# Adsorption and Stability of Suxamethonium and Its Major Hydrolysis Product Succinylmonocholine Using Liquid Chromatography-Electrospray Ionization Mass Spectrometry

**Hiroe Tsutsumi,* Mayumi Nishikawa, Munehiro Katagi, and Hitoshi Tsuchihashi**

*Forensic Science Laboratory, Osaka Prefectural Police Headquarters, 1–3–18 Hommachi, Chuo-ku, Osaka 541–0053, Japan*

(Received March 10, 2003; Accepted March 27, 2003)

For the purpose of reliable identification of suxamethonium (SUX) use, the stability and adsorption of SUX and its specific hydrolysis product succinylmonocholine (SMC) were investigated under various storage conditions using liquid chromatography-electrospray ionization mass spectrometry (LC-ESI MS). Significant and slight adsorption to glassware was observed for SUX and SMC, respectively, whereas there was no significant adsorption to plasticware by either SUX or SMC. The silanization methods for glassware have also been found to affect the adsorption of SUX to glassware. Additionally, alkaline conditions led to significant loss of both SUX and SMC due to their hydrolysis, but SMC showed greater stability than SUX under the present storage conditions, suggesting that SMC is a superior marker for SUX use when stored in alkaline conditions. On the other hand, there was no significant loss of SUX and SMC under acidic conditions even in human urine at room temperature or higher (20–38°C), or in distilled water.

**Key words** —— suxamethonium, succinylmonocholine, adsorption, stability, liquid chromatography-electrospray ionization mass spectrometry

## INTRODUCTION

Suxamethonium (SUX), a depolarizing neuromuscular blocking agent, has been extensively used as preanesthetic for surgical procedures and as a muscle relaxant in electroconvulsion therapy. Artificial respiration is required when it is administered because the undesirable side effect of respiratory depression frequently occurs. Misuse of SUX leads to suffocation and finally to death. Thus the use of SUX is restricted and it has been considered one of the most important drugs in forensic toxicology.

SUX has two molecules of acetylcholine connected back-to-back through the acetate methyl group. Because of the two quaternary ammonium moieties, SUX exhibits extremely high polarity. In addition, owing to its chemical instability, SUX is known to be easily and rapidly hydrolyzed in aqueous alkaline solution.[1] It is also well documented that SUX is rapidly metabolized in the human body by plasma cholinesterase (BuChE), initially to succinylmonocholine (SMC) and finally more slowly to succinic acid and choline (Fig. 1). It can be difficult to detect SUX in blood samples. Unlike clinical science, blood samples are rarely collected for the analysis of SUX in forensic toxicology. In addition, SUX was reported to be rapidly excreted in unchanged form in human urine at a small percentage of the administered dose,[2] and urine is therefore regarded as the most suitable specimen in which to confirm SUX use.

On the other hand, it is well known that approximately 10% of the SMC dose is excreted unchanged 30 min after its administration.[3] It is also well known that SMC shows less affinity to BuChE than SUX.[4] This suggests that SMC may be excreted in human urine at a higher concentration than SUX, and that SMC would be a significant marker for the confirmation of SUX use. However, only a few studies of

*To whom correspondence should be addressed: Forensic Science Laboratory, Osaka Prefectural Police Headquarters, 1–3–18 Hommachi, Chuo-ku, Osaka 541–0053, Japan. Tel.: +81-6-6268-1234, ext. 572; Fax: +81-6-6271-8066; E-mail: h-tutuoppe@iam.ne.jp

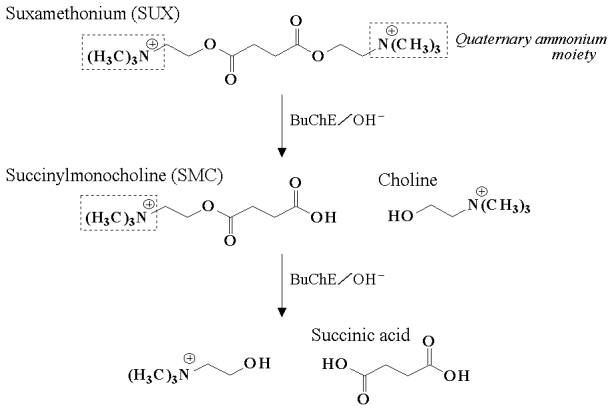


**Fig. 1.** Generalized Hydrolysis Pathway of SUX

the detailed properties and analytical method for SMC have been published.[1,4,5)]

In the analyses of unstable compounds such as SUX and SMC, the storage conditions of aqueous samples, including standard stock solution, used sample residues, and urine samples, are extremely important when the concentrations of the drugs are expected to be fairly low. It is well known that cationic compounds such as quaternary ammonium compounds readily adsorb to glassware.[6,7)] SUX and SMC are also expected to adsorb to a considerable extent. The stability of SUX has been well studied,[6,8–10)] and it is recommended that analytical samples be kept in acidic conditions at low temperature. However, detailed data on the adsorption of SUX and SMC have rarely been reported. More detailed research not only on stability but also on the adsorption of both SUX and SMC is required for accurate and reliable determination of these compounds in samples.

We investigated the adsorption and stability of both SUX and SMC using liquid chromatography-electrospray ionization mass spectrometry (LC-ESI MS), which has recently been applied successfully to the analysis of ionic compounds in forensic science.[11,12)]

## MATERIALS AND METHODS

**Chemicals** —— SUX chloride and hexamethonium bromide were purchased from Tokyo Kasei Co. (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. SMC iodide was synthesized[13)] by preparing choline iodide with succinic anhydride in our laboratory. The acetonitrile used was of HPLC grade, and all other chemicals used were of analytical grade.

The pH of 10 mM ammonium formate buffer was adjusted to appropriate values (pH 3, 4, 5, 7, 9, 10) with 10% aqueous formic acid solution or 10% aqueous ammonia solution. The standard solutions of both SUX and SMC were prepared in 10 mM ammonium formate buffer (pH 4) immediately before use. The sample solutions used for examination were prepared at the concentrations of 0.5 and 0.05 ppm by diluting the standard solution of 10 mM ammonium formate buffer of various pH values (pH 3, 4, 5, 7, 9, 10), and urine samples spiked with SUX (concentration 0.2 ppm) and SMC (concentration 10 ppm) were adjusted to pH 3, 6, 8, and 9 by diluting the standard solution with drug-free human urine. The silanization agents dimethyldichlorosilane and dimethylpolysiloxisane (siliconize L-25) were purchased from Shin-Etsu Chemical Co. (Tokyo, Japan) and Fuji Systems (Fukushima, Japan), respectively. Plasticware, including polypropylene microtubes (Eppendorf, Hamburg, Germany), polypropylene centrifuge vials (ELKAY, IL, U.S.A.) and polystyrene microplates (IWAKI, Chiba, Japan), and glassware, including brown sample vials and clear test tubes (Maruemu, Osaka, Japan) were used with/without silanization for to investigate adsorption. Two silanized glass vials (Agilent Technologies, Palo Alto, CA, U.S.A. and SPELCO, Please give city, PA, U.S.A.) were also used for comparison.

**Silanization Procedure with Dimethyldichlorosilane (Method A)** —— The glassware was immersed in a solution of 10% dimethyldichlorosilane in toluene for about 30 min. The prepared glassware was rinsed with toluene, and then immediately thereafter with methanol.

**Silanization Procedure with Dimethylpolysiloxisane (Method B)** —— After dimethylpolysiloxisane (siliconize L-25) was prepared in approximately 2% solution with distilled water at 70°C, the glassware was immersed in the solution for at least 10 sec. The glassware was rinsed with water and then dried for 24 hr.

**Instrumentation** —— The LC-ESI MS system consisted of a PU-980 HPLC pump (JASCO, Tokyo, Japan) and a platform quadrupole mass spectrometer equipped with an ESI interface (Micromass, Manchester, U.K.). ESI MS was performed in positive mode with the cone and the capillary voltages set at 25 V and 3.5 kV, respectively. LC separation

was accomplished on a TSK-GEL $\alpha$-2500 (2.0 mm i.d. × 150 mm, TOSOH, Tokyo, Japan) with acetonitrile-30 mM ammonium formate (70 : 30, v/v) as the mobile phase at a flow rate of 0.12 ml/min. Quantitative analysis was carried out employing the molecular ion of each analyte (*m/z* 145, 204 and 101 for SUX, SMC, and hexamethonium, respectively) in selected ion monitoring (SIM) mode.

**Sample Preparation of Urine Samples** —— Bond Elut® CBA cartridges (3-ml capacity, Varian, Harbor City, CA, U.S.A.) were prewashed successively with methanol (3 ml), distilled water (3 ml), and 10 mM ammonium formate buffer (pH 6, 3 ml). Urine samples (1 ml) mixed with 3 ml of 10 mM ammonium formate buffer (pH 6) were applied to the prepared cartridges. After the cartridges were washed with water (3 ml), the retained analytes (SUX) were eluted with 2 ml of 0.1 M HCl-methanol (50 : 50, v/v). Five-microliter aliquots were injected into the LC-MS system. For analysis of SMC, the urine samples were diluted with a 10-fold volume of 10 mM ammonium formate buffer, and 5-$\mu$l aliquots were directly injected into the LC-MS system, because satisfactory recovery was not achieved by preparation with a Bond Elut® CBA cartridge.

## RESULTS AND DISCUSSION

**Adsorption to Vessels**

To determine the adsorption of SUX and SMC, the differences in adsorption to the various vessels mentioned given above were compared. Two-milliliter aliquots of two concentrations of SUX and SMC standard solutions (0.5 and 0.05 ppm, pH 4) were stored in the various vessels for 2 hr. Then, 5-$\mu$l aliquots of each solution were collected and subjected to LC-MS and the concentrations of both SUX and SMC were determined.

As a preliminary experiment, adsorption of SUX to polypropylene microtubes was examined. A 2-ml aliquot of SUX standard solution (0.5 ppm, pH 4) was stored in one polypropylene microtube for 30 min, and then the solution was placed into another, identical polypropylene microtube. This step was then repeated. Five-microliter aliquots of the solutions were subjected to LC-MS, and the decrease in SUX and SMC concentrations was measured. The results revealed that neither adsorbed to the polypropylene microtubes. Then the adsorption of SUX and SMC to each vessel was estimated as the percentage of concentration in each vessel compared with that in the polypropylene microtube.

As summarized in Table 1, little loss of SUX was observed in two types of polypropylene vessel (microtubes and centrifuge vials), although approximately 10–20% loss was observed in polystyrene microplates. The loss of SUX in two types of glassware ranged from approximately 20% to 50%, and greater loss of SUX appeared with lower concentrations of solution, as suggested by Tozuka.[14]

On the other hand, less loss of SMC was observed for both plastic- and glassware compared with that of SUX. This is because compounds such as SUX and SMC do not interact with polypropylene due to the hydrophobicity of its surface, and SUX shows slight adsorption to polystyrene due to the small polarity of the phenyl group in polystyrene. It was also found that SUX exhibited significant adsorption to glassware but SMC exhibited only slight adsorption. This difference is due to the stronger interaction between the two quaternary ammonium moieties in SUX and silanols (Si-OH) on the glass surface than that between one quaternary ammonium moiety in SMC and the silanols, in addition to the repulsion between the carboxyl group in SMC and the silanols. The data on adsorption of hexamethonium to glass summarized in Table 1 confirms this above explanation.

**Silanization Effect on Suppression of Adsorption**

When ionic compounds are stored in glass vessels, silanization of the glassware has been employed. Differences between silanization methods were investigated. Glassware that had been treated with two silanizing methods (methods A and B) was employed. In glassware silanized by method A, a large amount of SUX was lost. Silanization by method B almost completely inhibited adsorption of SUX, and approximately 99% of SUX was recovered in 0.05 ppm solution (Table 2).

As shown in Table 2, two commercially available silanized glass vials (Agilent and SPELCO) were also used in the present study. In the former, adsorption was inhibited by approximately 10–20%, while in the latter adsorption was enhanced by more than 35% enhancement compared with that in the untreated glassware (Table 2).

It is generally believed that silanization of free silanols inhibits the adsorption of ionic compounds to glassware. In this study, two silanization methods were investigated, and there was a marked dif-

**Table 1.** Recovery (%) of SUX, SMC, and Hexamethonium (HEX) in Various Vessels

| Vessel | | Compound concentration (ppm) | | | | |
|---|---|---|---|---|---|---|
| | | SUX | | SMC | | HEX |
| | | 0.05 | 0.5 | 0.05 | 0.5 | 0.5 |
| Plasticware | Microtube (PP) | 100 | 100 | 100 | 100 | 100 |
| | Centrifuge vial (PP) | 105 | 96 | 101 | 106 | 98 |
| | Microplate (PS) | 78 | 87 | 95 | 102 | 101 |
| Glassware | Brown sample vial | 47 | 75 | 100 | 104 | 27 |
| | Clear test tube | 51 | 79 | 92 | 93 | 43 |

PP, polypropylene; PS, polystyrene. Each value is expressed as the percentage (%) of concentration in each vessel to that in the microtube (PP).

**Table 2.** Recovery (%) of SUX in Glassware Treated with Silanization

| Vessel | | SUX concentration (ppm) | |
|---|---|---|---|
| | | 0.05 | 0.5 |
| Glassware silanized with | Method A | 48 | 67 |
| | Method B | 86 | 99 |
| Commercially available silanized glass vial | Agilent | 67 | 86 |
| | SPELCO | 12 | 23 |
| Untreated glassware | | 47 | 75 |

The glassware silanized was brown sample vials. Method A, silanization with dimethyldichlorosilane; Method B, silanization with dimethylpolysiloxisane.

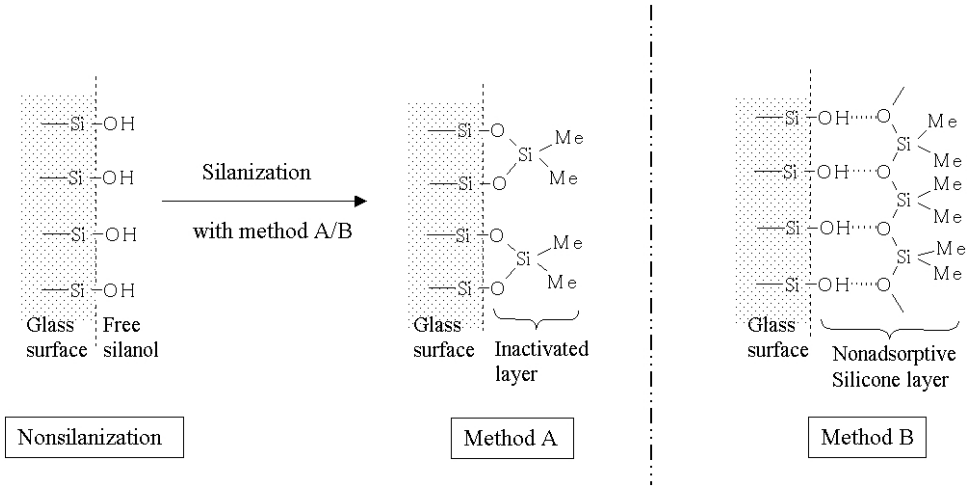


**Fig. 2.** Schematic Diagram of Silanizaition of Glassware with Method A or B

ference between the two methods. With method A, dimethyldichlorosilane reacted directly with free silanol on the glass and formed covalent bonds to mask the silanol, while with method B, dimethylpolysiloxisane bound to the glass surface with hydrogen bonds to form silicone polymer on the glass surface (Fig. 2). Additionally, the reaction in method A was more readily disturbed by water. This resulted in the formation of a thicker, denser nonadsorptive silicone layer with method B (Fig. 2), and eventually in different inhibition of adsorption of the two methods. There were also differences among commercially available silanized glassware using the different silanization methods. Thus it is recom-

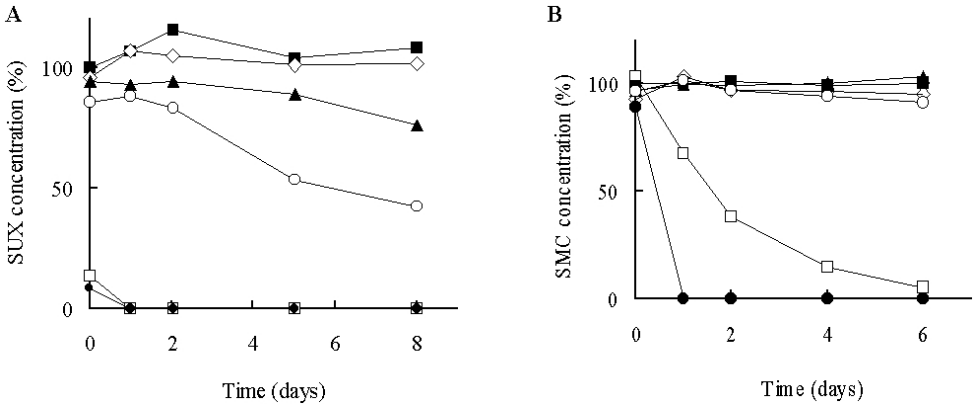


**Fig. 3.** Time Course of SUX (A) and SMC (B) Concentrations in Buffer at Various pH Values at Room Temperature
Symbols: ■, pH 3; ◇, pH 4; ▲, pH5; ○, pH 7; □, pH 9; ●, pH 10.

mended that polypropylene vessels or glassware silanized by dimethylpolysiloxisane be employed for preparation of SUX.

## Stability in Buffer Solution at Various pH Values and Temperatures

The influence of pH and temperature on the stability of SUX and SMC in buffer solution was examined. SUX and SMC standard solutions (0.5 ppm) at various pH (pH 3, 4, 5, 7, 9, 10) of buffer solution were stored at 38°C, at room temperature (about 20°C), in a refrigerator (4°C), and in a freezer (–18°C), and the loss of SUX and SMC were examined by LC-MS for a period of several days. As vessels, polypropylene microtubes in which SUX and SMC did not adsorb were used.

Figure 3 shows changes in SUX and SMC concentrations in buffer at pH 3, 4, 5, 7, 9, and 10 at room temperature. In alkaline solutions at pH 9 and 10, SUX readily degraded and no SUX was detectable after 1 day, whereas in acidic solutions at pH 3 and 4, SUX was stable and no significant degradation was seen for up to 8 days (Fig. 3A). On the other hand, SMC was not as greatly affected by pH as SUX: even at pH 9, the degradation of SMC was relatively slow and 10% of the initial amount remained unchanged after 4 days. At acidic pH of less than 7, SMC was stable without significant degradation even after 6 days (Fig. 3B).

Both SUX and SMC were affected by temperature at pH at which they were relatively unstable; higher temperature led to greater loss of SMC at pH 9, and at pH 7 no remained SUX undegraded at 38°C. However, most SUX (more than 90%) remained at –18°C and 4°C even after 7 days.

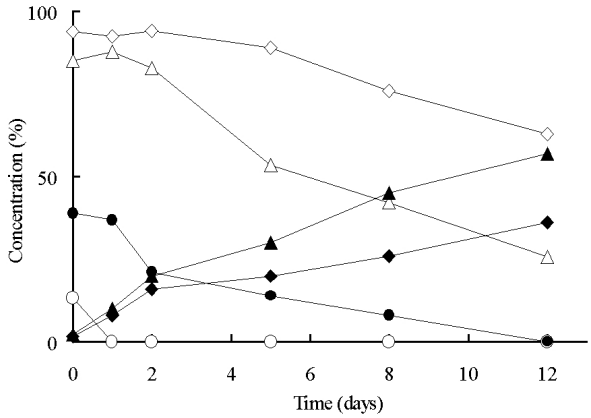


**Fig. 4.** Time Course of SMC Concentration Generated by Hydrolysis of SUX in Various pH Buffer Solutions at Room Temperature
Symbols: ◆, SMC at pH 5; ▲, SMC at pH 7; ●, SMC at pH 9; ◇, SUX at pH 5; △, SUX at pH 7; ○, SUX at pH 9.

The time course of SMC concentration generated by hydrolysis of SUX in the buffer solutions (pH 5, 7, and 9) at room temperature was quantified. At neutral or weakly acidic pH, the SMC concentration gradually increased with the decrease in SUX concentration. At pH 7, the concentration of SUX and generated SMC were almost equal after 8 days. On the other hand, in alkaline solution SUX was readily hydrolyzed to SMC as mentioned above. At pH 9, SUX was hydrolyzed almost completely within 1 day, but 40% of SMC generated by the hydrolysis of SUX remained unchanged after 1 day. The generated SMC remained detectable even after 8 days (Fig. 4).

There have been many studies on the stability of SUX. Ikarashi *et al*. reported that after incuba-

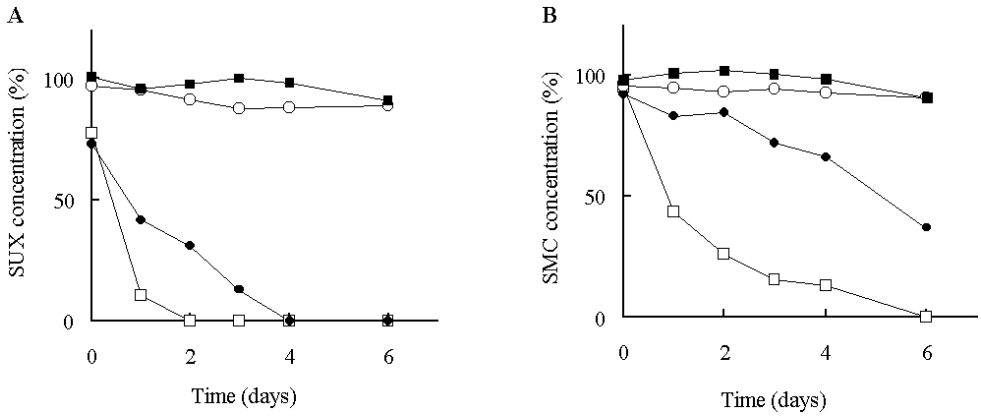


**Fig. 5.** Time Course of SUX (A) and SMC (B) Concentration in Human Urine at Various pH Values
Symbols: ■, pH 3; ○, pH 6; ●, pH 8; □, pH 9.

tion for 10 min at 37°C SUX was rapidly hydrolyzed at pH above 7.5.[8] Stevens *et al*. found that SUX stored even at 4°C was not detected after 4 months at pH of more than 7, and that at pH 5 at 4°C, loss of SUX was not found during 6 to 8 months.[6] It is also known that SUX at pH 3 at 4°C can safely be stored for 7 weeks[6] and for 4 months at –70°C even in human plasma.[6,10] However, no detailed research on the stability of SMC has been reported. In this study, our data were in agreement with the previously reported data on the stability of SUX. Additionally, it was revealed that SMC is more stable than SUX, suggesting that SMC would be detectable even when SUX was not.

**Stability in Human Urine**

As previously described in several papers, it is extremely difficult to detect SUX in blood samples. Even after pretreatment of blood with a cholinesterase inhibitor, SUX was detectable only within 15 min using HPLC with fluorescence detection,[15] within 4 min using HPLC with electrochemical detection,[10] and within 7 min using LC-MS-MS[6] after SUX administration. Urine is more suitable and advantageous for analysis to confirm the use of SUX, as mentioned in the Introduction.

To investigate the influence of the sample pH and temperature on the stability of SUX and its main specific metabolite SMC in urine samples, SUX- and SMC-spiked urine samples (0.2 and 10 ppm) were stored at room temperature. The concentrations of both SUX and SMC in the urine were measured for a period of 6 days using LC-MS. Sample preparation was performed according to the method described above in this paper.

The stability of SUX and SMC showed an almost identical dependence on pH in human urine to that in buffer. Under acidic conditions (pH 3) both were stable and no significant loss was observed, while under alkaline conditions both were hydrolyzed, but SMC was detectable for 3 days longer than SUX (Fig. 5). This suggests that the analysis of SMC is preferable if the collected urine sample is alkaline.

In conclusion, polypropylene vessels and silanized glassware prepared with dimethylpolysiloxane did not significantly adsorb SUX and SMC and were advantageous for the storage of aqueous samples. There was significant loss of both SUX and SMC under alkaline conditions, although SMC showed greater stability than SUX. This suggests that SMC is a superior marker of SUX use when stored under alkaline conditions. On the other hand, there was no significant loss of either SUX or SMC under acidic conditions even in human urine at room temperature or higher (20–38°C). It is therefore recommended that aqueous samples including standard stock solutions, used sample residues, and urine samples should be stored in polypropylene vessels or silanized glassware under acidic conditions (less than pH 4) at lower temperatures (refrigerated or frozen if possible). Careful sample preparation is required prior to analysis taking the properties of SUX and SMC described in this study into consideration.

## REFERENCES


1) Gibb, D. B. (1972) Suxamethonium — a review. *Anaesth. Intensive Care*, **1**, 109–118.
2) Moffat, A. C. (1986) Suxamethonium Chloride. In *Clarke's Isolation and Identification of Drugs, Second Edition* (Moffat, A. C., ed.), The Pharmaceutical Press, London, pp. 998–999.
3) Foldes, F. F., Vandervort, R. S. and Shanor, S. P. (1955) The fate of succinylcholine in man. *Anesthesiology*, **16**, 11–21.
4) Pitts, N. I., Deftereos, D. and Mitchell, G. (2000) Determination of succinylcholine in human plasma by high-pressure liquid chromatography with electrochemical detection. *Br. J. Anaesth.*, **85**, 592–598.
5) Agarwal, D. P. and Goedde, H. W. (1976) Thin-layer chromatographic separation of $^{14}$C-labelled succinyldicholine, succinylmonocholine and choline. *J. Chromatogr.*, **121**, 170–172.
6) Roy, J. J., Boismenu, D., Gao, H., Mamer, O. A. and Varin, F. (2001) Measurement of succinylcholine concentration in human plasma by electrospray tandem mass spectrometry. *Anal. Biochem.*, **290**, 238–244.
7) Mano, N., Oda, Y. and Asakawa, N. (1997) Qantitative method for biological active substance in biological sample by hyphenated MS technique. *J. Mass Spectrom. Soc. Jpn.*, **45**, 401–415.
8) Ikarashi, Y., Hada, T. and Way, E. L., *et al.* (1990) Determination of succinylcholine hydrolytic enzyme activity in human plasma. *J. Chromatogr.*, **533**, 23–33.
9) Stevens, H. M. and Moffat, A. C. (1974) A rapid screening procedure for quaternary ammonium compounds in fluids and tissues with special reference to suxamethonium (succinylcholine). *J. Forensic Sci. Soc.*, **14**, 141–148.
10) Gao, H., Roy, S. and Varin, F. (1998) Determination of succinylcholine in human plasma by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. B*, **718**, 129–134.
11) Tsuchihashi, H. and Nishikawa, M. (1995) Analysis of quaternary ammonium drug. *Jpn. J. Forensic Toxicol.*, **13**, 1–10.
12) Nishikawa, M., Katagi, M., Miki, A. and Tsuchihashi, H. (2003) Forensic toxicological determination of surfactant by liquid chromatography/electrospray ionization mass spectrometry and liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Health Sci.*, **49**, 138–148.
13) Phillips, A. P. (1953) Preparation of the monocholine ester of succinic acid and some related derivatives. *J. Am. Chem. Soc.*, **75**, 4725–4727.
14) Tozuka, Z. (1999) Pretreatment for quantitative analysis. *J. Mass Spectrom. Soc. Jpn.*, **47**, 229–245.
15) Lagerwerf, A. J., Vanlinthout, L. E. H. and Vree, T. B. (1991) Rapid determination of succinylcholine in human plasma by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.*, **570**, 390–395.