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# Determination of succinylcholine in human plasma by high-performance liquid chromatography with electrochemical detection

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**Abstract**

An alternative HPLC method for the quantification of the depolarizing neuromuscular blocking agent succinylcholine in human plasma is described. Drug spiked plasma and patient plasma samples were extracted using a $C_1$ solid-phase cartridge. Succinylcholine was separated on a Cyano column and quantitated using electrochemical detection at a potential of 450 mV and 750 mV. Mobile phase consisted of a mixture of phosphoric acid–acetonitrile–methanol (45:35:25) adjusted to an apparent pH of 5. Standard curves for the quantitation were linear in the range of 250–8000 ng/ml. Between-day and within-day relative standard deviations were 5.1% and 1.7%, respectively. Mean drug recovery and accuracy was 68% and 104%, respectively. 

*Keywords:* Succinylcholine

## 1. Introduction

Succinylcholine, 2,2′-[(1,4-dioxo-1,4-butanediyl) bis (oxy) ] bis [*N, N, N* - trimethylethanaminium] dichloride, is a short-acting depolarizing neuromuscular blocking agent. With two molecules of acetylcholine connected back-to-back through the acetate methyl group, succinylcholine is hydrolyzed in the blood by plasma cholinesterases. In vitro studies [1] have shown that up to 85% of succinylcholine is destroyed within the first half minute following its addition to blood with only 5% remaining after 2 min.

Succinylcholine has been extensively studied and is still currently used in anesthesia because of its fast onset of action. However, the pharmacokinetics of succinylcholine is poorly documented owing to a lack of analytical sensitivity. Quantitation of succinylcholine in dog plasma, urine and cerebrospinal fluid was first accomplished by Dal Santo [2] using radiolabeled drugs. Since then a number of methods have been described for the determination of succinylcholine in tissues and human plasma, such as thin-layer chromatography (TLC) [3,4], gas chromatography–mass spectrometry (GC–MS) [5–8] and high-performance liquid chromatography (HPLC)–fluorescence detection [9]. The GC–MS method proved to be the most sensitive with a lower limit of quantitation of 5 ng/g of succinylcholine in embalmed tissue [10] and 10 ng/ml in plasma [11].

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However, this apparatus is not readily available in a clinical setting. A lower limit of 2000 ng/ml was recently achieved using an HPLC–electrochemical detection (HPLC–ED) assay [12] coupled with an enzymatic post-column. This lower sensitivity remains insufficient for evaluating the pharmacokinetics of succinylcholine in humans. In the present report, an HPLC–ED assay with a sensitivity of 250 ng/ml in human plasma is described.

## 2. Experimental

### 2.1. Chemicals and reagents

Succinylcholine chloride was purchased from Sigma (St. Louis, MO, USA). Pipecuronium was kindly supplied by Organon Labs. (West Orange, NJ, USA). Organic solvents, tetramethyl-ammonium chloride (TMAH) and ammonium phosphate were of HPLC grade and all other chemicals were of analytical-reagent grade (Fisher Scientific, Montreal, Canada). Bio-filtered water was prepared by further purifying HPLC grade water (Anachemia Science, Rouses Point, NY, USA) by passage through a Sep-Pak $C_{18}$ cartridge (Millipore, Waters, Milford, MA, USA) immediately before use. All solutions were filtered through a 0.2-μm membrane (Type HVLP, Millipore).

### 2.2. Buffer and standard solutions

The TMAH buffer was prepared by dissolving 0.2756 g TMAH in 1 ml bio-filtered water and adding to 240 ml of methanol. A solution of 0.1 *M* of HCl was added to adjust the apparent pH to 3.0. The total volume was then completed up to 250 ml and filtered as described above.

Fresh stock solutions of pure succinylcholine standard (1 mg/ml) were prepared monthly in 0.01 *M* TMAH buffer at pH 3.0 and stored at 4°C. A working solution (0.1 mg/ml) was made weekly by further dilution.

A stock solution of pipecuronium (400 μg/ml) used as the internal standard (I.S.) was prepared in TMAH buffer and further diluted in a 0.1 *M* phosphate buffer (pH 5.0) to yield the working solution (100 ng/ml).

### 2.3. Chromatographic apparatus and conditions

The chromatographic system consisted of a Constametric III pump (LDC Milton Roy, Riviera Beach, FL, USA), a LP-21 pulse dampener (Scientific System [SSI], State College, PA, USA), a SIL-9A injector fitted with a 150-μl injection loop (Shimadzu, Kyoto, Japan) and a Coulochem II multi-electrode detector (Environmental Science, Bedford, MA, USA) linked to an 5010 analytical cell (Electrochemistry Separations Analysis, Bedford, MA, USA). The response time was set to 2 s. To find the optimal detection conditions, the potentials of both screen electrode (detector 1) and sample electrode (detector 2) were varied from 0 to 1.2 V. The 1000-mV output from detector 2 was connected to an C-R6A integrator (Shimadzu). A 12.5 cm×4.6 mm I.D. prepacked 5-μm Spherisorb CN column (Hichrom, Reading, UK) was used for the chromatographic separation of the analytes. In order to protect the analytical column and analytical cell, a guard column (Hichrom) was also equipped in the line. The SSI 0.45-μm in-line filters were placed before the guard column, the column and the analytical cell, respectively.

Four liters of mobile phase were prepared weekly using 0.03 *M* phosphoric acid (45%, v/v), acetonitrile (35%, v/v) and methanol (25%, v/v). The final apparent pH was adjusted to 5.00 with concentrated ammonium hydroxide. The solution was degassed under vacuum and pumped at a flow-rate of 2 ml/min. The mobile phase was allowed to recirculate during the equilibration period of the coulometer. However, to minimize the mobile phase contamination with TMAH, a solvent recycler (Alltech 345 Solvent Recycler, Alltech Associates, Deerfield, IL, USA) allowed the eluate to drain into the waste during a chromatographic run if the signal exceeded 1% of threshold value after a 10 s delay. The chromatographic system was operated at room temperature. The column was kept at the temperature of 35°C using a column heater (CH-30, Eppendorf North America, Madison, WI, USA).

### 2.4. Sample preparation

Bond Elut $C_1$ solid-phase extraction (SPE) cartridges (Analytichem International, Harbor City, CA,

USA) were conditioned with TMAH buffer (1×3 ml) and water (1×3 ml). Internal standard working solution (100 μl), plasma (1 ml) and water (1 ml) were added to the cartridges and then aspirated through the sorbent. The cartridges were sequentially washed with water (1×3 ml), acetonitrile (1×3 ml) and methanol (1×3 ml) under vacuum (8.5 kPa). Analytes were eluted into polystyrene culture tubes with 2×250 μl of 0.01 *M* TMAH buffer. Elution was carried out under gravity for 3 min, after completion a small vacuum (less than 20 kPa) was applied to the cartridges. A volume of 150 μl was injected onto the analytical column.

### *2.5. Calibration curves*

Cholinesterases in plasma samples were inactivated by echothiophate (2 μl of 2% solution per ml of plasma) to prevent the degradation of succinylcholine [13]. A pool of plasma containing 8000 ng/ml succinylcholine was serially diluted with drug-free plasma at concentrations ranging from 250–8000 ng/ml. Calibration curves were generated by plotting the analyte/I.S. peak-height ratio against the concentration of succinylcholine. Linear regression was carried out using a weight function of $1/X$.

### *2.6. Recovery*

The recovery of succinylcholine from human plasma was determined in quadruplicate at concentrations of 400, 1500 and 6000 ng/ml. Blank plasma spiked with known amounts of succinylcholine and 100 μl I.S. was extracted and compared with blank plasma extracts to which I.S. was added and subsequently spiked with the same amount of succinylcholine. The recovery was assessed by comparing the peak-height ratios of analyte/I.S. in the two sets of extracts.

The recovery of the I.S. at the concentration used for the assay was previously assessed by comparing the peak heights of 10 extracted samples with the 100% value determined using in vitro samples.

### *2.7. Precision and accuracy*

Intra-assay precision was assessed using quadruplicate spiked samples at three different concentrations ranging from 250 to 8000 ng/ml. Inter-assay precision was studied using spiked plasma samples (from the calibration curve) that were analyzed at five or more different days within a 30-day period.

For accuracy, drug-free plasma was spiked with succinylcholine to give 10 concentrations ranging from 250 to 8000 ng/ml and analyzed blindly. The estimated concentrations were then compared with the known concentrations of analyte to evaluate the percentage of bias.

### *2.8. Limit of quantitation*

Diluted solutions of succinylcholine were injected directly into the HPLC system to estimate the lower limit of detection with a signal-to-noise ratio of 3. The lowest concentration of the standard curve is defined as the lower limit of quantitation.

### *2.9. Stability*

The stability of succinylcholine in plasma was studied with drug spiked samples (1250 and 5000 ng/ml) stored at −70°C. Samples were assayed in triplicate on the day of preparation (day 0), one month and four months later.

### *2.10. Clinical samples*

The plasma concentration–time profile of succinylcholine was determined after administration of a 1 mg/kg dose in an anesthetized patient. Blood samples were collected at 0, 1, 1.5, 2, 3, 4, 6, 10, 14, 20 and 30 min in heparinized and enzyme-inhibited tubes and kept on an ice-water bath for less than 10 min. Centrifugation was applied at 3200 *g* for 5 min and the plasma was immediately flash-frozen on dry ice. Samples were stored at −70°C until analysis.

## 3. Results and discussion

The best oxidative potentials proved to be 450 and 750 mV for detector 1 and 2, respectively. The hydrodynamic voltammogram at detector 2 showed that the current attained a plateau phase at potentials higher than 900 mV. However, voltages greater than 800 mV were associated with a decrease in signal-to-

Table 1
Recovery and within-assay variability of succinylcholine in human plasma

| Concentration added (ng/ml) | Recovery (%) | R.S.D. (%) |
|---|---|---|
| 400 | 69±2.0 | 2.9 |
| 1500 | 67±0.7 | 0.9 |
| 6000 | 67±0.9 | 1.3 |

Results are expressed as mean±S.D., $n=4$.

noise ratio and a lengthier equilibration period. A voltage of 750 mV was therefore chosen as the selective oxidation potential for the second electrode. The first electrode was set at 450 mV to screen for possible oxidizable impurities in the mobile phase or the biological sample without any significant loss of analyte. As expected, increasing the temperature of the HPLC column from room temperature to 35°C improved the sensitivity of the assay.

The retention times of succinylcholine and I.S. were highly dependent on the pH and ionic strength of the mobile phase. Without any change in sensitivity, a faster elution could be achieved when increasing the ionic strength from 0.01 to 0.03 *M* of phosphoric acid in buffer or adjusting the pH from 4.5 to 5.0. Greater ionic strength resulted in increased background noise whereas resolution was decreased at lower pH (<4.5).

Six concentrations of succinylcholine ranging from 250 to 8000 ng/ml in plasma were used to establish a calibration curve for each analysis. Intra-assay and inter-assay precision was 1.71% (Table 1) and 5.06% (Table 2), respectively. The mean slope and intercept of the five calibration curves used for between day precision (Table 2) were 0.000244 (R.S.D. 15%) and −0.004577 (R.S.D. 74%), respectively. The mean $r^2$ was 0.9927 ranged from 0.9863 to 0.9990. A mean accuracy of 104±5.4% was observed for succinylcholine (Table 3).

Table 2
Between-assay variability for succinylcholine

| Concentration (ng/ml) | Extrapolated concentration (ng/ml) | R.S.D. (%) |
|---|---|---|
| 250 | 244±3 | 1.2 |
| 500 | 530±36 | 6.8 |
| 1000 | 965±80 | 8.3 |
| 2000 | 1922±86 | 4.5 |
| 4000 | 4012±188 | 4.7 |
| 8000 | 8098±400 | 4.9 |

Results are expressed as mean±S.D., $n=5$.

Table 3
Accuracy of the assay for succinylcholine

| Spiked concentration (ng/ml) | Estimated concentration (ng/ml) |
|---|---|
| 0 | 0 |
| 300 | 316 |
| 400 | 377 |
| 625 | 654 |
| 800 | 781 |
| 1250 | 1282 |
| 1850 | 2079 |
| 2500 | 2618 |
| 3750 | 4179 |
| 5000 | 5202 |
| 7500 | 8066 |
| Mean accuracy (%) | 104 |
| S.D. | 5.4 |

The lower limit of detection for succinylcholine was 50 ng on column. The lower limit of quantitation for succinylcholine in plasma was 250 ng/ml.

SPE cartridges such as Cyano, Carboxy, Phenyl and $C_1$ were tested for the best option. Succinylcholine was not recovered from Cyano or Carboxy columns, whereas the Phenyl column yielded approximately 20% recovery. The $C_1$ column was associated with the highest recovery (68%). Although lower than that obtained for vecuronium and mivacurium in our laboratory [14,15], the recovery of succinylcholine was constant and reproducible at all concentrations within the range of the calibration curve (Table 1). The underlying cause remains unclear. The pH environment was also found to influence the recovery of succinylcholine. In preliminary experiments, recovery of succinylcholine chloride spiked into plasma adjusted at different pH (4.4, 5.4, 6.4 and 7.4) gradually decreased as the pH decreased. It was observed that concentration of the eluate to half volume with SpeedVac or evaporation under a light stream of nitrogen further decreased the recovery by 52% and 48%, respectively. Therefore, it is recommended not to concentrate the eluate. In our experience, up to 150 μl of eluate can be loaded on column without loss of sensitivity.

Upon repetitive injections of succinylcholine working solutions (about 40), we observed a 50%

loss in sensitivity which, in our opinion, resulted from the accumulation of TMAH in the system. To prevent this phenomenon, a solvent recycler was added to the system which significantly increased the viability of the conditions. Guard column analytical column and cell were disconnected from the system and reconditioned whenever a decrease of more than 10% of the original response was observed. Sequential rinsing with water, methanol, acetonitrile, methanol and water was found to be effective in restoring optimum sensitivity for the cell. Accordingly, this cell washing procedure is recommended after approximately 70 injections of plasma extracts. Working with bio-filtered water and high-quality solvents, as well as careful degassing and filtering of mobile phase through a 0.2-μm membrane, allowed reproducible analyses.

Several chemicals were tested as potential internal standard. In our conditions, the retention times of pipecuronium, mivacurium, vecuronium, rocuronium and its 17-desallyl metabolite proved to be 19, 21, 21, 18 and 12 min, respectively. However, pre-acidification of plasma with 2 *M* $H_2SO_4$ which was required for stabilization of rocuronium and its 17-desallyl metabolite resulted in a 50% decrease in the recovery of succinylcholine. In order to optimize retention time and recovery, pipecuronium was finally chosen as the internal standard.

Succinylcholine is sensitive to pH and temperature. Foss and Benezra [13] reported that the rate of hydrolysis of a 5% solution stored at room temperature was gradually increased as the pH was changed from 3 to 11. In this study, succinylcholine stock solutions proved to be stable at 4°C in 0.01 *M* TMAH buffer (pH 3.0) for at least two months. In addition, to minimize the influence of temperature the eluate from the SPE column should be kept in an ice-water bath before injection. In stabilized plasma, succinylcholine was found to be stable for at least four months when stored at −70°C (Table 4).

Fig. 1 shows representative chromatograms of extracts of drug-free plasma with or without spiked succinylcholine under the described conditions. The retention times of succinylcholine and pipecuronium (I.S.) were 9.2 and 19.8 min, respectively (Fig. 1B Fig. 1C). The total time for a chromatographic run, including the time needed for the baseline to return to its pre-injection value, was less than 25 min.

Table 4
Stability of succinylcholine stored at −70°C

| Time (day) | Concentration (ng/ml) | |
|---|---|---|
| | 1250 | 5000 |
| 0 | 1222±37 | 5038±64 |
| 30 | 1269±35 | 5015±67 |
| 120 | 1256±24 | 5057±13 |

Results are expressed as mean±S.D., $n=3$.

This analytical method was applied to determine the concentration–time profile of succinylcholine in one patient after the administration of an intravenous (i.v.) bolus of 1.0 mg/kg succinylcholine bromide. Pre-dose plasma sample was free of endogenous or drug interferences under the anesthetic procedure described (Fig. 1A). The plasma concentration–time profile of succinylcholine for this patient shows rapid

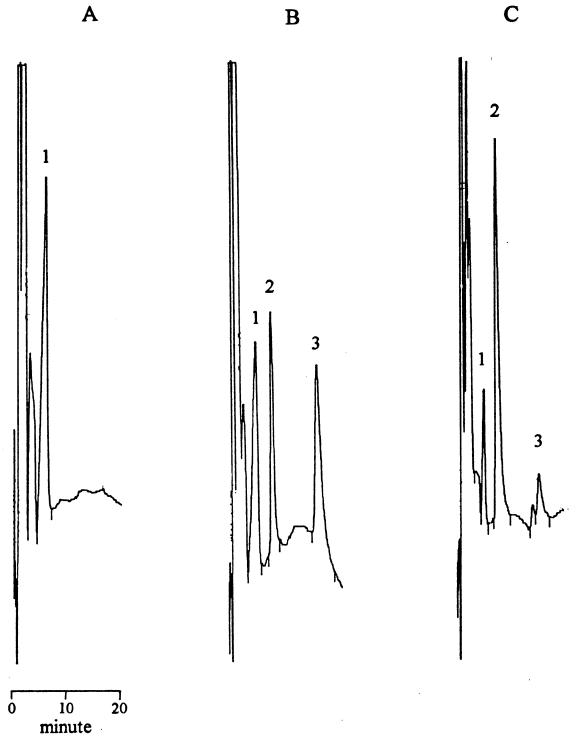


Fig. 1. HPLC chromatogram for: (A) drug-free plasma, (B) drug-free plasma spiked with 3000 ng/ml of succinylcholine chloride and (C) plasma sample collected at 60 s after the i.v. administration of 1.0 mg/kg succinylchlorine bromide in an anesthetized patient. Peaks: 1=tetramethyl-ammonium chloride; 2=succinylcholine and 3=internal standard.

decay from 25.33 μg/ml to 0.11 μg/ml within the first 3 min. Plasma concentration was not detectable after 4 min of the i.v. bolus administration.

## 4. Conclusions

A highly selective HPLC assay with ED has been developed for determination of succinylcholine in plasma samples. This sensitive and reproducible assay provides a time-saving and less expensive alternative to currently available methods. The sensitivity of the analytical cell is crucial to achieve the lowest limit of quantitation of 250 ng/ml for succinylcholine.

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