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Short communication

Analysis of epinephrine from fifteen different dental anesthetic formulations by capillary electrophoresis

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Abstract

A robust method for the quantification of epinephrine from 15 different commercial dental anesthetic formulations is developed using CE. This work presents an extension to a method reported earlier. The solvability of several anesthetic compounds was improved through appropriate dilutions and the addition of sodium dodecyl sulfate to the separation background electrolyte. By controlling the mobility of the analyte at different pH values, a dilute solution of epinephrine is focused into a sharp zone with the injection of about 150 nl of anesthetic solution into the capillary. This on-column concentration technique extended the concentration detection limit of epinephrine to about 5.0×10^{-7} *M* using a commercially available UV detector. A correlation plot between the measured and listed epinephrine concentration for the 15 dental anesthetic solutions demonstrated excellent accuracy of this method.

Keywords: Pharmaceutical analysis; Epinephrine

1. Introduction

Epinephrine, a natural catecholamine that acts as an important neurotransmitter of the sympathetic nervous system, is often used in pharmaceutical preparations that make up local anesthetic solutions [1,2]. Regular monitoring of solutions is vital since epinephrine has a limited shelf life due to air oxidation. Antioxidants, such as sodium metabisulfite, are commonly added to anesthetic formulations in order to minimize epinephrine oxidation.

Capillary electrophoresis (CE) is particularly well suited for the analyses of pharmaceutical products because of its small sample volume requirement, reduced overall costs and minimal environmental impact. However, there has been only one reported methodology for epinephrine quantification in pharmaceutical formulations using CE [3]. Recently, an assay for epinephrine was developed for anesthetic solutions containing lidocaine as the local anesthetic [4]. The method allowed direct sample injection with no sample pretreatment. However, the separation protocol was unable to analyze other dental anesthetic solutions that contained carticaine, bupivacaine or prilocaine. Poor solubility of these local anesthetics, under the alkaline background electrolyte (BGE) conditions used for the separation, resulted in sample precipitation inside the capillary. The current manuscript serves to extend the previous method to quantify epinephrine from fifteen different anesthetic solutions containing epinephrine. Precipitation of the local anesthetics in the capillary was avoided through

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dilution and/or the addition of sodium dodecyl sulfate (SDS) to the separation BGE.

2. Experimental

2.1. BGE preparation and chemicals

The aqueous BGE used for CE separation consisted of 160 m*M* borate (40 m*M* Borax, Sigma, St. Louis, MO, USA) and 1 m*M* ethylenediaminetetraacetic acid (EDTA, BDH, Toronto, Canada). The pH of the BGE was adjusted to 10.2 by using 0.1 *M* NaOH (BDH). SDS and epinephrine (epinephrine bitartrate) were purchased from Sigma. Dilutions were made using either the separation BGE, or a solution containing 220 m*M* glucose (Raylo, Alberta, Canada) and 30 m*M* *para*-hydroxybenzoic acid (Eastman, New York, NY, USA). Dental anesthetic solutions were provided by Astra Pharma Canada (Mississauga, Canada). The dental anesthetic solutions contained lidocaine (Xylocaine, nine different products, 5–20 mg/ml), bupivacaine (Sensorcaine, three different products, 2.5–5 mg/ml), carticaine (Astracaine, two different products, 40 mg/ml) or prilocaine (Citanest, one product, 40 mg/ml) as the local anesthetic. Solutions also contained various additives and impurities such as sodium metabisulfite, sodium chloride, methylparaben, 2,6-xylidine, *o*-toluidine and hydroxybenzoic acid. The concentrations of epinephrine in dental anesthetic solutions (5–20 μg/ml) are about 500- to 8000-times lower than the local anesthetic (2.5–40 mg/ml).

2.2. Capillary electrophoresis system

Separations were performed on a P/ACE 5000 automated CE system [Beckman Instruments (Canada), Mississauga, Canada]. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 57 cm×75 μm I.D.×365 μm O.D. were used. New capillaries were first rinsed with 1.0 *M* NaOH for 5 min under high pressure (20 p.s.i.; 1 p.s.i.= 6894.76 Pa), followed by rinsing with the separation BGE for 10 min. The capillary was then left to equilibrate overnight in the separation BGE prior to use. Each separation was preceded by a 1.5-min high-pressure (20 p.s.i.) rinse with 0.1 *M* NaOH, followed by a 4-min rinse with the separation BGE. The samples were then introduced using a 25-second low-pressure injection (0.5 p.s.i.) and the separation was carried out for 15 min at 15 kV under a temperature of 25°C. Absorbance was monitored at 280 nm and data were collected and processed using System Gold.

3. Results and discussion

Borate was selected as the BGE because of its ability to selectively complex with epinephrine (because of its vicinal dihydroxyl functionality) from the other components contained in the anesthetic solution. Fig. 1 depicts a typical electropherogram obtained from the direct injection of 150 nl of Xylocaine 1.5% using a 160 m*M* borate BGE, containing 1 m*M* EDTA at a pH 10.2. Excellent separation is obtained since epinephrine migrates with a negative electrophoretic mobility due to borate complexation, whereas lidocaine co-migrates with the electroosmotic flow. Sample injection in CE is typically no greater than 1% of the capillary length (about 10 nl) because of band broadening. Despite the relatively large sample volume injected (about 150 nl), epinephrine elutes as a focused band. In contrast, lidocaine elutes as a broad sample plug. This type of selective analyte focusing is interesting since it occurs naturally with the BGE conditions selected. In addition, analyte focusing occurs even with the presence of high concentrations of salt. Good concentration sensitivity for epinephrine is achieved due to the large sample volumes injected and efficient analyte focusing. The limit of detection for epinephrine, when 150 nl, of sample was injected, is 5×10^{-7} *M* using a commercial UV absorbance detector. The concentration sensitivity for epinephrine can be further extended by using larger injection volumes, in conjunction with longer and wider capillaries. On-column concentration of epinephrine, via a dynamic pH junction between acidic sample and basic BGE zones, is hypothesized to be operative in this system [4,5].

The direct injection of three other dental anesthetic formulations (Astracaine, Sensorcaine and Citanest) with the borate BGE resulted in sample precipitation. Efforts were made to improve the solvability of these

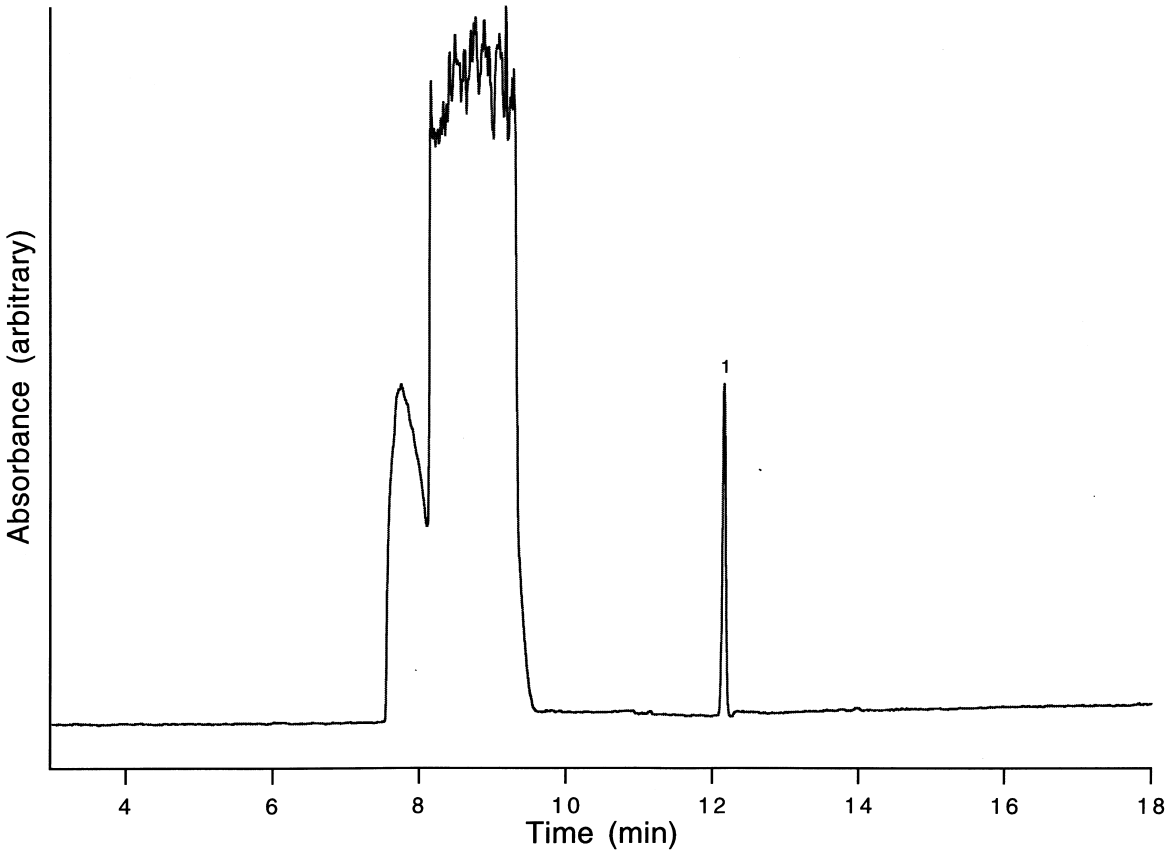

Fig. 1. Electropherogram showing the separation of epinephrine (1) from excess lidocaine by directly injecting Xylocaine 1.5% dental anesthetic solution that contains 5 μg/ml of epinephrine. Peak 1 is epinephrine.

local anesthetics in the alkaline conditions of the original BGE system. The addition of acetonitrile or methanol to the borate BGE prevented sample precipitation, but resulted in poor separation and loss of epinephrine focusing. Similarly, the pH of the borate BGE was lowered incrementally from 10.2 to 9.0, which improved the aqueous solubility of the dental anesthetic solutions, but decreased the separation and focusing of epinephrine.

It was observed that dilution of the dental anesthetic solutions prior to injection prevented sample precipitation, while retaining excellent separation. Fig. 2 shows the electropherograms obtained from Citanest 4% and Sensorcaine 0.5%. Citanest 4% was diluted with the borate BGE, followed by vortexing for several seconds. The high insolubility of Sensorcaine 0.5% in the borate BGE required dilution with a viscous and acidic solution made from glucose and hydroxybenzoic acid. This solution was used since it resembles the original matrix of the dental anesthetic solution. Astracaine solutions that were diluted with the preceding solution were soluble in the separation BGE, but a large background (anesthetics) signal overlapped with epinephrine elution. The use of 50 m*M* SDS in the borate BGE resulted in a good separation of epinephrine from other components in the solution (Fig. 3). Dilution of Astracaine solutions was also carried out with the 50 m*M* SDS borate BGE. Table 1 summarizes the sample preparations for the dental anesthetic solutions used in this report. Relative standard deviation based on six replicates averaged 2.5% for the dental anesthetic solutions. The accuracy of the epinephrine assay was evaluated by using a plot of the measured values (y) vs. the

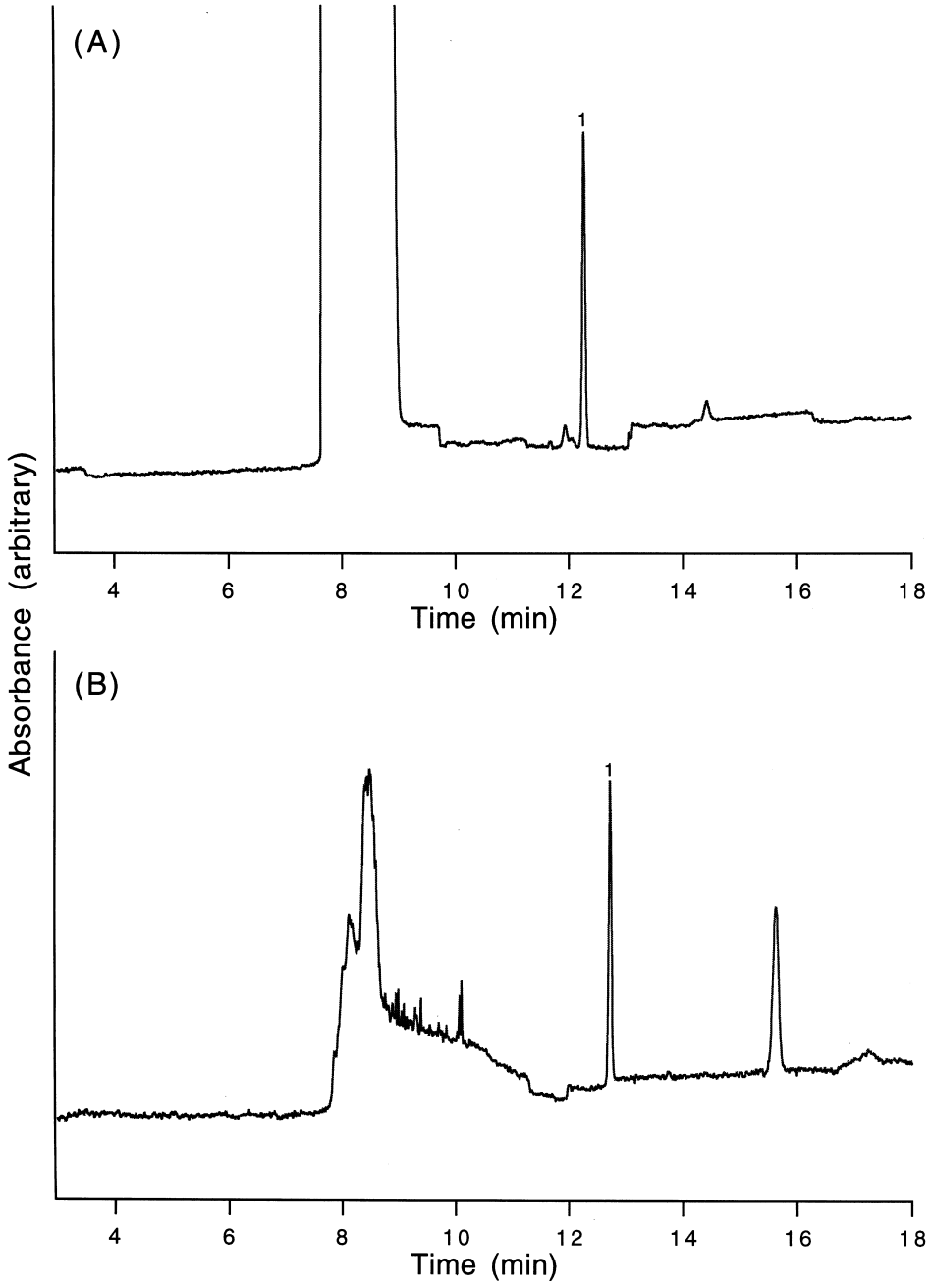

Fig. 2. Electropherograms depicting the separation of epinephrine from excess prilocaine and bupivacaine with (A) two-fold dilution of Citanest 4% in the separation BGE and (B) three-fold dilution of Sensorcaine 0.5% in glucose–hydroxybenzoic acid solution prior to injection. Both dental anesthetic solutions contain 5 μg/ml of epinephrine. Peak 1 is epinephrine.

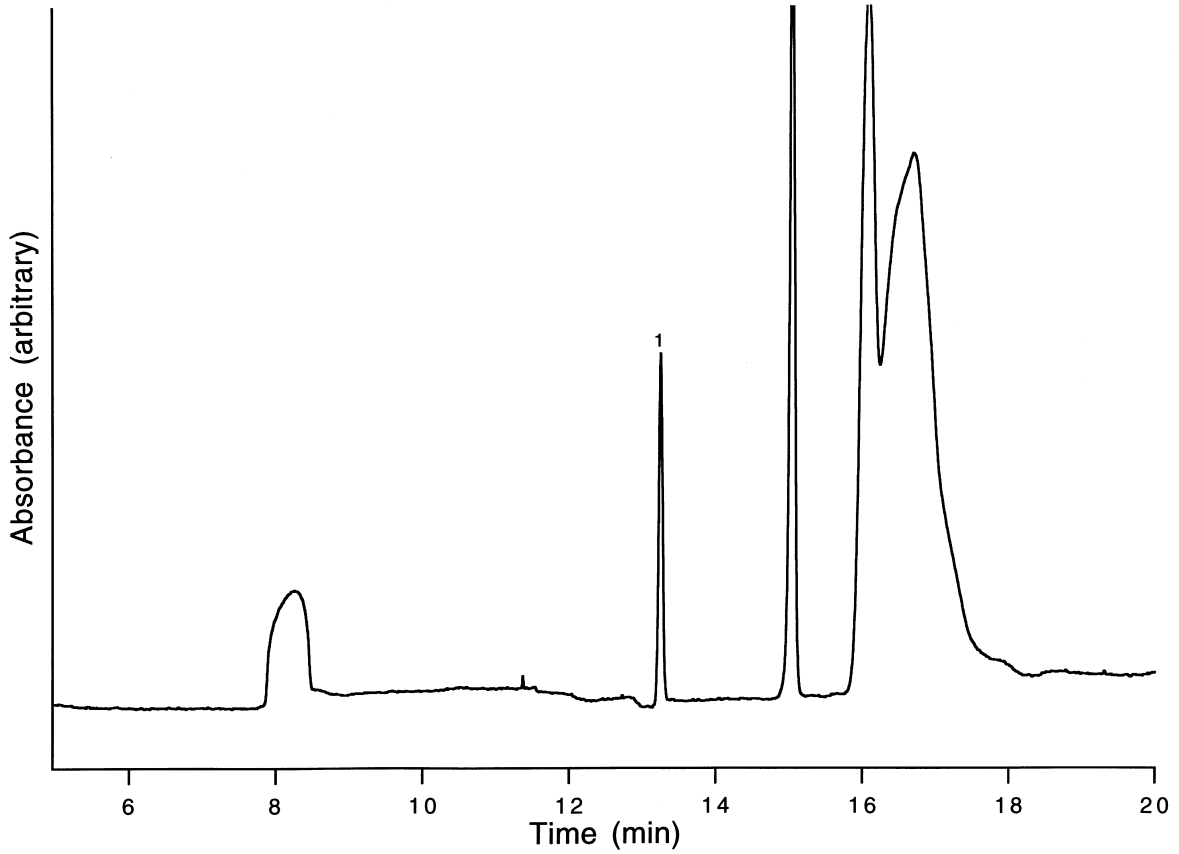

Fig. 3. Electropherogram showing the separation of epinephrine from excess carticaine, using 50 m*M* SDS in borate BGE. A two-fold dilution of Astracaine 4% that contains 5 μg/ml of epinephrine was performed using the separation BGE prior to injection. Peak 1 is epinephrine.

listed concentrations (x), for all of the dental anesthetic solutions analyzed. The correlation plot, $y = 0.980x - 0.227$, has an R^2 of 0.997, which demonstrated excellent accuracy and reliability.

4. Conclusion

A robust method for the quantification of epinephrine from fifteen dental anesthetic solutions by CE

Table 1
Sample preparation used for dental anesthetic solutions prior to analysis by CE

Dental anesthetic solution	Sample dilution
Xylocaine	No dilution required
Citanest	Two-fold dilution with the separation BGE
Sensorcaine	Three-fold dilution with the glucose–hydroxybenzoic acid solution
Astracaine	Two-fold dilution with the separation BGE

is developed. Good selectivity, precision, accuracy and reliability is demonstrated with this novel separation protocol. Appropriate dilution of six of the 15 anesthetic formulations was necessary to prevent precipitation, whereas the remaining solutions were injected directly into the capillary. The use of the anionic surfactant, SDS, to the optimized separation BGE was required to improve the separation of Astracaine solutions. The focusing effect is observed to arise naturally for epinephrine even with the presence of high concentrations of salt and other components contained in the sample solutions. This allowed the injection of large amounts of sample, resulting in improved concentration sensitivity for epinephrine analysis by CE with UV detection.

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