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Short communication

Determination of bromide in canine plasma using ion chromatography

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ABSTRACT

A new ion chromatographic procedure has been developed and validated for the determination of bromide in canine plasma. Following a simple dilution, samples were separated on a Metrosep A Supp 5 column. The mobile phase was an isocratic mixture of 2.2 mM Na_2CO_3, 1.0 mM $NaHCO_3$, and 1% acetonitrile, with a flow-rate of 0.7 ml/min. The procedure produced a linear curve over the concentration range of 50–2500 μg/ml. The development of the assay permitted the determination of therapeutic levels after oral administration of potassium bromide to dogs being treated for epilepsy.

1. Introduction

Primary epilepsy is the most common neurologic disorder in dogs. It is defined as an imbalance in excitatory and inhibitory neurotransmission with no discernible underlying disease. Epileptic seizures are thought to arise from neurons with an increase in cell membrane excitability or imbalance of inhibitory and excitatory neurotransmitters [1]. Estimates of the incidence of canine epilepsy range from 2 to 3% of total referral teaching hospital admissions. At present two drugs are considered the mainstay of treatment for canine epilepsy: phenobarbital and potassium bromide (KBr). Phenobarbital has long been the first drug of choice in treating canine epileptics. However, it may cause blood dyscraiss and hepatotoxicity, which can be fatal. Also, within the 2–3% admitted population approximately 20–50% will eventually be classified as 'refractory epileptics' and will demonstrate inadequate seizure control on phenobarbital therapy [2].

Bromide is a halide anticonvulsant that offers an effective alternative to phenobarbital and other barbiturates for treatment of epilepsy in dogs. It reduces the frequency, severity and intensity of seizure episodes in dogs with refractory seizures. Potassium bromide is commonly administered in combination with phenobarbital however; it is gaining popularity as a single agent with some clinicians advocating its use as the first line treatment in epileptic dogs.

Bromide treatment is often initiated in epileptic dogs in response to development of unacceptable adverse effects during high-dose phenobarbital treatment. Like serum phenobarbital concentrations, measurement of serum bromide concentrations is important for management of epileptic dogs treated with bromide. Bromide concentrations can vary for a given dose, because elimination of bromide varies with renal function and chloride content in the diet [3]. Therefore, dogs with renal insufficiency should have KBr concentrations monitored frequently. Because bromide and chloride compete for renal elimination a high chloride diet increases bromide elimination. This variability in disposition among animals renders the relationship between dose and plasma or serum drug concentrations unpredictable in the individual patient. Therefore monitoring drug levels is an important tool for anticonvulsant safety and efficacy in dogs. Thus an easy, cost-effective assay that requires minimal sample handling needs to be available.

A few ion chromatography methods have been developed to measure bromide in biological fluids [4–11]. Some of the methods involve preconcentration of sample [6], post-column reaction detection [7,10,11], sample filtration [5] or use of mass spectrometry [8]. We describe a simple, specific and reproducible method to determine the concentration of bromide in canine plasma samples.

2. Experimental

2.1. Reagents and chemicals

Sulfuric acid (H_2SO_4, analytical reagent grade), acetonitrile (HPLC grade), sodium carbonate (Na_2CO_3, ACS reagent grade) and sodium bicarbonate ($NaHCO_3$, ACS reagent grade) were purchased from Fisher Scientific (Pittsburg, PA, USA). The bromide stock solution (1000 μg/ml) was purchased from Ultra Scientific (Kingstown, RI, USA).

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2.2. Chromatography

The chromatography system consisted of a 709 IC Pump, 732 IC Conductivity Detector, 733 IC Separation Center, 752 Pump Unit, 762 IC Interface, a 788 IC Filtration sample processor and an anion self-regenerating suppressor Module MSM (Metrohm-Peak, Houston, TX, USA). Samples are injected by the 788 filtration processor which contains an inline filter. The range of the detector was 100 μS/cm and the full scale was 10.0 μS/cm. The bromide was separated on a Metrosep A Supp 5 (100 mm × 4.0 mm, 5 μm) column with a precolumn Metrosep A SUPP 4/5 Guard Column. Metrodata IC Net 2.1(Metrohm-Peak, Houston, TX, USA) was used for data acquisition and processing.

The mobile phase was an isocratic mixture of 2.2 mM Na_2CO_3, 1.0 mM $NaHCO_3$ and 1% acetonitrile pumped at a rate of 0.7 ml/min. All determinations were performed at ambient temperature. The buffer was prepared fresh daily using double-deionized water and filtered through a 0.22 μm membrane and degassed before use. The suppressor regeneration solution consisted of 200 mM H_2SO_4 and 2% acetonitrile. If column contamination does occur there is a cleaning procedure recommended by the manufacturer.

2.3. Preparation of calibration standards

Working standards were prepared fresh daily from a 1000 μg/ml bromide stock solution in water. The stock solution was aliquoted into vials to prevent cross contamination. All solutions were stored at 4 °C. Appropriate amounts of stock solution were added to plasma then placed in 17 mm × 108 mm Metrohm autosampler tubes (Metrohm-Peak, Houston, TX, USA) and filled to 10 g with double-deionized water using a Sartorius H120 digital balance (Brinkman Instruments, Westbury, NY, USA). The final concentrations were 50, 100, 250, 350, 500, 1000 and 2500 μg/ml for calibration standards, and 150, 300, 750 and 1500 μg/ml were used for quality control samples. Linearity was assessed by linear regression analysis using Graphpad Prism 3.0 (GraphPad Software, San Diego, CA, USA). The calibration curve had to have a correlation coefficient of 0.99 or better. The acceptance criterion for each back-calculated standard concentration was 10% deviation from the nominal value except the lower limit of quantification (LLOQ) which was set at 20%.

2.4. Sample preparation

Previously frozen plasma samples were vortexed and diluted 1:100 by placing 100 μL in a 17 mm × 108 mm Metrohm autosampler tube. Then the tube was filled to 10 g with double-deionized water using a Sartorius H120 digital balance.

3. Results

Endogenous plasma components did not interfere with elution of bromide. Blank plasma samples for specificity testing were prepared in the same manner as study samples. Six different blank plasma samples were used in the pre-validation process. Fig. 1 shows chromatograms of a blank plasma sample (A), a 250 μg/ml plasma standard (B) and a canine sample after oral administration of KBr (C). The bromide peak elutes at 7.09 min and is well separated from interfering matrix components.

The plasma peak area versus concentration was plotted and produced a linear curve for the concentration range used (50–2500 μg/ml) with the correlation coefficients ranging from 0.998 to 0.999. The mean slopes, r^2, and intercept values are reported in Table 1. Intra-assay R.S.D. for plasma spiked with specific concentrations of bromide ranged from 0.7 to 1.8% (Table 1).

Table 1
Intra-assay accuracy, precision and assay linearity for bromide in plasma

Concentration added (μg/ml)	Concentration measured (μg/ml) (mean ± S.D.)	R.S.D. (%)
Intra-assay variability ($n = 6$)		
10	9.8 ± 0.9	9.3
150	150 ± 1.9	1.3
300	300 ± 2.2	0.7
750	760 ± 13.4	1.8
1500	1500 ± 12.3	0.8
Assay linearity ($n = 6$)	Mean ± S.D.	R.S.D. (%)
Intercept	−56.4 ± 4.0	6.3
Slope	1047.5 ± 9.0	0.9
r^2	0.9995 ± 0.0005	0.05

S.D.: standard deviation; n: number of samples.

The LOQ was 10 μg/ml and its R.S.D. was 9.3% (Table 1). The inter-assay R.S.D. ranged from 2.9 to 6.5%. The individual values are reported in Table 2. The recovery of bromide from spiked canine plasma was compared with the directly injected analyte at concentrations of 150, 300, 750 and 1500 μg/ml. Values ranged from 97 to 102% (Table 2). Testing of autosampler and short-term stability of standards for 24 h indicated that bromide is stable. Values for 150, 300, 750 and 1500 μg/ml standards after 24 h in the autosampler were 130, 270, 700 and 1430 μg/ml and 140, 270, 720 and 1460 μg/ml for 24 h short-term refrigerated storage.

Fluoride, chloride, phosphate, sulfate and oxalate, may also be quantitated using the same method. Intra-assay R.S.D. ranged from 0.7 to 3.1% for fluoride, 0.1–1.1% for chloride, 0.5–2.9% for sulfate, 0.1–0.7% for phosphate and 2.4–3.4% for oxalate. Inter-assay R.S.D. ranged from 1.5 to 4.5% for fluoride, 1.0–1.5% for chloride, 2.5–4.6% for sulfate, 1.8–3.9% for phosphate and 1.3–3.3% for oxalate. Recoveries ranged from 96 to 100% for all five compounds. Correlation coefficients ranged from 0.998 to 0.999 for all curves.

4. Discussion

The method presented here for therapeutic drug monitoring is fast, easy, reproducible and cost-effective. Bromide was quantified in canine plasma by performing a simple dilution followed by analysis with ion chromatography. The procedure requires only a 100 μl sample volume making it useful for toy breeds.

Buchberger's [7] ion chromatography procedure for bromide analysis involves the use of a post-column reaction detector. The method requires the use of methanesulphonic acid and 4,4-bis-(dimethylamino) diphenylmethane which may not typically be found in most labs and requires an additional pump for the post-column reagent. Serum samples also had to undergo ultrafiltration before they could be analyzed and bromide levels could only be approximated in a small range because of the parabolic nature of the standard curve produced by the method. Miller and Cappon [10] use 1 ml of serum and ultrafiltration. The Michigami et al. [5] method

Table 2
Inter-assay variability and recovery for bromide in plasma ($n = 5$)

Concentration added (μg/ml)	Concentration measured (μg/ml)	R.S.D. (%)	Recovery (%)
150	146	6.5	97
300	300	3.6	101
750	770	3.7	102
1500	1510	2.9	100

n: number of days.

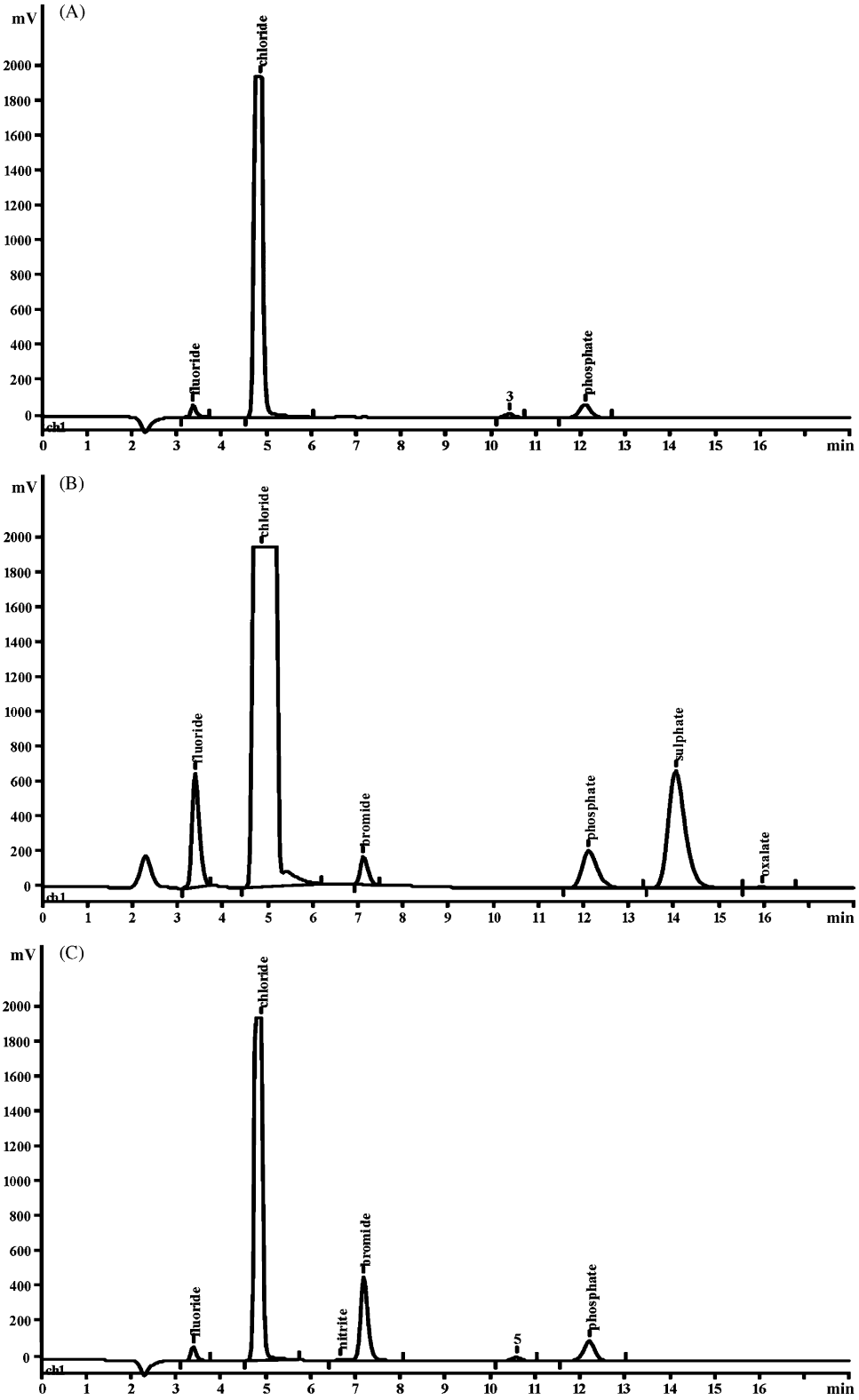

Fig. 1. Chromatograms for bromide. (A) Blank plasma sample; (B) plasma standard (250 μg/ml); (C) plasma sample after an oral dose of KBr (1030 μg/ml).

requires a 0.5 ml sample which must be diluted, filtered through an ultrafiltration membrane, transferred to a calibrated 5 ml flask and diluted again before analysis. Their method also requires the use of a coated cetylpyridinium chloride column which must be prepared, conditioned and tested before use. The column also has to be regenerated after the analysis of roughly 70 samples by washing with cetylpyridinum chloride again.

De Jong and Burggraaf [4] determine inorganic phosphate, bromide, nitrate and sulfate in serum. The peaks elute on the tail of a large peak making integration much more difficult and less accu-

rate. The method also has a 30 min run time. No other validation information was listed in the paper. Quinones et al. [8] used acetonitrile to precipitate proteins which then required evaporation with nitrogen and dilution with water. This method requires the use of mass spectrometry a sophisticated and complex instrumentation that is not widely available. Dijkgraff-ten Bolscher et al. [9] also use acetonitrile to precipitate proteins in 1 ml of plasma. The supernatant is then removed evaporated and re-dissolved in mobile phase. Although the peaks are small there appears to be interfering compounds present from the extraction. Wong et al. [11] uses ultrafiltration to remove plasma proteins and uses diluted bromide to measure extracellular water volumes in minipigs. Moore et al. [6] uses microconcentrator tubes for sample preparation. After centrifugation the sample passes through a cation exchange column into a 50 volumetric flask containing buffer then diluted with water.

Our procedure eliminates the use of acetonitrile, nitrogen evaporation, sample microconcentration, ultrafiltration consumables and mass spectrometry. It is a rugged procedure with the column still in use after 2000 injections and the guard column replaced roughly every 300 injections. The inline filter in the 788 autosampler is usually replaced after 300 injections. If the samples are extremely lipemic the inline filter may have to be replaced sooner. Fluoride, chloride, phosphate, sulfate and oxalate, may also be quantitated using the same method and nitrate may be separated. The limit of detection and recovery are more than adequate for use in therapeutic drug monitoring of bromide.

In conclusion the method developed is simple, rapid, specific and inexpensive. It can be used routinely to determine bromide in canine plasma samples. The sample size makes it useful in situations where sample size is limited. The assay was found to be suitable for the determination of therapeutic bromide levels in patients treated at this facility.

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