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SHORT COMMUNICATION

Evaluation of the chemical stability of succinylcholine chloride stored in syringes under room-temperature conditions

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Abstract

Background: Succinylcholine chloride is essential for achieving neuromuscular blockade during emergency airway management. The manufacturer specifies a 14-day stability period for 20 mg/mL vials at room temperature, which limits implementation of prefilled-syringe protocols. **Aim:** This study aims to investigate the chemical stability of succinylcholine chloride after transfer from manufacturer vials to polypropylene syringes during extended room-temperature storage. **Methods:** A validated high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method was developed to quantify percent recovery. Ten 20 mg/mL succinylcholine chloride vials were used: five samples were stored refrigerated (2–8°C; controls; $n = 5$), and five were transferred to polypropylene syringes and stored at room temperature (20–25°C; $n = 5$). Samples were analyzed over 90 days. The HPLC-UV method met United States Pharmacopeia criteria for accuracy, precision (<5% error and % relative standard deviation), and linearity ($R^2 > 0.99$). **Results:** Succinylcholine chloride maintained >90% of the initial concentration over 90 days in polypropylene syringes stored at room temperature (93.13% on day 90) and in refrigerated controls (93.97% on day 90). No significant differences were observed between storage conditions ($p>0.05$). All samples remained physically stable, with no visible color change or precipitate. **Conclusion:** Despite its ester bonds, succinylcholine chloride remained chemically stable in polypropylene syringes for up to 90 days at room temperature. **Relevance for Patients:** These findings support the extended stability of prefilled succinylcholine syringes, which may improve emergency preparedness; however, sterility validation remains necessary before clinical implementation.

Keywords: Succinylcholine chloride; Neuromuscular blocking agents; Drug stability; High-performance liquid chromatography–ultraviolet detection; Prefilled syringes; Emergency medicine

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1. Introduction

Rapid sequence intubation (RSI) is one of the most time-critical procedures in emergency medicine, requiring immediate availability of injectable neuromuscular blocking agents. Succinylcholine chloride is the preferred agent for RSI due to its rapid

onset and short duration with predictable recovery.[1] In contrast, rocuronium has a longer duration and requires a reversal agent.[2] As such, the ready availability of the preferred neuromuscular blocking agent (succinylcholine chloride) during an intubation may significantly impact patient outcomes.[1,2]

The structure of succinylcholine chloride contains two ester bonds, notorious for hydrolytic instability (Figure 1), yet several studies have indicated that succinylcholine chloride retains its chemical integrity well beyond the 14-day beyond-use date (BUD) indicated by the manufacturer.[3] This is particularly relevant given that a 14-day BUD could constrain emergency preparedness, especially when prefilled syringes would be a viable tool to ensure rapid medication availability.

Previous stability investigations of succinylcholine chloride have indicated that its chemical stability indeed exceeds 14 days. Adnet *et al.*[4] investigated the degradation rate of succinylcholine chloride using nuclear magnetic resonance spectroscopy, and reported a 1.2%/month degradation rate for the 20 mg/mL solution when stored at room temperature. Another research team examined succinylcholine chloride, among other drugs, when stored at ambient temperatures, often exceeding the United States Pharmacopeia (USP) definition of room temperature (20–25°C).[5,6] They found that succinylcholine chloride, in its manufacturer's ampule, retained 89% of the theoretical concentration after 12 months.[5] Similarly, Merlin *et al.*[7] demonstrated that succinylcholine chloride, when stored in a prehospital setting in the manufacturer's vials, protected from light, retained >90% potency, and was not significantly vulnerable to changes in temperature. However, by analyzing degradation products by mass spectrometry, they demonstrated that degradation was apparent even at the study's initiation, evidenced by detectable levels of succinyl monocholine and choline. These studies align with an earlier investigation demonstrating the resistance of succinylcholine chloride to various manipulations, including the addition of preservatives and sterilization by the hospital pharmacist.[8]

To facilitate workflow, some hospitals may desire to aliquot drug products into syringes. The ability to prefill syringes and store them at room temperature enables timely dosing and may help mitigate dosing errors that could occur when withdrawing drug product from the bulk container.[9,10] A 2024 systematic review of prefilled syringe applications reported consistent reductions in medication errors, adverse events, preparation time, and drug waste.[11] For succinylcholine chloride, these advantages are particularly pronounced given the high stress and time-sensitive scenarios that characterize its use.[1] Despite data supporting the chemical stability of succinylcholine chloride against chemical degradation, most health care systems would not authorize transferring a drug product to a secondary container without data to support such manipulation. Vials of succinylcholine chloride may be stored at 2–8°C for 30 days or at room temperature for up to 14 days, per the manufacturer's labeling.[3] Therefore, we aim to examine the stability of succinylcholine chloride when transferred from the manufacturer's container into 1-mL polypropylene syringes.

The percentage recovery of the aliquoted drug was monitored for 90 days using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, a gold standard for drug stability analysis.[12-14] The 90-day study period was selected to support emergency department and emergency medical service (EMS) preparedness while providing data for BUD decisions.

2. Materials and methods

2.1. Equipment and chromatographic conditions

All chromatographic analyses were performed using an HPLC system with UV detection (LC-20ADXR pumps, SIL-20ACXR autosampler, CBM-20A controller, CTO-20A column oven, and SPD-20A UV detector,

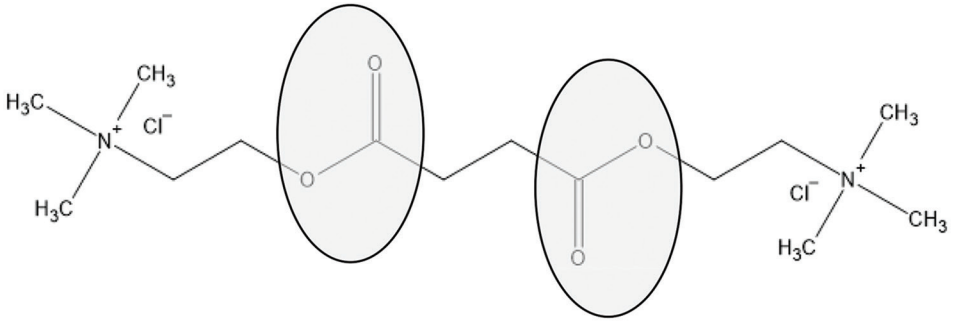

Figure 1. Chemical structure of succinylcholine chloride. The two ester bonds (circled) contribute to the compound's hydrolytic instability.

Shimadzu Scientific, Japan). The detection wavelength was optimized at 218 nm based on succinylcholine's UV absorbance characteristics.[15] The mobile phase consisted of liquid chromatography–mass spectrometry (LC–MS) grade 0.01% trifluoroacetic acid in water and LC–MS grade acetonitrile (60:40) (Honeywell Burdick and Jackson, USA) at a flow rate of 0.400 mL/min. Chromatographic separation was achieved using an HPLC column (150 × 4.6 mm; 3.5-µm particle size) (XBridge C18, Waters Corporation, USA). Succinylcholine chloride calibration standards (5–25 mg/mL) were prepared daily using a USP-grade reference standard (USP Reference Standard, USA) in a 50:50 acetonitrile: water mixture. Anectine® (succinylcholine chloride injection, 200 mg/10 mL) was used for the stability investigation (Sandoz, USA). All samples were filtered using a 13-mm 0.2-µm syringe filter (Whatman® Puradisc, Cytiva, USA) before analysis. Sample injection volume was 30 µL, with autosampler (Fisher Scientific, Waltham, USA) purging (2-propanol) between injections.

2.2. HPLC with UV detection method validation procedures

Validation was performed in accordance with USP General Chapter <1225>, focusing on system suitability, precision, accuracy, and linearity.[16] For system suitability, each 20 mg/mL calibration sample chromatogram (100% assay level) was required to meet the criteria of resolution ≥2.0, tailing factor ≤2.0, and column efficiency (theoretical plates) ≥2,000. Benchmarks for precision and accuracy were < 5% relative standard deviation (RSD) and <5% error, respectively. For these experiments, three replicates of each concentration, 15 mg/mL (75% assay level), 20 mg/mL (100% assay level), and 25 mg/mL (125% assay level), were evaluated over three days. Finally, linearity over the range of 5–25 mg/mL was evaluated on each day of the stability investigation. The linearity target was a coefficient of determination (R^2) > 0.99.

2.3. Stability study setup

The stability study was designed to encompass unit-dosing in syringes and room-temperature storage. Ten vials of succinylcholine chloride from a single lot (AA0058, expiration 04/2025) were obtained to eliminate the possibility of cross-lot variability. Vials were randomly allocated into two storage conditions: 5 vials remained in the manufacturer's original container and stored under refrigerated conditions (2–8°C) as controls, while 5 vials were aliquoted into 1-mL polypropylene syringes and stored at room temperature (20–25°C), representing the experimental group. Sampling time points (0, 24, 48 h; 7, 14, 21, 30, 60, 90 days) were selected to capture both early stability patterns and long-term degradation trends. At each time point, samples from each of the five replicate vials/syringes per condition were analyzed in triplicate. For these analyses, succinylcholine concentrations were determined by comparing peak areas to a freshly prepared calibration curve generated from reference-standard calibration solutions. Calculated concentrations were expressed as percentage recovery relative to day 0 samples (designated as 100% initial concentration). Statistical comparisons between experimental and control samples were performed using a two-way analysis of variance (ANOVA) (p<0.05) using GraphPad Prism software (Version 9.5.1, GraphPad Software, Inc., USA). These analyses did not assess bioequivalence.

3. Results

3.1. HPLC with UV detection method validation results

The HPLC–UV method was adapted from an LC–MS assay previously used for the quantification of various neuromuscular blocking agents, including succinylcholine chloride.[17] An example chromatogram of succinylcholine chloride, with a retention time of about 3.5 min at the 100% assay level, is shown in Figure 2. Validation metrics are summarized in Table 1. Notably, the tailing factor, theoretical plates, and resolution all meet the USP <1225> benchmarks, indicating symmetrical peaks well separated from other formulation components. Linearity assessment revealed excellent correlation between concentration and detector response across the 5–25 mg/mL range, with R^2 exceeding the acceptance criteria of 0.99. The calibration range was chosen so that 20 mg/mL corresponded to 100% assay concentration for the stability study, representing the concentration of the commercial product. As such, the samples could be introduced into the HPLC vials for analysis without manipulation, eliminating a source of error. Precision and accuracy evaluation demonstrated the method's suitability for stability investigations, with % RSD and % error well within the ≤5% threshold.

3.2. Chemical stability over 90 days

Analytical performance remained robust throughout the stability investigation, with all system suitability criteria consistently satisfied. The chromatogram shown in Figure 3 illustrates the typical separation quality achieved at study initiation. Complete resolution of the succinylcholine chloride peak from formulation components was maintained across all samples, ensuring accurate quantification throughout the 90 days.

All samples maintained excellent physical stability throughout the 90-day study period, with no visual

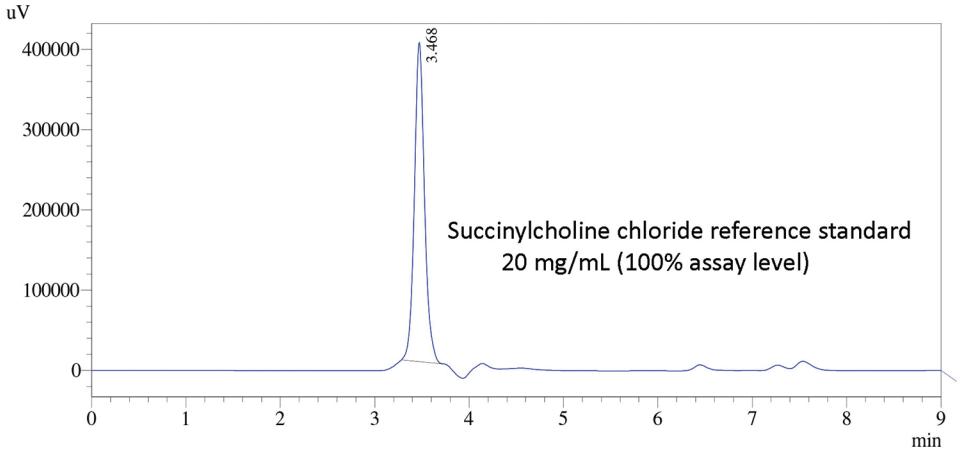

Figure 2. Representative high-performance liquid chromatography chromatogram of succinylcholine chloride reference standard

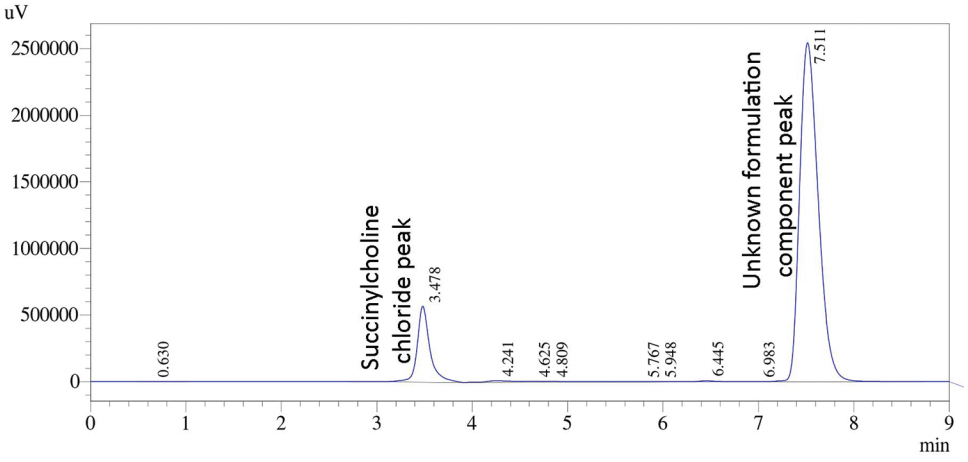

Figure 3. High-performance liquid chromatography chromatogram of the study sample at Day 0. The representative chromatogram shows separation from formulation excipients.

Table 1. High-performance liquid chromatography with ultraviolet detection method validation data

Validation criteria	Results
System suitability	Average tailing factor=1.18 Average theoretical plates (N)=4,112 Average resolution=9.275
Linearity	R^2=0.9974 Calibration range: 5–25 mg/mL
Accuracy (% error)	75% assay level=2.08 100% assay level=0.85 125% assay level=1.07
Precision (% relative standard deviation)	75% assay level=2.93 100% assay level=3.24 125% assay level=1.88

Note: Validation parameters were evaluated over three days, according to United States Pharmacopeia Chapter<1225>.

evidence of precipitation, crystallization, or discoloration. In addition, succinylcholine chloride demonstrated remarkable chemical stability throughout the 90-day study period, with both storage conditions maintaining >90% recovery (Table 2, Figure 4). Room-temperature syringe samples retained 93.13% recovery at day 90, representing only a 6.87% degradation over the 3-month period. Refrigerated controls were very similar (93.97% at day 90), indicating that the temperature differential between storage conditions had minimal impact on chemical degradation. The stability profile showed gradual, linear degradation over time, without evidence of accelerated degradation phases. This pattern supports the previously cited predictable degradation kinetics.[4]

Statistical analysis using two-way ANOVA confirmed

Table 2. Succinylcholine chloride concentration (mg/mL) data throughout the 90-day stability study

Storage period	Experimental samples (room-temperature syringe storage; 20–25°C)	Control samples (refrigerated manufacturer container; 2–8°C)
Initial concentration	20.48±0.44	20.48±0.44
24-h storage	20.31±0.63 (99.16)	20.42±0.38 (99.76)
48-h storage	20.27±0.33 (98.96)	20.43±0.43 (99.76)
7-d storage	20.11±0.40 (98.20)	20.15±0.60 (98.40)
14-d storage	19.99±0.25 (97.59)	20.06±0.18 (97.93)
21-d storage	19.82±0.28 (96.71)	19.93±0.28 (97.29)
30-d storage	19.55±0.30 (95.48)	19.66±0.22 (95.98)
60-d storage	19.12±0.36 (93.37)	19.31±0.34 (94.27)
90-d storage	19.07±0.25 (93.13)	19.25±0.26 (93.97)

Note: Values represent mean concentration (mg/mL)±standard deviation (% recovery) from n=5 replicate samples analyzed in triplicate injections at each time point. Percentages in parentheses indicate percent recovery relative to Day 0 concentration (defined as 100%).

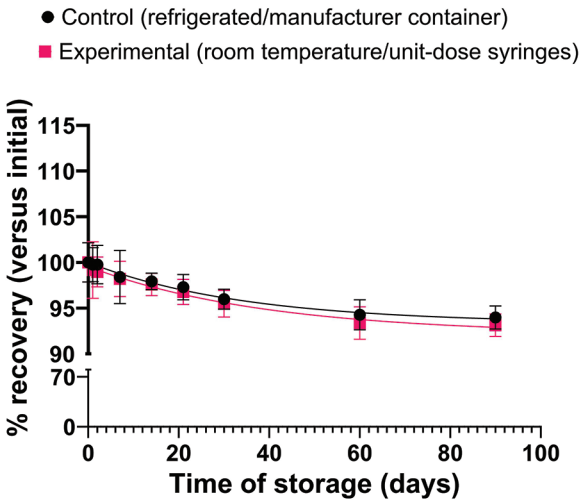

Figure 4. Succinylcholine chloride percent recovery relative to Day 0 throughout a 90-day storage period. Data are presented as mean recovery ± standard deviation (n = 5 samples with triplicate injections at each time point).

no significant differences between storage conditions at any time point (p>0.05), with confidence intervals remaining narrow throughout the study, indicating consistent performance across all replicate samples. This analysis assessed whether there were significant differences between storage conditions, but it is insufficient to demonstrate bioequivalence.

4. Discussion

Our findings show > 90% recovery of injectable succinylcholine chloride over 90 days, consistent with 89% potency after 12 months[5] and with > 90% potency in prehospital settings at elevated temperatures previously shown.[7] The degradation rate of succinylcholine chloride reported in this study is approximately 2.3% per month, similar to what was reported by Adnet *et al.*[4] Our work supplements prior findings by demonstrating that chemical stability extends to succinylcholine chloride transferred to secondary containers. These results suggest that the manufacturer's 14-day room-temperature limit may be conservative regarding chemical stability. These data support the chemical stability of succinylcholine chloride when stored in syringes for up to 90 days. In addition, collecting these data from room-temperature syringes aligns with realistic emergency department and EMS storage conditions. Demonstrating chemical stability in secondary containers is a necessary first step toward potentially extending BUD, thereby reducing medication waste. Furthermore, prefilled syringes could improve emergency response times, reducing dosing errors in critical emergencies.[9,11]

The choice of polypropylene syringes for this work was based on literature supporting this polymer's compatibility with small-molecule drugs.[18-20] The absence of a statistically significant difference between syringe-stored samples and those in the manufacturer's container (p>0.05) is consistent with polypropylene compatibility for succinylcholine chloride storage. However, alternative syringe materials, different concentrations, or different storage conditions may yield different results and would require separate validation.

Analytical validation confirmed the method's suitability for stability assessment, with all USP <1225> parameters consistently meeting acceptance criteria throughout the 90-day investigation. Unlike previous LC–MS investigations that detected early formation of succinyl monocholine and choline,[7] UV detection at 218 nm did not reveal observable degradation products. This difference likely reflects the inherent sensitivity limitations of UV detection compared to mass spectrometry, or potentially indicates that degradation product concentrations remained below the method's detection threshold. Future investigations monitoring impurity formation will require more advanced equipment, such as LC–MS, to detect low-level degradation products. Future studies should evaluate additional syringe materials and extend the study duration beyond 90 days.

Finally, the most significant limitation of this investigation is the absence of sterility testing. A recent systematic review indicates that among 24 studies involving prefilled syringes, only four (17%) evaluated sterility and

contamination.[11] Extended beyond-use dating requires both chemical stability and microbiological safety, the latter being paramount for parenteral medications. This study establishes a critical foundation, but future studies monitoring product sterility to ensure microbial integrity of the injectable product will be needed to fully implement succinylcholine in secondary containers.

5. Conclusion

This investigation demonstrates that succinylcholine chloride maintains >90% recovery when stored in 1-mL polypropylene syringes at room temperature for 90 days, exceeding the manufacturer's 14-day recommendation. The absence of significant differences between room-temperature syringe storage and refrigerated storage indicates that container transfer does not compromise drug stability. The chemical stability validation eliminates a key barrier to implementing prefilled syringe protocols, which have been shown to reduce medication errors (10–73%), decrease adverse event rates, and minimize waste (from 46–92% to 0–15%).[11] The chemical stability demonstrated here, if complemented by appropriate sterility validation, could support improvements in emergency airway management workflows while maintaining the chemical integrity essential for effective neuromuscular blockade.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Timothy Coffey, Stacy Brown
Data curation: Jeffrey Klein
Formal analysis: Stacy Brown
Funding acquisition: Stacy Brown
Investigation: Jeffrey Klein
Methodology: Stacy Brown, Jeffrey Klein
Project administration: Timothy Coffey, Stacy Brown
Software: Stacy Brown
Resources: Timothy Coffey, Stacy Brown
Supervision: Stacy Brown
Validation: Stacy Brown, Jeffrey Klein
Visualization: Timothy Coffey
Writing-original draft: Stacy Brown, Jeffrey Klein
Writing-review & editing: Stacy Brown

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

Further disclosure

The HPLC-UV method development and validation that supported this study were previously presented (prior to initiation of the stability study) as a poster at the Appalachian Student Research Forum (ASRF), April 5, 2024, Johnson City, Tennessee, United States, titled "Chemical Potency of Succinylcholine Chloride Stored in Room-Temperature Syringes."

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