of 2 weeks. The running buffer was composed of 90 mmol/L sodium tetraborate decahydrate and 10 mmol/L NaCl. The resulting pH was 9.24. The running buffer was prepared daily. The used internal standard was potassium thiocyanate (Merck, Darmstadt, Germany), which was prepared daily in deionized water at the concentration of 2.0 mmol/L and then used as diluent of the serum samples.

2.3. Instrumentation

A capillary electropherograph P/ACE-MDQ (Beckman Coulter, Fullerton, CA, USA) fitted with a diode array detector operating at 200 nm wavelength was used. Control of the instrumentation, data acquisition and data reporting were performed by using the software 32 Karat (Beckman Coulter). Uncoated fused-silica capillaries (60 cm total length, 50 cm effective length, 50 μm I.D.) provided by Composite Metal Services (The Chase, Hallow, Worcestershire, UK) were used.

2.4. Analytical methods

2.4.1. Capillary electrophoresis

All samples were diluted 1:10 with the internal standard solution (potassium thiocyanate 2 mmol/L) and directly injected. Injection was by application of 0.5 psi (3.45 kPa) pressure for 5 s.

The electrophoretic separations were carried out under constant voltage of 20 kV in reverse polarity mode (i.e. cathode at the injection end of the capillary). The temperature of the capillary was kept constant at 25.0 °C.

Conditioning of fresh capillaries was carried out by rinsing with 1 M sodium hydroxide (10 min), 0.1 M sodium hydroxide (10 min), water (5 min) and running buffer (5 min) by applying a 50 psi (344.74 kPa) pressure at the injection end. Between consecutive runs the capillary was washed with 0.1 M sodium hydroxide (1 min), water (1 min) and then with the electrolyte buffer (2 min) by applying 30 psi (206.84 kPa) pressure at the injection end. At the end of the day, the capillary was rinsed with water (50 psi (344.74 kPa), 10 min). Before use, buffers and rinsing solutions were degassed by sonication under reduced pressure and filtered through a 0.45 μm cellulose membrane filter (Sartorius, Hannover, Germany).

Bromide concentrations in unknown samples were calculated from the ratio bromide area/internal standard (I.S.) area by interpolation on a standard curve constructed by plotting the bromide concentrations of six standard sera added with potassium bromide in the range from 2.5 to 60.0 mmol/L against the respective bromide area/I.S. area ratios.

3. Results and discussion

The use of a basic running buffer in uncoated silica capillaries causes a strong electroosmotic flow (EOF), when an electric field is applied, directed towards the cathodic end of the capillary. Under the used "reversed polarity" conditions (cathode at the injection end of the capillary), the EOF was directed towards the injection end of the capillary. Thus, most of the sample components (neutrals, cations and anions with a mobility lower than the EOF), after injection and voltage application, were pushed back out of the capillary, whereas small anions with mobility higher than that of the EOF could reach the detector. Under these conditions, the selectivity of the electrophoretic separation was highly increased, thus permitting the direct injection of serum samples, without interferences from the different serum components. Analytical selectivity was also increased by direct UV detection since only a limited number of small inorganic ions, including bromide and other halide ions, absorb UV radiation. Among wavelengths in the low UV range (200, 210, 214 and 220 nm), 200 nm proved to be the best compromise between signal and noise for bromide detection.

Further potential interference from matrix components has been reported to arise from chloride ions present in serum at

concentrations around 100 mmol/L. In order to avoid baseline disturbances caused by the excess of chloride in the sample, the running buffer was added with sodium chloride (10 mmol/L), which balanced the chloride concentration of the injected samples (serum diluted 1:10).

Under the described conditions, the bromide ions migrated to the detector in a sharp peak with efficiency of 208,000 theoretical plates/meter in about 4.5 min. In order to normalize peak areas for the injected volumes, an internal standard, potassium thiocyanate, was added to the sample diluent. Thiocyanate was clearly separated from the bromide peak having a migration time of about 7 min. Although the cyanide peak looked slightly asymmetrical in shape (fronting), this phenomenon did not hamper I.S. area measurement.

The injection of serum produced very clean electropherograms with a steady baseline within the migration time interval of the anions of interest (Fig. 1).

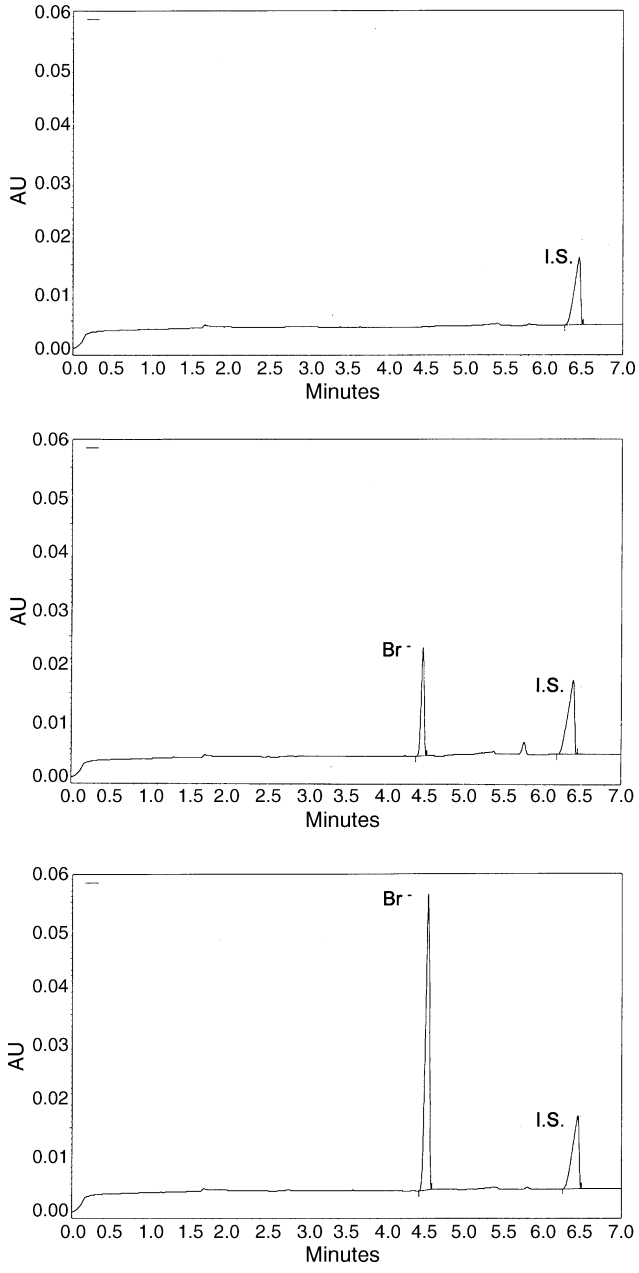

Fig. 1. Top: capillary electropherogram from blank serum (I.S. potassium thiocyanate 2 mmol/L); middle: capillary electropherogram from blank serum added with 12.5 mmol/L potassium bromide; bottom: capillary elecropherogram of a serum sample from a patient under potassium bromide treatment; calculated bromide concentration: 29.8 mmol/L. [Run conditions: uncoated fused-silica capillary (50 μm I.D., 50 cm effective lenght); running buffer, 90 mmol/L sodium tetraborate and 10 mmol/L NaCl; voltage: 20 kV, temperature: 25 °C; detection: UV absorption at 200 nm wavelength. For further details see text.]

3.1. Method validation

The limit of detection of the described method, calculated as the lowest concentration producing a signal-to-noise ratio about 5, was 0.05 mmol/L. The limit of quantification, calculated as the bromide concentration producing peak area ratios (Br^-/I.S.) with a relative standard deviation $\leq$10% was 0.1 mmol/L. The linearity of determination in serum, taking into account the therapeutic range of bromide (about 10–30 mmol/L), was tested on 6 points in the range from 2.5 to 60 mmol/L.

The resulting equation was $y = 0.0746x - 0.0372$; $R^2 = 0.9995$ (x = bromide concentration; y = bromide peak area/I.S. peak area).

The imprecision was estimated on 7 "intra-day" replicate injections of sera at bromide concentrations of 5, 20 and 60 mmol/L. In addition the same samples were stored frozen in aliquots and analyzed on 7 non-consecutive days to assess the "day-to-day" imprecision. In short, relative deviation standards of quantitative determinations were always <2% in both intra-day and day-to-day experiments. Migration time precision was excellent, when expressed as a ratio between bromide migration time and I.S. migration time, being always <1%. The slight overestimation observed in the recovery studies looks negligible in terms of clinical significance. The details of recovery and imprecision studies are shown in Table 1. Also absolute migration time repeatability was excellent with R.S.D. of 0.47% for bromide and 0.43% for thiocyanate in intra-day tests showing the stability of both EOF and ion mobility.

The injection of chloride, fluoride, iodide, nitrite and nitrate, cyanide, sulphate, carbonate, citrate, phthalate, acetate and oxalate at concentration >100 mmol/L did not give any interference on bromide determination.

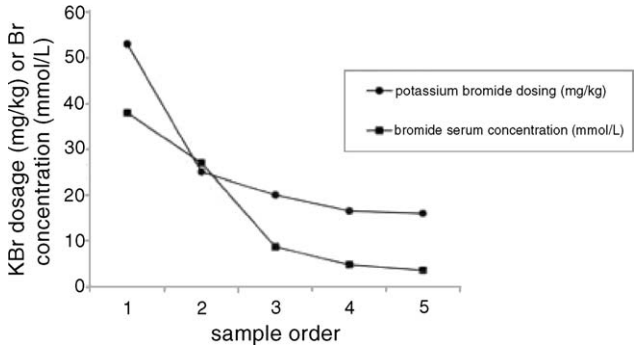

Fig. 2. Time course of bromide serum concentrations and potassium bromide dosing in an infant undergoing potassium bromide treatment (potassium bromide administered orally every day; sampling before treatment).

Table 1
Evaluation of accuracy and imprecision

Expected concentration (mmol/L)	Measured concentration (mmol/L)	Recovery (%)	Recovery R.S.D. (%)	Migration time[a] R.S.D. (%)
Intra-day ($n=7$)				
5.00	5.18	103.60	0.96	0.28
20.00	20.65	103.20	0.94	0.38
60.00	61.30	102.10	0.59	0.30
Day-to-day ($n=7$)				
5.00	5.18	103.60	1.07	0.66
20.00	20.71	103.50	1.06	0.71
60.00	61.72	102.90	0.97	0.65

[a] Relative values: bromide migration time/I.S. migration time.

The analysis of 20 clinical samples from three infants (one female, two males) suffering from refractory epilepsy and treated orally with potassium bromide at doses in the range from 150 to 675 mg/day gave bromide concentrations ranging from 3.4 to 38.1 mmol/L (see Fig. 1, for example). As shown in Fig. 2, seriate samples from the same patient undergoing potassium bromide therapy at decreasing dosages (daily oral administration) showed bromide concentrations changing in agreement with the therapy.

4. Conclusions

In the present study, for the first time, capillary electrophoresis has been applied for monitoring of potassium bromide therapy in critical patients. The advantage of this approach is based on its extremely simple application requiring reduced sample amounts (as little as 100 μL) and very limited sample treatment. No dedicated instrument or reagents are needed, thus keeping costs low and practicability high. In addition, the analysis is rapid and can easily be automated on any commercial capillary electropherograph.

On the basis of these considerations, capillary electrophoresis can be proposed as the method of choice for bromide analysis in serum samples, especially for therapeutic drug monitoring purposes.

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