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Determination of suxamethonium in a pharmaceutical formulation by capillary electrophoresis with contactless conductivity detection (CE-C^4D)

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ABSTRACT

A simple method based on capillary electrophoresis with a capacitively coupled contactless conductivity detector (CE-C^4D) was developed for the determination of suxamethonium (SUX) in a pharmaceutical formulation. A hydro-organic mixture, consisting of 100 mM Tris–acetate buffer at pH 4.2 and acetonitrile (90:10, v/v), was selected as background electrolyte (BGE). The applied voltage was 30 kV, and the sample injection was performed in the hydrodynamic mode. All analyses were carried out in a fused silica capillary with an internal diameter of 50 μm and a total length of 64.5 cm. Under these conditions, a complete separation between SUX, sodium ions and the main degradation products (choline) was achieved in less than 4 min. The presence of acetonitrile in the BGE allowed a reduction of SUX adsorption on the capillary wall. The CE-C^4D method was validated, and trueness values between 98.8% and 101.1% were obtained with repeatability and intermediate precision values of 0.7–1.3% and 1.2–1.6%, respectively. Therefore, this method was found appropriate for controlling pharmaceutical formulations containing suxamethonium and degradation products.

1. Introduction

Suxamethonium (SUX) chloride (also known as succinylcholine) is a medication widely used in emergency medicine and anesthesia to induce muscle relaxation. It is used to make endotracheal intubation possible and acts as a depolarizing neuromuscular blocker [1]. SUX has two quaternary ammonium groups contributing to the very high polarity of the compound (Fig. 1). The chemical instability of SUX is well known [2]; it is rapidly hydrolyzed in aqueous alkaline solution to succinylmonocholine (SMC) and choline, and further hydrolyzed to choline and succinic acid. The chemical structures of these compounds are shown in Fig. 1. The adsorption on different surfaces, as well as the stability of SUX and its major hydrolysis product (SMC), was investigated by Tsutsumi et al. [2]. An adsorption of SUX to glassware (not to plasticware) occurred, and the sufficient stability of the samples was demonstrated in acidic conditions and in distilled water.

Several analytical methods were previously reported for the determination of suxamethonium [1–6]. The lack of a chromophore required other detection techniques in place of direct UV absorbance. Most of them used HPLC coupled with mass spectrometry (MS) [2,4] or electrochemical detection [1,3]. The separation of SUX and its degradation products by HPLC was often insufficient, and analysis times of more than 20 min were needed. Because these studies did not demonstrate a complete separation between SUX and its degradation products, a highly selective detector like MS was needed to counterbalance the low resolution of the analytical separation. Capillary electrophoresis (CE) with indirect UV detection is an alternative to analyze quaternary ammonium compounds [7–10]. SUX can be analyzed by CE, since it is a small molecule with a high polarity. With the CE-indirect UV analysis, it is well known that peak shapes and sensitivity depend on the relative mobilities of the analyte and the background electrolyte (BGE) [10]. Thus, in order to obtain a good detection signal, a BGE with mobility matching that of the counter-ions is required. The search for a suitable BGE in indirect optical detection remains a compromise between matching electrophoretic mobilities, concentrations, maximum absorption wavelength, molar absorptivity, and charge of the analyte [11]. Generally, indirect UV can provide an acceptable means of detection, however, with strongly reduced sensitivity [12]. Another approach to determine SUX was achieved by CE coupled with attenuated total internal reflectance infrared microspectroscopy (FT-IR) [6]. However, no simple method that sufficiently separated SUX and its degradation products in a pharmaceutical formulation has been described in the literature.

SUX possesses high conductivity due to the presence of quaternary ammonium groups allowing a conductimetric detection. During the past few years, contactless conductivity detection (CCD)

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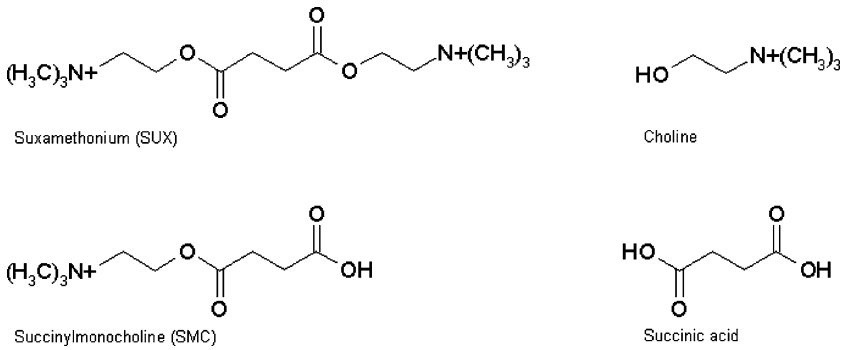

Fig. 1. Structures of suxamethonium and degradation products.

has become a good alternative to optical detection techniques in CE [12], and a capacitively coupled contactless conductivity detector (C^4D) was developed by Zemann [11,13]. The detector presents two metal tube electrodes, placed around the capillary. An oscillation frequency between 75 and 300 kHz is applied to one of the electrodes, and a signal is produced when an analyte zone with a different conductivity passes through the retention gap [14]. Inorganic cations and anions have been successfully analyzed by CE-C^4D, but the method is also suitable for organic ions such as alkylammonium cations [15]. In comparison with MS, C^4D can be considered a simple and inexpensive detection technique for routine analysis.

In this study, a CE-C^4D method was developed and validated to determine suxamethonium in a formulation and was applied to the quantitation of SUX in commercially available pharmaceutical products (Lysthenon and Succinolin).

2. Experimental

2.1. Chemicals

Succinylcholine chloride dihydrate, choline chloride, potassium chloride, and Tris(hydroxymethyl)–aminoethane (Tris) were purchased from Fluka (Buchs, Switzerland). Succinic acid was purchased from Sigma–Aldrich (Steinheim, Germany). Water and NaCl (0.9%) used for pharmaceutical preparations were obtained by Bichsel Laboratories (Interlaken, Switzerland). Acetic acid (glacial, 100%), methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Succinylmonocholine was obtained by the degradation of a succinylcholine solution at 10 mg mL^{-1} in an alkaline solution [2]. Lysthenon (2% and 5%) was purchased from Nycomed Pharma SA (Dübendorf, Germany) and Succinolin was obtained from Amino AG (Neuenhof, Switzerland).

2.2. BGE preparation

Different BGEs (phosphate, citrate, 2-(N-morpholino) ethanesulfonic acid/histidine, lactate, acetate, at several pH and ionic strengths) were used for the development of the method. The final BGE was a hydro-organic buffer corresponding to a mixture of an aqueous BGE (100 mM Tris–acetate buffer at pH 4.2) and acetonitrile (90:10, v/v). The aqueous BGE was prepared by an adequate dilution of the concentrated acid solution, and a solution of Tris at 1 M was added to adjust the solution to pH 4.2. The solution was then diluted to the final volume with distilled water. The BGE was degassed in an ultrasonic bath for 10 min before use.

2.3. Instrumentation and capillaries

CE experiments were carried out with an $HP^{3D}CE$ system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler and a power supply able to deliver up to 30 kV. $HP^{3D}CE$ was coupled to a TraceDec detector (Innovative Sensor Technologies GmbH, Strasshof, Austria). The conductivity sensor consisted of two electrodes separated by a detection gap of 1 mm, positioned along the capillary by sliding it into the desired position (14.5 cm from the cathode). A CE ChemStation (Agilent) was used for CE control and data handling, and a C^4D Tracemon (Innovative Sensor Technologies) was used for conductivity detector control and data acquisition. Analyses were performed in uncoated fused silica (FS) capillaries from BGB Analytik AG (Böckten, Switzerland) with an internal diameter (i.d.) of 50 μm, an outside diameter (o.d.) of 375 μm and a total length of 64.5 cm (effective length of 50 cm). Capillaries coated with poly(vinyl alcohol) (PVA) from Agilent (Waldbronn, Germany) with 50 μm i.d. and 32.5 cm total length were also tested. All experiments were performed in the cathodic mode. The capillary was thermostated at 25 °C in a high velocity air stream, and a voltage of 30 kV was applied. The generated current was between 5 and 50 μA depending on the buffer solution. Samples were kept at ambient temperature in the autosampler and injected in the hydrodynamic mode to fill approximately 1% of the effective capillary length (40 mbar for 10 s). The final configuration of the C^4D was set at an output frequency of 75 kHz, an output voltage of 80 Vpp, 50% of gain and an offset of ~50. The detector acquisition corresponded to the CE mode of 19.8 Hz. Before first use, FS capillaries were sequentially rinsed with methanol, 0.1 M NaOH, water, methanol, 0.1 M HCl, water and BGE for 5 min. A voltage of 30 kV was then applied for 60 min with the BGE. The TraceDec was set to run for 1 h before the first analysis in order to obtain a constant signal. Prior to each sample injection, the capillary was rinsed by pressure (940 mbar) for 3 min with fresh BGE. When not in use, the capillary was rinsed with water and methanol. As the electrophoresis process altered the running buffer pH by electrolysis and subsequently changed the migration times, the separation buffer was refreshed every six runs.

2.4. Method validation

A validation was performed to estimate the potential of the method for the quantitative analysis of suxamethonium in a pharmaceutical formulation. The validation was based on the guidelines of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) [16] and carried out over three series. Each series involved the injection of a freshly prepared BGE, two calibration standards (CS), four validation standards or quality control (QC) samples at 80%, 100% and 120% each, water and methanol rinsing of

the capillary, and instrument shut-off. Potassium chloride was used as the internal standard (IS). The calculations were performed using normalized area (area/migration time) ratios of suxamethonium on the internal standard.

2.5. Sample preparation

A stock standard solution was prepared by dissolving suxamethonium in water for CS and in 0.9% NaCl for QC in order to obtain a concentration of 10 mg mL^{-1}, and was stored at 4 °C until use. The stock standard solution for QC corresponds to a commonly presented dilution of SUX in the hospital. The IS stock solution was prepared by dissolving potassium chloride in water at a concentration of 10 mM. For CS and QC, three concentration levels were prepared at 80%, 100% and 120% of the target value by diluting the appropriate volume of SUX stock solution in distilled water. Potassium chloride was added as an internal standard to obtain a final concentration of 0.4 mM. Sample solutions were stable for more than 2 days at 4 °C, and no degradation was observed for the tested analytes during analysis. To study the separation of SUX and its degradation products, samples with 10 mg mL^{-1} of SUX, choline, succinic acid and SMC were prepared in 0.9% NaCl.

2.6. Application to pharmaceutical products

Suxamethonium was determined in the commercially available pharmaceutical products Lysthenon® (2% and 5%) from Nycomed Pharma SA (Dübendorf, Germany) and Succinolin® (5%) from Amino AG (Neuenhof, Switzerland). Therefore, the formulations were diluted in distilled water to obtain a final concentration of 0.4 mg mL^{-1} of SUX with 0.4 mM of the IS corresponding to the 100% STD. The quantitative analysis was repeated five times ($N = 5$) for each formulation.

3. Results and discussion

SUX used in emergency medicine and anesthesia is administrated as an isotonic formulation. For determining this compound, a CE-C^{4}D method was developed and validated.

3.1. Method development

3.1.1. Buffer selection

The selection of the BGE was based on conductivity detection of suxamethonium and sufficient selectivity toward degradation products and sodium. In C^{4}D, the response arises from the difference in conductivity between the analytes and BGE co-ions. For obtaining the highest signal-to-noise ratio, the largest possible difference of the conductance of the analyte and electrolyte is required. Nevertheless, CE requires the use of electrolytes with a higher ionic strength compared to the sample zone in order to take advantage of the stacking effect. The compromise consists of using an amphoteric or low conductance buffer at high ionic strength [13]. Furthermore, the BGE requires a pH inferior to 7, in order to avoid the hydrolysis of suxamethonium during the analysis [2]. Among the different tested BGEs, the Tris–acetate buffer presented the best compromise for performing the complete separation of suxamethonium and its degradation products. This BGE possesses a low conductivity and can be used at an ionic strength of 100 mM without generating a high current (~20 μA). The pH range of this system was between 4 and 5, where SUX was stable.

Potassium chloride was chosen as the internal standard because it presents a much higher mobility than the other compounds (SUX, SMC, choline and sodium).

As expected with the Tris–acetate buffer, the effective mobilities of the compounds were in the following decreasing order: potassium, sodium, SUX, choline and SMC. Choline was detected close to SUX and SMC migrated afterwards, at 6 min. In these conditions, succinic acid did not interfere with the cation analysis. The other compounds were detected in less than 4 min as presented in Fig. 2.

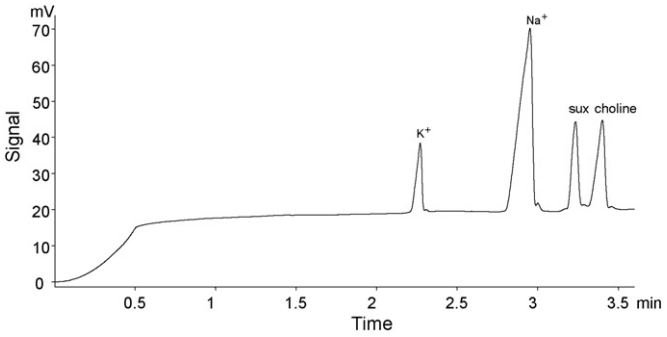

Fig. 2. Electropherogram obtained for the CE-C^{4}D analysis of a sample containing SUX (0.2 mg mL^{-1}), choline (0.2 mg mL^{-1}) and K$^+$ (0.4 mM) in an aqueous solution (presence of Na$^+$ at 3 mM). BGE: 100 mM Tris–acetate at pH 4.2, acetonitrile (90:10, v/v). All other experimental conditions are described in Section 2.3.

3.1.2. Influence of pH

The BGE conductivity depends mainly on the pH of the solution. Initial experiments were performed at pH 4.8 in order to work at the highest buffer capacity. However, the BGE conductivity decreased at lower pH values, while the detection of suxamethonium was improved. Acetate/Tris buffer solutions with different pH were tested in the buffer region, and pH 4.2 was selected since the signal to noise ratio of SUX was significantly enhanced (data not shown).

3.1.3. Influence of the buffer concentration

The first analyses were performed with a buffer concentration of 20 mM to reduce the background conductivity. Nevertheless, to improve the resolution between sodium and suxamethonium, buffer solutions with higher molarities were tested. Investigations of the effect of Tris–acetate ionic strength on the electrophoretic mobilities of organic anions showed that ion association and/or complexation equilibria could occur with this buffer system [17]. The electrophoretic mobility of ions was influenced by interactions with buffer components, which can enhance the selectivity in CE [18,19]. Acetic acid is a weak complexing agent that could interact with cations present in the system such as suxamethonium, choline, sodium or potassium. In this work, the resolution between sodium and SUX was improved by increasing the buffer concentration. Therefore, a 100 mM Tris acetate buffer at pH 4.2 was selected and, under these conditions, the generated current was still acceptable (inferior to 30 μA).

Different oscillation voltages and oscillation frequencies of the C^{4}D were tested (data not shown). An oscillation voltage of 80 Vpp and a frequency of 75 kHz gave the best results with the selected BGE.

The LOD of the method was estimated (*ca.* 10 μg mL^{-1}) and was much lower than the target value (*i.e.* 200 μg mL^{-1}) obtained after dilution of the pharmaceutical formulation.

3.1.4. Adsorption on the capillary wall

As described in the literature, quaternary ammonium groups can interact with the capillary walls [7,8,20], and a significant adsorption of SUX to glassware has been reported [2].

With the 100 mM Tris–acetate buffer at pH 4.2, deterioration of the SUX peak shape occurred after several runs (Fig. 3A). The capillary was flushed with the buffer solution for 5 min, and then a voltage of 30 kV was applied for 60 min before starting the

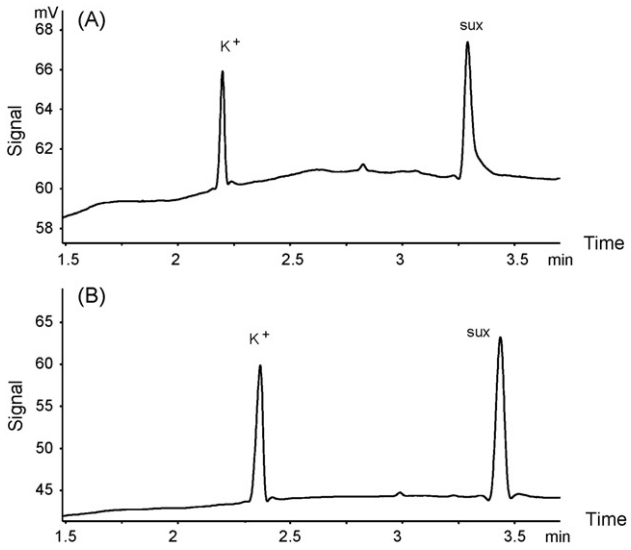

Fig. 3. Electropherograms obtained for the CE-C^{4}D analysis of a sample containing K^+ (0.4 mM) and SUX (0.2 mg mL^{-1}) in water using the BGE (A) 100 mM Tris–acetate at pH 4.2 and BGE (B) 100 mM Tris–acetate at pH 4.2, acetonitrile (90:10, v/v). All other experimental conditions are described in Section 2.3.

analysis. This procedure contributed to a constant peak shape, but a tailing was observed and the symmetry was insufficient (0.54).

To avoid the adsorption of the compound onto the capillary wall, different strategies could be applied. The use of higher temperatures or extreme pH values could help to decrease adsorption [21], but due to the low stability of suxamethonium, this approach was not investigated. In the literature, the use of organic modifiers in the BGE is often recommended [7,8,20] since they change the viscosity and the solvation ability of the carrier electrolyte, thus inducing better peak shapes. As an example, methanol, acetonitrile and tetrahydrofuran were tested as buffer additives in order to disrupt micelle formation within the sample of cationic surfactants (quaternary ammoniums) and to reduce the ability of surfactants to strongly adsorb onto the capillary walls [20]. In our case, the peak shape of suxamethonium was not influenced by methanol, introduced in the BGE at 10% and 20% (data not shown). However, 10% acetonitrile in the BGE gave a better peak shape (symmetry of 1.16), compatible with a quantitative determination of suxamethonium. Electropherograms with and without acetonitrile in the BGE are shown in Fig. 3.

Another approach to reduce adsorption on the capillary wall was the use of PVA-coated capillaries. These have been used to overcome adsorption problems of proteins [21] and quaternary ammoniums [22]. The peak shape of SUX was improved by using PVA capillaries and the aqueous acetate/Tris buffer (data not shown). Nevertheless, PVA capillaries are more expensive than ordinary uncoated capillaries. Therefore, the hydro-organic solution was preferred for further routine analyses of suxamethonium.

3.2. Method validation

The developed method was validated according to the SFSTP recommendations. Quantitative performance was estimated in three separate series ($j = 3$) with the V2 protocol [16]. This protocol involves three concentration levels ($k = 3$) with two repetitions ($n = 2$) for calibration standards and three concentration levels ($k = 3$) with four repetitions ($n = 4$) for validation or quality control samples.

Table 1
Validation results: trueness, repeatability and intermediate precision of the developed CE-C^{4}D method for the analysis of suxamethonium in a pharmaceutical formulation.

Theoretical concentration of suxamethonium	Trueness	Repeatability (CV)	Intermediate precision (CV)
80%	98.8%	1.1%	1.2%
100%	100.2%	1.3%	1.3%
120%	101.1%	0.6%	1.6%

The calibration curve was obtained for each series with conventional least-squared linear regression using the three concentration levels (80%, 100% and 120% of the target value). After establishing the calibration curves for each series, concentrations of the QC were computed from the analytical response to obtain trueness, repeatability and intermediate precision. Trueness was expressed as the ratio between the theoretical and average measured values at each concentration level. Repeatability and intermediate precision were expressed as the coefficient of variation (CV%) of the ratio of the intra-day standard deviation (s_r) and between-day standard deviation (s_R), respectively, on the theoretical concentrations as described in [23]. The s_r and s_R values were obtained thanks to ANOVA analysis. As reported in Table 1, the trueness and precision values were in accordance with regular recommendations for the analysis of pharmaceutical formulations over the tested concentration range. The CV (repeatability and intermediate precision) was lower than 2%, with trueness between 98.8% and 101.1%. To visualize the overall method variability, the accuracy profile was built combining trueness and intermediate precision as the confidence interval [24]. As presented in Fig. 4, the total error did not exceed the acceptance limits (±5%) for all concentration levels. Consequently, the developed CE-C^{4}D method could be considered accurate for SUX over the tested range.

3.3. Application to pharmaceutical products

In order to demonstrate the applicability of the CE-C^{4}D method to real samples, quantitation of SUX was achieved on three commercially available pharmaceutical products: Lysthenon® (2% and 5%) from Nycomed Pharma SA (Dübendorf, Germany) and Succinolin® (5%) from Amino AG (Neuenhof, Switzerland). The concentration of SUX was calculated with reference to a calibration curve constructed the same day. CS at three concentration levels were replicated twice, and conventional least-squared linear regres-

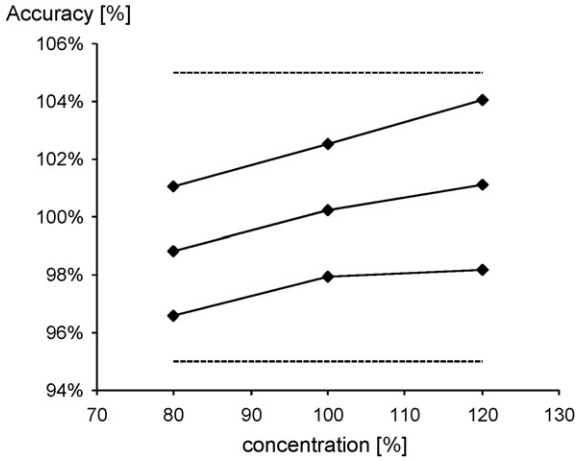

Fig. 4. Accuracy profile of the developed CE-C^{4}D method for the determination of suxamethonium in a pharmaceutical formulation using a linear regression model. The dashed lines represent the acceptance limits of 95% and 105%.

sion was applied. Since five independent analyses ($N = 5$) were performed on each pharmaceutical formulation, the result of the analysis could be expressed as

$$cnf(x) = \bar{x} \pm t_{\mathrm{d.f.},\alpha} \sqrt{\frac{s_r^2}{N} + s_g^2} \quad (1)$$

where N is the number of analyses performed during the routine analysis and $\bar{x}$ is the mean result. The $t_{\mathrm{d.f.},\alpha}$ (student constant depending on d.f. and α set at 5%), s_r^2 and s_g^2 variance values were determined during validation with the regular ANOVA-based variance decomposition [24]. The analysis repetition was useful to obtain a smaller confidence interval, since most of the variability came from repeatability (s_r^2). In Lysthenon (2%), a SUX concentration of $20.2 \pm 0.2\ \mathrm{mg\,mL^{-1}}$ was determined. Lysthenon (5%) contained $50.0 \pm 0.5\ \mathrm{mg\,mL^{-1}}$ and Succinolin contained $51.3 \pm 0.5\ \mathrm{mg\,mL^{-1}}$ of SUX. The indicated concentrations of the pharmaceutical products were confirmed to be in the authorized specifications of ±5% of the target value (19–21 $\mathrm{mg\,mL^{-1}}$ and 47.5–52.5 $\mathrm{mg\,mL^{-1}}$, respectively) by the developed CE-C^4D method.

4. Conclusions

A simple method was developed for the quantitative determination of suxamethonium in pharmaceutical formulations by capillary electrophoresis with a capacitively coupled contactless conductivity detector. The developed method exhibited very good quantitative performance in terms of accuracy and precision with an analysis time of less than 4 min for SUX and its main degradation product (choline). The problem of adsorption onto the capillary wall could be reduced by the addition of acetonitrile. The results demonstrate that the CE-C^4D analysis is very useful for the determination of SUX in commercial products and can be used as a routine technique in quality control for compounds possessing quaternary ammonium groups.

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