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Validation of an improved ion chromatography method for the limit of choline test in the USP Succinylcholine Chloride monograph

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Keywords

Dionex IonPac CS19 column, suppressed conductivity detection, pharmaceutical, USP monograph, drug substance, drug product, impurity, United States Pharmacopeia, ICS-5000+, ICS-6000

Goal

To validate an improved ion chromatography method developed for the limit of choline test in the United States Pharmacopeia (USP) Succinylcholine Chloride monograph

Introduction

Succinylcholine chloride, also known as suxamethonium or suxamethonium chloride, is a United States Food and Drug Administration (FDA) approved intravenous (IV) medication used as a skeletal muscle relaxant during procedures of short duration (e.g., endotracheal intubation, endoscopic examinations, electrically or pharmacologically induced convulsive therapy) after general anesthesia has been induced.[1,2]

Pharmaceutical companies have tried using the ion chromatography (IC) method to test the limit of choline in succinylcholine chloride according to the USP Succinylcholine Chloride monograph,[3] but they observed a problem. These companies reported that after about 6–7 h of analysis a large peak elutes. This interferes with subsequent analyses. They also reported that during the 6–7 h there is a loss of choline retention time. We confirmed these observations and developed a new method to solve the problem. The modified IC method has been proposed for the limit of choline test in the USP Succinylcholine Chloride monograph.[4]

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This application note reports the method development and then the evaluation of the improved IC method for the limit of choline test. The evaluation follows the guidelines given by the International Conference on Harmonization (ICH) and the USP, which are outlined in the ICH Guideline Q2A and Q2B Validation of Analytical Procedures,[5,6] the USP General Chapter <1225> Validation of Compendial Methods,[7] and USP General Chapter <621> Chromatography.[8] A Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system with a Thermo Scientific™ Dionex™ IonPac™ CS19 anion-exchange column (USP L97) and a Thermo Scientific™ Dionex™ CERS™ 500 Cation Electrolytically Regenerated Suppressor for suppressed conductivity detection were used to execute the method.

Experimental

Equipment

- A Dionex ICS-5000+ HPIC system* was used in this work. It includes:
  - Eluent Generator
  - Pump
  - Column Heater
  - Degasser
  - Conductivity Detector with cell
- Thermo Scientific™ Dionex™ AS-AP Autosampler, with 250 µL syringe (P/N 074306), 1.2 mL buffer line assembly (P/N 074989), 5 µL injection loop
- Thermo Scientific™ Dionex™ EGC 500 MSA Methanesulfonic Acid Eluent Generator Cartridge (P/N 075779)
- Dionex CERS 500 Cation Electrolytically Regenerated Suppressor (2 mm) (P/N 082543)
- Thermo Scientific™ Dionex™ CR-CTC 500 Continuously Regenerated Cation Trap Column (P/N 075551)
- Thermo Scientific™ Chromeleon™ 7.2 Chromatography Workstation

*This method can be run on any system supporting an electrolytic suppressor or any Thermo Scientific™ Dionex™ ion chromatography system using a chemically regenerated suppressor. Please note that this method was not tested with a chemically regenerated suppressor. This method can also be run with manually prepared MSA but was tested using electrolytically generated MSA.

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Choline chloride USP reference standard (Sigma-Aldrich® Cat# 1133547-500MG, Lot R060C0)
- Succinylcholine chloride (Acros Organics™ 96%, Cat# AC460110050)
- Potassium chloride (Mallinckrodt®, 99.7%, Cat# 6858)

Conditions

Table 1. Chromatography conditions of the original USP monograph method[3] to test the limit of choline in succinylcholine chloride

Columns:	Thermo Scientific™ Dionex™ IonPac™ CS19 2-mm Analytical, 2 × 250 mm (P/N 076028) Thermo Scientific™ Dionex™ IonPac™ CG19 2-mm Guard, 2 × 50 mm (P/N 076029)
Eluent:	6.4 mM (0.62 g/L) Methanesulfonic acid (MSA)
Eluent Source:	Dionex EGC 500 MSA cartridge with Dionex CR-CTC 500 continuously regenerated cation trap column (may not have been used by the method contributor)
Flow Rate:	0.25 mL/min
Injection Volume:	5 µL (full loop)
Column Temperature:	35 °C
Detection:	Suppressed conductivity, Dionex CERS 500 (2 mm) Suppressor, recycle mode, 5 mA current
Detection/ Suppressor Compartment:	30 °C
Cell Temperature:	35 °C
System Backpressure:	~2700 psi
Noise:	< 3 nS/min
Run Time:	18 min

Table 2. Chromatography conditions of the improved IC method to test[4] the limit of choline in succinylcholine chloride

Columns:	Dionex IonPac CS19 2-mm Analytical, 2 × 250 mm (P/N 076028) Dionex IonPac CG19 2-mm Guard, 2 × 50 mm (P/N 076029)		
Eluent:	Methanesulfonic acid (MSA)		
	Time (min)	*MSA (mM)*	*Curve*
	-3	6	5
	0	6	5
	14	6	5
	15	50	5
	33	50	5
	34	6	5
	40	6	5
Eluent Source:	Dionex EGC 500 MSA cartridge with CR-CTC 500 continuously regenerated cation trap column		
Flow Rate:	0.25 mL/min		
Injection Volume:	5 µL (full loop)		
Column Temperature:	30 °C		
Detection:	Suppressed conductivity, Dionex CERS 500 (2 mm) Suppressor, recycle mode, 37 mA current		
Detection/ Suppressor Compartment:	30 °C		
Cell Temperature:	30 °C		
System Backpressure:	~2900 psi		
Noise:	< 2 nS/min		
Run Time:	43 min		

Preparation of solutions and reagents

Note: Do not use glassware to prepare the solutions. Polymeric containers made of high-density polyethylene (HDPE) are recommended.

Stock standard solutions 1000 µg/mL

Accurately weigh 100.0 mg of pure anhydrous salts (choline chloride using USP reference standard, potassium chloride using 99.7 % salt) into 125 mL polypropylene bottles, and dissolve in 100 mL (100.00 g) of DI water to make 1000 µg/mL stock solutions. Keep stock standard solutions at 4 ˚C.

Choline chloride calibration standard, 0.2, 2, 4, 8, 16, 25, 50 µg/mL

To prepare calibration standard solutions, dilute the choline chloride stock standard solution (1000 µg/mL) to the appropriate concentrations with DI water.

System suitability solution

Mix the stock standard solutions 1.00 mL (1.00 g) of choline chloride and 0.50 mL (0.50 g) of potassium chloride stock) and 98.5 mL (98.5 g) of DI water to make the system suitability solution containing 10.0 µg/mL of choline chloride and 5.0 µg/mL of potassium chloride. Keep the solution at 4 °C.

Sample preparation

Succinylcholine chloride sample solution, 2.000 mg/mL

Accurately weigh 200.0 mg of succinylcholine chloride into a 125 mL polypropylene bottle and dissolve in 100 mL (100.0 g) DI water. Store at 4 °C immediately following preparation.

Spiked succinylcholine chloride sample solution

Accurately weigh 400.0 mg of succinylcholine chloride into a 125 mL polypropylene bottle and dissolve in 100 mL (100.0 g) DI water to make 4.000 mg/mL succinylcholine chloride sample stock. Mix the 4.000 mg/mL succinylcholine chloride sample stock, choline chloride standard, and DI water to make the 1, 2, 4, and 8 µg/mL of choline chloride spiked in 2.000 mg/mL sample solution. (For example, to make 2 µg/mL of choline chloride spiked in 2.000 mg/mL sample, mix 4 mL of the 4.000 mg/mL sample stock, 1 mL of 16 µg/mL choline chloride standard, and 3 mL of DI water.) These are 0.04% to 0.3% of choline chloride in succinylcholine chloride.

Robustness study

Following the guidelines of USP General Chapter <1225>, Validation of Compendial Methods[6] and USP General Chapter <621>, Chromatography[7], the robustness of this method was evaluated by examining the results of the suitability standard (concentration, retention time (RT), peak asymmetry of choline, and resolution between potassium and choline) after imposing a small variation (±10%) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature).

The following variations were tested:

- Flow rate at 0.275 mL/min, 0.25 mL/min, 0.225 mL/min
- Column temperature at 27 °C, 30 °C, 33 °C
- Eluent: Methanesulfonic acid (MSA) concentrations: ±10% as shown in Table 3.

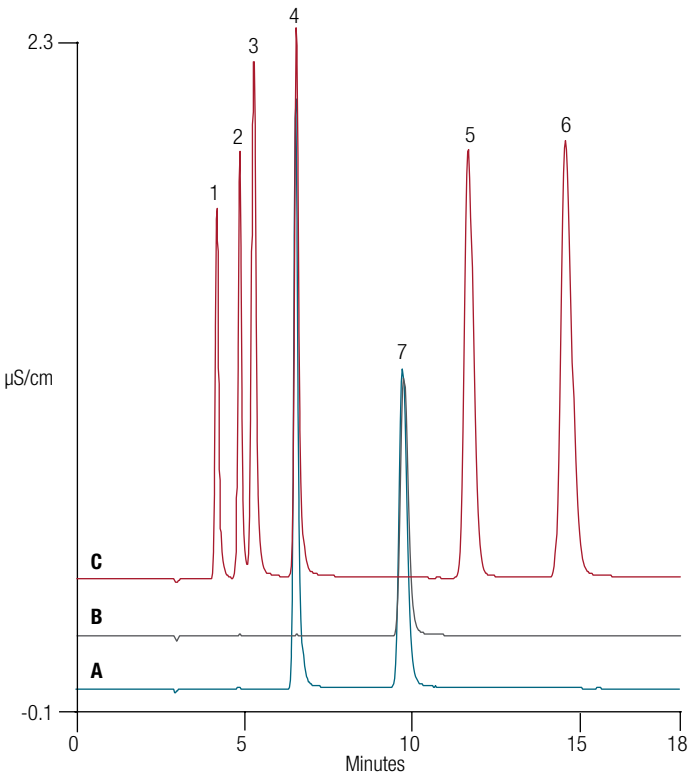
Table 3. Eluent concentrations for robustness

Time (min)	MSA (mM)	+10% MSA (mM)	-10% MSA (mM)
-3	6	6.6	5.4
0	6	6.6	5.4
14	6	6.6	5.4
15	50	55	45
33	50	55	45
34	6	6.6	5.4
40	6	6.6	5.4

Results and discussion

Separation of choline from cations

Figure 1 shows the separation of choline (retention time about 10 min) from common cations using the chromatography conditions of the original USP monograph method (Table 1); choline is well separated from the common cations (lithium, sodium, ammonium, potassium, magnesium, and calcium).



Columns:	Dionex IonPac CG19, 2 × 50 mm Dionex IonPac CS19, 2 × 250 mm
Eluent:	6 mM Methanesulfonic acid (MSA)
Flow Rate:	0.25 mL/min
Inj. Volume:	5 µL (Full loop)
Column Temp.:	35 °C
Sampler Temp.:	4 °C
Detection:	Suppressed conductivity, Dionex CERS 500 (2 mm) Suppressor, 30 °C, 37 mA, recycle mode
Samples:	A: System suitability solution B: 8 µg/mL USP Choline Chloride standard C: Mixed common cations

Peaks:	A	B	C (µg/mL)
1. Lithium	0	0	0.25
2. Sodium	0	0	1.00
3. Ammonium	0	0	1.25
4. Potassium	0	50	2.50
5. Magnesium	0	0	1.25
6. Calcium	0	0	2.50
7. Choline	8.0	10.0	0

Figure 1. Separation of choline from common cations using the isocratic IC method described in the USP Succinylcholine Chloride monograph

It was confirmed that choline retention was less after each injection of the sample containing succinylcholine chloride. It was also confirmed that the baseline shifted upward ~ 6.5 h after the injection of the first succinylcholine chloride sample (data not shown). This interferes with subsequent sample injections. An improved IC method (Table 2) was developed based on the assumption that the baseline rise and retention time problem of the original USP method are caused by the retention of succinylcholine on the column

Figure 2 shows separation of choline from succinylcholine and common cations with the improved IC method for choline analysis. Similar to the original method, choline is well separated from the common cations. With the addition of a 50 mM MSA wash for 18 min to each injection (Table 2), succinylcholine is eluted at about 26 min. The total run time of this method is 43 min, which includes an additional 3 min of column re-equilibrium at the starting conditions. By eluting succinylcholine, choline retention time is stable during choline analysis and no baseline upset (rise) is observed after 6 to 7 h of sample analysis. The remainder of this application note will report data from the evaluation of this method and discuss this evaluation.

Calibration, limit of detection (LOD) and limit of quantitation (LOQ)

First, a calibration curve for choline chloride was established at seven levels from 0.2 to 50 µg/mL. Figure 3 shows the calibration plot for choline. A linear relationship was observed for peak area to concentration with a coefficient of determination (r^2) of 0.9998 (Figure 3 and Table 4).

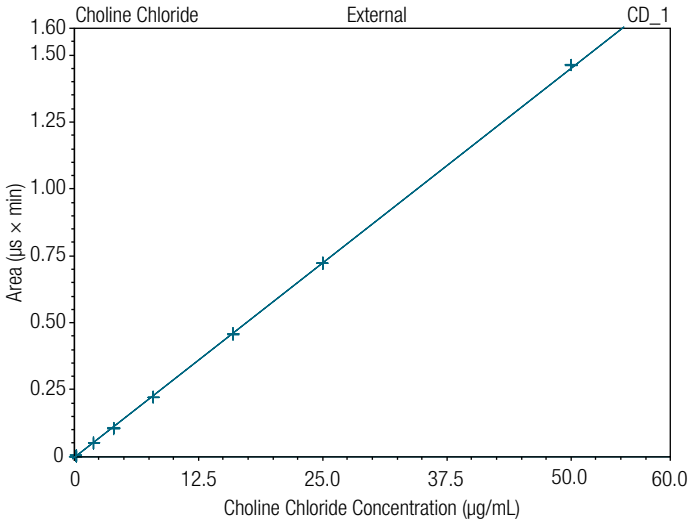
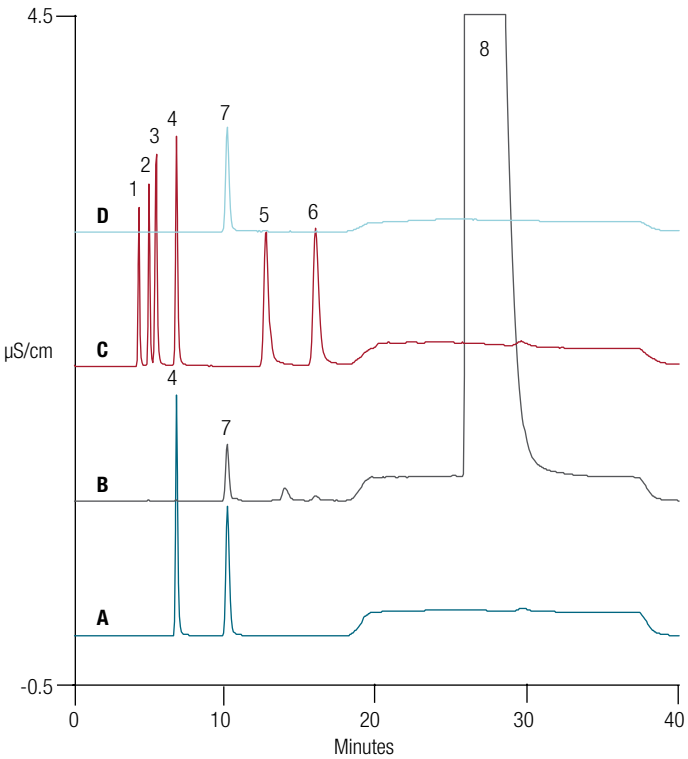


Figure 3. Calibration plot for choline chloride analysis (n=3)



Columns:	Dionex IonPac CG19, 2 × 50 mm Dionex IonPac CS19, 2 × 250 mm
Eluent:	Methanesulfonic acid (MSA): 6 mM 0–14 min; 6 to 50 mM, 14–15 min; 50 mM, 15–33 min; 50 to 6 mM, 33–34 min; 6 mM 34–40 min.
Flow Rate:	0.25 mL/min
Inj. Volume:	5 µL (Full loop)
Column Temp.:	35 °C
Sampler Temp.:	4 °C
Detection:	Suppressed conductivity, Dionex CERS 500 (2 mm) Suppressor, 30 °C, 37 mA, recycle mode
Samples:	A: System suitability solution B: 2 mg/mL of succinylcholine chloride sample C: Mixed common cations D: 8 µg/mL USP Choline Chloride standard

Peaks:

	A	B	C	D (µg/mL)
1. Lithium	0	0	0.25	0
2. Sodium	0	0	1.00	0
3. Ammonium	0	0	1.25	0
4. Potassium	5.0	0	2.50	0
5. Magnesium	0	0	1.25	0
6. Calcium	0	0	2.50	0
7. Choline	10.0	3.0	0	8.0
8. Succinylcholine	0	2000	0	0

Figure 2. Separation of choline from succinylcholine and common cations using an improved IC method

Table 4. Calibration, LOD, and LOQ for choline

Calibration Standards (µg/mL)	Calibration Type	r^2	Response Factor (µS × min)/(µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
0.2–50	Linear, through origin	0.9998	0.0291	0.06	0.2

The LOD and LOQ were determined by seven injections of the 0.20 mg/L choline standard. The baseline noise was determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peak of interest. The LOD and LOQ were determined for the concentration at the signal-to-noise ratio 3× and 10× (Table 4). Using this method, the LOD was determined to be 0.06 mg/L and the LOQ was 0.2 mg/L.

System suitability

The system suitability solution contains 10.0 µg/mL of choline chloride and 5.0 µg/mL of potassium chloride. Table 5 shows the system suitability results from three different sequences over three days. The method has relative standard deviation (RSD) of 0–0.1% for retention time and 0.2–1% for peak area. The resolution between choline and potassium is 10. These results surpass the suitability requirements of RSD, not more than (NMT) 3% for choline, and resolution, not less than (NLT) 5.0, between choline and potassium in the current Succinylcholine Chloride USP monograph and the proposed revision.[3,4]

Sample analysis and precision

As a linear relationship of peak area to concentration was established for choline chloride from 0.2 to 50 µg/mL, the proposed monograph revision IC method uses a single standard point of 8 µg/mL choline chloride standard to determine the percentage of choline in the portion of succinylcholine chloride taken.

The percentage of choline in the portion of succinylcholine chloride taken was calculated as follows:

$$\text{Result (\%)} = \left(\frac{ru}{rs}\right) \times \left(\frac{Csd}{Cu}\right) \times \left(\frac{Mr1}{Mr2}\right) 100$$

ru= Peak area of choline from the succinylcholine chloride *Sample* solution

rs= Peak area of choline from the choline chloride *Standard* solution

Csd= Concentration of USP Choline Chloride RS in the *Standard* solution (8 µg/mL)

Cu= Concentration of succinylcholine chloride in the *Sample* solution (1000 × 2.000 mg/mL)

Mr1= Molecular weight of choline, 104.17

Mr2= Molecular weight of choline chloride, 139.62

Table 5. System suitability data of the improved IC method over three days

Day	Retention Time				Peak Area				Resolution	
	Potassium		Choline		Potassium		Choline			
	Average (min)	RSD	Average (min)	RSD	Average (µS × min)	RSD	Average (µS × min)	RSD	Average	RSD
1	6.7	0.1	10.1	0	0.31	1	0.28	0.4	10	0.1
2	6.7	0	10.0	0	0.31	0.2	0.28	0.2	10	0.2
3	6.7	0	10.1	0	0.31	0.2	0.28	0.3	10	0.2

The USP monograph requires that succinylcholine chloride contain no more than 0.3% of choline, which is equal to 8 µg/mL choline chloride in 2.00 mg/mL of succinylcholine chloride.

The method reproducibility and precision were evaluated by running the test for choline in the succinylcholine chloride sample. Table 6 lists the results over three separate days. Three sample solutions, 2.000 mg/mL of succinylcholine chloride in water, were independently prepared on each day. Each sample solution was tested with multiple (n=3) injections. The succinylcholine chloride sample contained 0.11% choline, which passes the acceptance criteria of NMT 0.3%. Table 6 also shows that the method is precise with intraday precision from 0.5% to 1.2% and interday precision of 3.4%

Method accuracy

Method accuracy was validated by spiked recovery of choline in a succinylcholine chloride sample over four concentration levels and over three days, with three replicates of each concentration (Table 7). The method was shown to be accurate with good recovery (average from 104% to 118%) for a low level of choline (0.04% to 0.3%) spiked in succinylcholine chloride.

Robustness

Using the system suitability solution containing 10.0 µg/mL of choline chloride and 5.0 µg/mL of potassium chloride, robustness of the improved IC method was evaluated by measuring the influence of small variations (±10%) in chromatography parameters (e.g., flow rate, eluent concentration, and column temperature) on the measured choline concentration, RT, peak asymmetry, and resolution between potassium and choline. The peak asymmetry was measured using the USP formula. The resolution was determined relative to the previous peak in the chromatogram using the USP formula. The system suitability solution was injected three times at each chromatographic condition. These experiments were run on two columns from different lots. Table 8 summarizes the robustness test results. Although choline RT changed when the chromatography condition changed, the choline peak asymmetry and resolution between choline and potassium only change a small amount (<4%), and the measured choline concentrations were about the same (<1.1% variation) for all chromatography conditions. These results indicate the method was robust to changes in chromatography conditions.

Table 6. The percentage of choline in the succinylcholine chloride sample

	Day 1		Day 2		Day 3	
	Average (%)	RSD	Average (%)	RSD	Average (%)	RSD
Sample 1	0.11	0.6	0.11	0.4	0.12	3.3
Sample 2	0.11	0.5	0.11	0.2	0.12	0.2
Sample 3	0.11	0.5	0.11	0.1	0.12	0.1
Average	0.11	0.5	0.11	0.3	0.12	1.2
Overall average = 0.11% RSD = 3.4%						

Table 7. Recovery of choline spiked in succinylcholine chloride

Choline Spiked in 2.000 mg/mL Succinylcholine Chloride Sample	Day 1		Day 2		Day 3		Average Recovery (%)
	Recovery (%)	RSD	Average (%)	RSD	Average (%)	RSD	
0.04%	127	1	98	0.8	130	1.1	118
0.07%	104	0.5	99	0.4	114	0.3	106
0.15%	103	0.2	101	0.4	108	0.3	104
0.3%	104	0.1	103	0.3	107	0.3	105

Table 8A. Robustness of the improved IC method for choline in succinylcholine chloride for Column A*

Parameter		Retention Time		Asymmetry		Resolution	
		Min	Diff. (%)		Diff. (%)		Diff. (%)
Flow Rate (mL/min)	0.275	9.15	-9	1.28	1.3	9.7	-2.6
	0.25	10.06		1.27		9.9	
	0.225	11.17	11	1.29	2.1	10.2	2.4
Eluent Conc. (mM)	5.445	10.84	8	1.26	-0.5	10.3	4.0
	650	10.06		1.27		9.9	
	6.655	9.43	-6	1.28	0.8	9.5	-3.8
Column Temp. (°C)	27	10.39	3	1.28	0.8	9.8	-0.8
	30	10.06		1.27		9.9	
	33	9.77	-3	1.27	0.3	9.9	0.3

*Injected sample: 10.0 µg/mL of choline chloride and 5.0 µg/mL of potassium chloride in water; average of three injections for each condition

Table 8B. Robustness of the improved IC method for choline in succinylcholine chloride for Column B*

Parameter		Retention Time		Asymmetry		Resolution	
		Min	Diff. (%)		Diff. (%)		Diff. (%)
Flow Rate (mL/min)	0.275	9.76	-9	1.23	-2	10.1	-2
	0.25	10.71		1.26		10.2	
	0.225	11.88	11	1.27	1	10.5	0
Eluent Conc. (mM)	5.445	11.55	8	1.23	-2	10.0	-3
	650	10.71		1.26		10.2	
	6.655	9.99	-7	1.26	0	10.6	3
Column Temp. (°C)	27	11.05	3	1.25	-1	10.2	0
	30	10.71		1.26		10.2	
	33	10.34	-3	1.25	-1	10.3	1

*Injected sample: 10.0 µg/mL of choline chloride and 5.0 µg/mL of potassium chloride in water; average of three injections for each condition

Conclusion

This study described a modified IC method for the Limit of Choline Test in the USP Succinylcholine Chloride monograph. The IC method, with the addition of a 50 mM MSA wash for 18 min to each injection to elute succinylcholine, meets the parameters specified in the USP Succinylcholine Chloride monograph and was validated following USP and ICH guidelines. The study showed that the IC method is reproducible, has a linear calibration, and is sensitive for choline determination in succinylcholine chloride. The method is precise, accurate, and robust. Therefore, it is suitable to replace the current limit of choline method in the Succinylcholine Chloride USP monograph, which was found to be problematic.

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