



Short communication

A sub-minute electrophoretic method for simultaneous determination of naphazoline and zinc



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ABSTRACT

This paper reports for the first time, a method for simultaneous determination of naphazoline (NPZ) and zinc (Zn) using an analytical separation technique (capillary electrophoresis with capacitively coupled contactless conductivity detection -CE-C⁴D). A single run is possible every 55 s (sampling rate = 65 h⁻¹). The separation by CE-C⁴D was achieved on a fused silica capillary (50 cm length – 10 cm effective, 50 μm i.d.) with a background electrolyte (BGE) composed by 20 mmol L⁻¹ of 2-(morpholin-4-yl)ethane-1-sulfonic acid (MES) and 20 mmol L⁻¹ of histidine (HIS) (pH 6.0). Detection limits were estimated at 20 and 30 μmol L⁻¹ and recovery values for spiked samples were 98 and 102% for NPZ and Zn, respectively. The developed procedure was compared to HPLC (NPZ) and FAAS (Zn) and no statistically significant differences were observed (95% confidence level).

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1. Introduction

Naphazoline (NPZ) is an imidazoline derivate used as a topical, fast and long-acting decongestant, by nasal and ocular routes [1]. Its therapeutic action results from direct stimulation of β-adrenergic receptors causing local vasoconstriction [2]. Excessive doses of NPZ can cause central nervous system depression, bradycardia, and hypotension, by the inhibition of central sympathetic activity [3]. NPZ has enhanced effects when used in conjunction with Zn salts due to a synergistic effect [4].

Ideally, the quality control of pharmaceutical formulations containing more than one active principle should be carried out in a single analytical run. However, the determination of NPZ and Zn in a single analytical procedure is a challenge task due to their different properties. NPZ is an organic compound and can be determined by potentiometry [5], voltammetry [6], photometry [7], high performance liquid chromatography (HPLC) [8], capillary electrophoresis [9,10] and atomic absorption/emission spectrometry (indirect method) [11]. Procedures for Zn determination are based on spectrophotometry [12], atomic spectroscopy [13], capillary electrophoresis [14], polarography [15], and neutron activation analysis [16]. To our knowledge, a single procedure for simultaneous determination of NPZ and Zn was previously reported. It is

based on a batch injection analysis with square-wave voltammetric detection [17]. The possibility of contamination of the working electrode during the analysis can be considered a disadvantage of this method.

Capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C⁴D) is a separation technique widely used for simultaneous determination of charged molecules [18–21]. This system requires simple instrumentation and often provides a short analysis time, low-cost, high separation efficiency and low consumption of reagents and samples [22,23]. The possibility of simultaneous determination of organic and inorganic species (e.g. drugs and their counter-ions) can also be considered an advantage [24–27]. In addition, the universal character of the contactless detector is often useful for determination of chromophoric and non-chromophoric compounds in a single run. Three strategies are commonly employed to prepare the background electrolyte [19]: (1) use of a low mobility co-ion and counterion for analysis of high mobility species (most common strategy) [18,21,28]; (2) use of a high mobility co-ion and counterion for analysis of low mobility species [23,29]; (3) use of background electrolyte with intermediate mobility for analysis of species with higher and lower mobility (positive and negative peaks are detected in the same electropherogram) [30–32]. The universal detection capability and the operation in contactless mode also have a crucial role in miniaturized CE systems [33–37].

In this work, a new and fast method for simultaneous determination of NPZ and Zn based on CE-C⁴D is reported. For the first

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time, the possibility of simultaneous determination of NPZ and Zn is shown using an analytical separation technique.

2. Materials and methods

2.1. Reagents and samples

Deionized water ($R \geq 18\text{M}\Omega\text{cm}$) from a Millipore Direct-Q3 system (Bedford, MA, USA) was used to prepare all aqueous solutions. Acetonitrile and phosphoric acid (HPLC grade) were purchased from Merck (Darmstadt, Germany), metallic Zn from Synth (Diadema – Brazil), 2-(morpholin-4-yl)ethane-1-sulfonic (MES) acid and histidine (HIS) from Vetec and naphazoline hydrochloride (NPZ) from Sigma-Aldrich (St. Louis, United States). A 1000mg L^{-1} Zn stock solution was prepared by dissolving 100 mg of metallic Zn in 3 mL of HCl 65% (v/v) and diluted to a final volume of 100 mL with deionized water. NPZ stock solution was freshly prepared just before the experiments by dissolution in deionized water.

Pharmaceutical samples (eye drops) were purchased from local drug stores, labeled to contain NPZ and ZnSO_4 in concentrations of 0.15 and 0.30mg mL^{-1} , respectively. Samples were submitted to different dilutions: 2-fold and 3-fold for samples 1 and 2, respectively.

2.2. CE measurements

Electrophoresis separations were performed in a homemade CE equipment with a compact and high-resolution capacitively coupled contactless conductivity detector (C^4D) [18,19]. The detector was positioned surrounding the capillary at 10 cm from the inlet site. The fused-silica capillary used in all experiments was 50 cm long (effective length of 10 cm) and $50\ \mu\text{m}$ i.d. \times $375\ \mu\text{m}$ o.d. (Agilent, Folsom, CA, USA). Prior to use, the capillary was flushed with deionized water for 10 min, 0.1mol L^{-1} NaOH for 10 min, again with deionized water for 10 min and finally with background electrolyte (BGE) for 10 min. The samples were injected hydrodynamically for 1 s at 25 kPa. The total analysis time for each injection (used to obtain the sampling rate) consists of the time necessary for the sample injection, vial replacements, and separation (analysis).

2.3. Reference procedures

HPLC/UV–vis measurements were performed on a Shimadzu LC-10 VP chromatograph equipped with an UV–vis detector (SPD-10AV), a LC column (Phenomenex 110A° Gemini-C18, 250 mm \times 4.6 mm, $5\ \mu\text{m}$), a manual injector ($20\ \mu\text{L}$) and a pump (LC-10AD-VP). Mobile phase was composed by a mixture (32:68, v/v) of methanol and 0.01mol L^{-1} H_3PO_4 (pH adjusted to 2.8 by adding triethylamine), under a flow-rate of 1.0mL min^{-1} . Measurements were performed at 280 nm (adapted from [38]).

Flame atomic absorption spectrometric measurements were performed on a Varian SpectrAA 220 spectrometer, under the following conditions: effective bandwidth 0.7 nm; wavelength 213.9 nm; HCL current 10 mV; hollow cathode lamp (HCL) L1788-30NE; flame temperature about 2700 K; mixture $\text{C}_2\text{H}_2/\text{air}$ 1.25/5.25 L/min (adapted from [39]).

3. Results and discussion

3.1. CE optimization

The separation efficiency of NPZ by CZE depends on the presence of its charged species in solution. The presence of these species can be predicted by the NPZ dissociation constant, which is estimated at 6.30×10^{-11} ($\text{pK}_a = 10.2$) [40,41], indicating that there is the pre-

Table 1

Optimized conditions for the proposed CE- C^4D procedure.

Parameters	Evaluated range	Optimized value
BGE concentration (mmol L^{-1})	10–30	20
Separation voltage (kV)	15–25	25
Injection time (s) at 25 kPa	0.75–2.0	1.0
Temperature ($^{\circ}\text{C}$)	20–30	25

Table 2

Analytical features of the proposed CE- C^4D procedure (confidence interval = 95%).

Analytical feature	NPZ	Zn
Migration time (s)	32.4 ± 0.3	40.2 ± 0.5
Resolution	5.65 ± 0.11	3.49 ± 0.14
Correlation coefficient	0.997	0.999
Linear range ($\mu\text{mol L}^{-1}$)	100–500	300–1500
LOD ($\mu\text{mol L}^{-1}$)	20	30
LOQ ($\mu\text{mol L}^{-1}$)	67	100
Analytical frequency (h^{-1})	72	72
Intra-day RSD (n = 10)	2.0%	1.2%
Inter-day RSD (n = 3)	4.7%	5.5%

dominance of its cationic form below pH 10. Zn is found as Zn^{2+} in acid solutions. The concentration of the bivalent cation decreases by increasing pH due the formation of $\text{Zn}(\text{OH})^+$, $\text{Zn}(\text{OH})_2(\text{s})$, $\text{Zn}(\text{OH})_3^-$ and $\text{Zn}(\text{OH})_4^{2-}$. Precipitation of $\text{Zn}(\text{OH})_2(\text{s})$ is observed above pH 8, which is therefore inappropriate for CE separation. Considering this information, proper pH for NPZ and Zn separation should be set below 7.0.

As previously reported [42,43], Zn determination by CE- C^4D can be performed by using a pH 6.0 MES/HIS buffer (BGE) with equal molar ratio prepared in water/methanol mixture. In this background buffer, Zn is chelated with HIS (through the imidazole rings) forming $\text{Zn}-(\text{HIS})_2^+$ cationic species [44,45]. The complex formation reduces the Zn mobility increasing peak resolution from sodium, which is usually present in high concentrations in eye drop samples. Simulations carried out using Peakmaster® software [46] revealed that low resolution is achieved for sodium and Zn peaks (absence of complexation). In addition, the use of low mobility counter-ion in CE background buffers (e.g. MES/HIS) is a common practice to avoid temperature increasing and increased noise level in the detector [19].

Based on these effects, different concentrations of MES/HIS (10, 20 and 30mmol L^{-1}) with and without methanol addition were evaluated for NPZ and Zn CE separation. The BGE composed of 20mmol L^{-1} of MES and HIS without methanol addition (pH = 6) showed the best results (stability, resolution, sample throughput) for the simultaneous determination of NPZ and Zn by CE- C^4D . Table 1 shows the optimized instrumental parameters of the proposed CE- C^4D method.

3.2. Analytical features and application

Fig. 1 shows the first, fifth and tenth electropherogram obtained for ten successive injections of a standard solution containing $300\ \mu\text{mol L}^{-1}$ of NPZ and $930\ \mu\text{mol L}^{-1}$ of Zn using 20mmol L^{-1} MES/HIS (pH = 6.0) as background electrolyte.

These data reveal that the proposed method is stable (intra-day RSD < 2% and inter-day < 5.5%; related to peak areas), fast (~ 65 injections h^{-1}), and present high resolution (> 3.5). Linear responses ($r > 0.997$ without use of IS) were observed in the ranges of 100–500 and $300\text{--}1500\ \mu\text{mol L}^{-1}$ for NPZ and Zn, respectively. Limits of detection were determined at 20 and $30\ \mu\text{mol L}^{-1}$ for NPZ and Zn, respectively. A summary of the analytical characteristics are shown in Table 2.

A comparison between electropherograms obtained for a standard and sample solution (both diluted in water) is shown in Fig. 2.

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