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# HPLC Method Development and Validation for Preservative Content (Methyl Paraben) For Succinylcholine Chloride Injection

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**Keywords:** Succinylcholine chloride injection, Preservative content, HPLC

**ABSTRACT**

Aim of this research work was to develop and validate HPLC method for preservative content (Methyl paraben) for Succinylcholine chloride injection. The method is found to be specific. The method is also stability indicating as evidenced by forced degradation studies. The method is found to be linear in the specified range. The method is robust w.r.to flow and temperature variations. System suitability is established and recorded. Hence, this method can be used for routine analysis.

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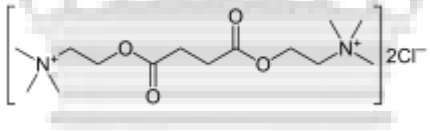
## INTRODUCTION

Analytical methods validation is an important regulatory requirement in pharmaceutical analysis. High-Performance Liquid Chromatography (HPLC) is commonly used as an analytical technique in developing and validating assay methods for drug products and drug substances (1). Method validation provides documented evidence and a high degree of assurance that an analytical method employed for a specific test, is suitable for its intended use. Over recent years, regulatory authorities have become increasingly aware of the necessity of ensuring that the data submitted to them in applications for marketing approvals have been generated using validated analytical methodology. The International Conference on Harmonization (ICH) has introduced guidelines for analytical methods validation (2, 3). Both United States Food and Drug Administration (USFDA) as well as United States Pharmacopoeia (USP) refer to ICH guidelines (4-7).

Product Name: Succinylcholine chloride

Chemical Name: 2, 2'-[(1,4-dioxobutane-1,4-diyl) bis(oxy)]bis (N,N, Ntrimethylethanaminium)

Molecular Structure:




Molecular formula: $C_{14}H_{30}N_2O_4$

Molecular Weight: 290.399 g/moL

CAS Registry Number: 306-40-1

Succinylcholine Chloride Injection, USP is a sterile, nonpyrogenic solution to be used as a short-acting, depolarizing, skeletal relaxant. The solutions are for I.M. or I.V. use. Succinylcholine Chloride, USP is chemically designated $C_{14}H_{30}N_2O_4$ and its molecular weight is 290.399.

Succinylcholine is a diquaternary base consisting of the dichloride salt of the dicholine ester of succinic acid. It is a white, odorless, slightly bitter powder, very soluble in water. The drug is incompatible with alkaline solutions but relatively stable in acid solutions. Solutions of the drug lose potency unless refrigerated.

Succinylcholine chloride Injection contains methylparaben as preservatives. Unused solution should be discarded. Product not requiring dilution (multiple-dose fliptop vial) contains sodium chloride to render isotonicity, contains sodium hydroxide and/or hydrochloric acid for pH adjustment, pH is 3.6 (3.0 to 4.5).

**Solubility:**

It is highly soluble in water (1 gram in about 1 mL), soluble in alcohol (1 gram in about 350 mL), slightly soluble in chloroform, and practically insoluble in ether.

**Mode of action:**

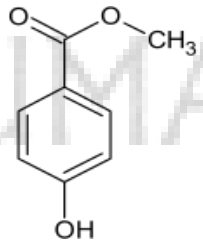
The mechanism of action of Succinylcholine involves what appears to be a "persistent" depolarization of the neuromuscular junction. This depolarization is caused by Succinylcholine mimicking the effect of acetylcholine but without being rapidly hydrolysed by acetyl cholinesterase. This depolarization leads to desensitization.

**Methyl paraben:**

Preservative : Methyl paraben

Chemical Name : Methyl 4-hydroxybenzoate

Molecular Structure :




Molecular formula : $C_8H_8O_3$

Molecular Weight : 152.15 $g \cdot mol^{-1}$

CAS Registry Number: 99-76-3

**Uses:**

Methyl paraben is an anti-fungal agent often used in a variety of cosmetics and personal-care products. It is also used as a food preservative and has the E number E218.

Methyl paraben is considered generally recognized as safe (GRAS) for food and cosmetic antibacterial preservation. Methyl paraben is readily metabolized by common soil bacteria, making it completely biodegradable.

Methyl paraben is readily absorbed from the gastrointestinal tract or through the skin. It is hydrolyzed to p-hydroxybenzoic acid and rapidly excreted in urine without accumulating in the body. Acute toxicity studies have shown that methyl paraben is practically non-toxic by both oral and parenteral administration in animals.

**1.0 Purpose:**

The purpose of this report is to outline the Analytical Method Development and validation of preservative content (Methyl paraben) method for Succinylcholine chloride in drug product of Succinylcholine chloride injection as per USP/ICH guideline.

**2.0 Scope:**

This report for Analytical Method Development is applicable to Analytical Research Department, Laboratory. This development exercise is applicable for Succinylcholine chloride injection.

**3.0 Equipments/Instruments:**

| Sr.No | Components | Details |
|---|---|---|
| 1 | Volumetric flasks | Glass, Class-A |
| 2 | Beakers | Glass |
| 3 | Measuring cylinder | Glass, Class-A |
| 4 | Pipettes | Glass, Class-A (both bulb and Graduated) |
| 5 | Balance | Balance with Sensitivity of 0.01mg |
| 6 | Column | Thermo Hypersil ODS,150 mm x 4.6 mm,5 µ or equivalent |
| 7 | HPLC system | Diode array detector/UV Detector |

**Note:** Ensure that the relevant equipments should be within calibrated state.

## 4.0 Reagents, Standard and Samples:

### 4.1 Reagents:

| Sr.No | Reagents | Batch No. | Grade | Use before |
|---|---|---|---|---|
| 1 | Heptane sulphonic acid | X-737547 | AR | 23 JUNE 2020 |
| 2 | Acetic acid | G14A/1314/1507/31 | AR | 18 JUNE 2018 |
| 5 | Acetonitrile | 4504290615 | HPLC | 18 JULY 2018 |

### 4.2 Standard:

| Sr.No | Working Standard | Batch No | Potency | Use before |
|---|---|---|---|---|
| 1 | Methyl Paraben | K46339557 | 99% | 31JAN2018 |

### 4.3 Sample:

| Sr.No | Sample |
|---|---|
| 1 | Succinylcholine Chloride injection |

Note: All the materials used were within the expiry date and stored at recommended storage conditions.

## 5.0 Procedure:

The following parameters are considered for Analytical Method Development of preservative contents method in the drug product of Succinylcholine chloride Injection.

- System suitability
- Specificity
- Specificity by forced degradation study
- Precision
- System precision
- Method precision
- Linearity
- Robustness

**5.1 System Suitability:**

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

Injected blank (diluent) (1 injection), standard solution of methyl paraben 40 ppm (5 injections), and checked the following system suitability.

| S.No | Acceptance criteria | Result |
|---|---|---|
| 1 | The tailing factor for peak from the standard solution is | 1.05 |
| 2 | The theoretical plates for the peak from the standard solution is | 7737 |
| 3 | %RSD for Methyl paraben peak in standard solution is | 0.3 |

**5.2 Specificity:**

Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.

It was performed the specificity parameter of the method by injecting blank, 4-Hydroxy Benzoic acid, Standard solution and sample solution and recorded the Retention times of blank, 4-Hydroxy Benzoic acid, Standard solution and Sample solution.

**RESULTS**

| S.No. | Solutions | Retentions times (min) |
|---|---|---|
| 1 | blank | ND |
| 2 | 4-Hydroxy Benzoic acid | 1.320 |
| 3 | Standard solution (methyl paraben) | 2.220 |
| 4 | sample solution (succinylcholine containing methyl paraben) | 2.220 |

**5.3 Specificity by Degradation Studies:**

**Specificity by forced degradation:**

Forced degradation of Succinylcholine chloride Injection is carried out, to confirm that during stability study or throughout the shelf life, any degradation product if found should not interfere with the main peak of Methyl paraben. In addition, the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic and dry heat) for each of the degradants.

**Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask and diluted to volume with diluent and mixed well.

**1.0N HCl Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask added 1.0 mL of 1.0N HCl Sample Solution and kept it at room temperature for 4hrs. Neutralised with 1.0 mL of 1.0N NaOH solution made upto the volume with diluent and mixed well. Similarly performed with 0.1N HCl Sample Solution also with respectively.

**1.0N NaOH Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask added 1.0 mL of 1.0N NaOH Sample Solution and kept it at room temperature for 4hrs. Neutralised with 1.0 mL 1.0N HCl of solution made upto the volume with diluent and mixed well. Similarly performed with 0.1N NaOH Sample Solution also with respectively.

**3% Peroxide Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask added 1.0 mL of 3% Peroxide Sample Solution and kept it at room temperature for 4hrs made upto the volume with diluent and mixed well. Similarly performed 1% Peroxide Sample Solution also with respectively.

**Thermal Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask and kept it at 80°C for 4hrs, made upto the volume with diluent and mixed well.

**UV-Light Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask and kept it at UV-Cabinet for 4hrs, made upto the volume with diluent and mixed well.

**1.0N NaOH Sample Solution:** Transferred 1.0 mL of Succinylcholine chloride injection sample into 25.0 mL volumetric flask added 1.0 mL of 1.0N NaOH Sample Solution and kept it at room temperature for 30 min. Neutralised with 1.0 mL 1.0N HCl of solution made upto the volume with diluent and mixed well.

| S.No | Condition | % of Methyl paraben |
|---|---|---|
| 1 | CONTROL | 104.2 |
| 2 | SAMPLE_1N HCL | 103.8 |
| 3 | SAMPLE_0.1N HCL | 104.0 |
| 4 | SAMPLE_1NaOH_4HRS | 0.0 |
| 5 | SAMPLE_0.1NaOH | 101.2 |
| 6 | SAMPLE_1%$H_2O_2$ | 103.1 |
| 7 | SAMPLE_3%$H_2O_2$ | 103.4 |
| 8 | SAMPLE_THERMAL | 99.7 |
| 9 | SAMPLE_UV | 102.1 |
| 10 | SAMPLE_1NAOH_30MIN | 51.4 |

**5.4 Precision:**

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous test. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient if variation) of series of measurements.

**5.4.1. System Precision:**

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area response of six determinations should be measured and % relative standard deviation should be calculated.

Injected Blank (diluent) (1 injection), standard solution (6 injections), and check the following parameters.

**RESULTS:**

| S.No | Area response |
|---|---|
| 1 | 26251202 |
| 2 | 26018664 |
| 3 | 26228070 |
| 4 | 26095134 |
| 5 | 26110358 |
| 6 | 26247091 |
| **Average** | **26158419.83333** |
| **Standard Deviation** | **97127.731818** |
| **RSD** | **0.4** |

**5.4.2. Method Precision:**

In method precision, a homogenous test of a single batch should be analysed six times. This indicates whether a method is giving consistent results for a single batch.

Analyse the sample of Succinylcholine chloride injection as per analytical procedure.

**Injection profile:**

| S.No | Sample ID | % Assay |
|---|---|---|
| 1 | Sample Preparation_1 | 99.4 |
| 2 | Sample Preparation_2 | 99.4 |
| 3 | Sample Preparation_3 | 99.7 |
| 4 | Sample Preparation_4 | 99.7 |
| 5 | Sample Preparation_5 | 99.7 |
| 6 | Sample Preparation_6 | 99.6 |
| **Average** | | **99.6** |
| **Standard Deviation** | | **0.156187754** |
| **RSD** | | **0.2%** |

Inject separately each of the following solutions into the chromatograph.

**5.5 Linearity:**

The linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Performed the Linearity with Methyl paraben.

Recorded the area response at each level and calculate slope, intercept, correlation coefficient and regression coefficient (R square). Test the intercept for statistical equivalence to zero.

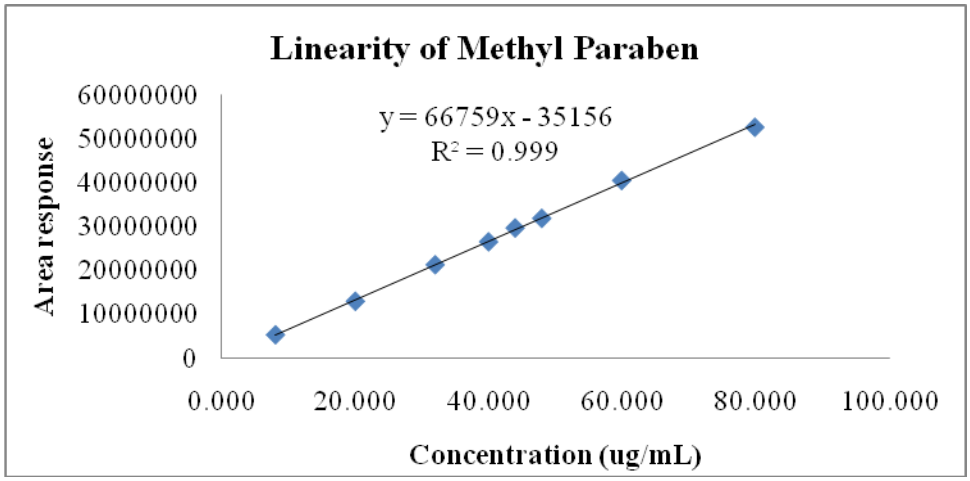
**Linearity Stock for Succinylcholine chloride:**

Weighed and transferred 20.18 mg of Methyl Paraben into 100 mL volumetric flask, made up to the volume with diluent and mixed well to dissolve.

| S.No | Volume taken from Stock (mL) | Made upto the volume with diluents (mL) | Level (%) |
|---|---|---|---|
| 1 | 0.4 | 10 | 20 |
| 2 | 1.0 | 10 | 50 |
| 3 | 1.6 | 10 | 80 |
| 4 | 2.0 | 10 | 100 |
| 5 | 1.1 | 5 | 110 |
| 6 | 1.2 | 5 | 120 |
| 7 | 3.0 | 10 | 150 |
| 8 | 4.0 | 10 | 200 |

**RESULTS:**

| Level (%) | Concentration of Methyl paraben (in µg/mL) | Made upto the volume with diluents (mL) |
|---|---|---|
| 20 | 7.991 | 5273016 |
| 50 | 19.978 | 12946708 |
| 80 | 31.965 | 21309153 |
| 100 | 39.956 | 26563018 |
| 110 | 43.952 | 29729764 |
| 120 | 47.948 | 31927935 |
| 150 | 59.935 | 40593475 |
| 200 | 79.913 | 52775126 |
| **Slope** | | 667593.7 |
| **STYEX** | | 405047.5 |
| **Intercept** | | -35155.9 |
| **r** | | 0.999688 |
| **$r^2$** | | 0.999377 |



**5.6 Robustness:**

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate in method parameters which provides an indication of its reliability during normal usage.

**Robustness parameters:**

- Change in column temperature ± 5ºC
- Change in flow rate ± 0.1ml/min

**RESULTS**

| Robustness | | | | |
|---|---|---|---|---|
| **Conditions** | **RT (min)** | **Area Ratio** | **Tailing factor for standard-1** | **Theoretical plate count for standard-1** |
| Original condition | 9.78 | 1.00 | 1.00 | 18148 |
| Flow 0.8 mL/min | 10.22 | 1.01 | 1.01 | 18068 |
| Flow 1.2 mL/min | 9.36 | 1.00 | 1.01 | 18349 |
| Temperature_25°C | 12.27 | 1.01 | 1.01 | 19900 |
| Temperature_35°C | 8.11 | 0.98 | 0.99 | 17249 |

**6.0 Method Description:**

**Reagents:**

| | |
|---|---|
| Heptane sulphonic acid | AR grade |
| Acetic acid | AR grade |
| Acetonitrile | HPLC grade |

**Buffer solution:**

Weigh 1.7 g of Heptane sulphonic acid into 1.0 Liter volumetric flask and add about 500 mL of water, mix well and add 15.0 mL of glacial acetic acid and make upto the volume with water and mix well. Filter through the 0.45 µ membranes filter.

**Mobile phase:**

Mix the ration of 650 mL of buffer solution and 350 mL of Acetonitrile, sonicate for 5 minutes to degas.

**Diluent:** Mobile phase

**Chromatographic conditions**:

Column : Thermo Hypersil BDS, C18, 150 X 4.6 mm, 5 µm
Flow rate : 1.5mL/minute
Wavelength : 254 nm
Injection volume : 10 µL
Temperature : 25°C
Run time : 6.0 minute

**Standard stock solution (200 ppm):**

Accurately weigh and transfer about 20.0 mg of Methyl paraben standard to a 100 mL volumetric flask. Make upto the volume with diluent and mix well.

**Standard solution (40 ppm):**

Pipette 10.0 mL of standard stock solution to a 50 mL volumetric flask, dissolve in and dilute to volume with diluent. Mix well.

**Sample preparation (40 ppm):**

Transfer 1.0 mL of sample into 25.0 mL volumetric flask and dilute to volume with diluent and mix well.

**Procedure:**

Separately inject blank (1 injection) and standard solution (5 injections) into the chromatograph and check the system suitability as follows.

- The tailing factor of methyl paraben in standard solution should NMT 2.0.

- The theoretical plates of methyl paraben in standard solution should NMT 2000.
- The RSD for 5 replicate injections of methyl paraben peak should NMT 2.0%.

If the system suitability parameters are within the limit then the sample preparation (in duplicate) into the chromatograph, record the chromatograms and measure the responses.

**Calculation:**

$$\% \text{ of Preservative contents} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{VT} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = Methyl paraben area response from sample preparation chromatogram.

AS = Average area response of methyl paraben standard chromatogram.

WS = Weight of methyl paraben for standard solution preparation in mg.

DS = Dilution of methyl paraben from standard solution.

DT = Dilution of sample preparation.

VT = Volume taken for sample preparation in ml.

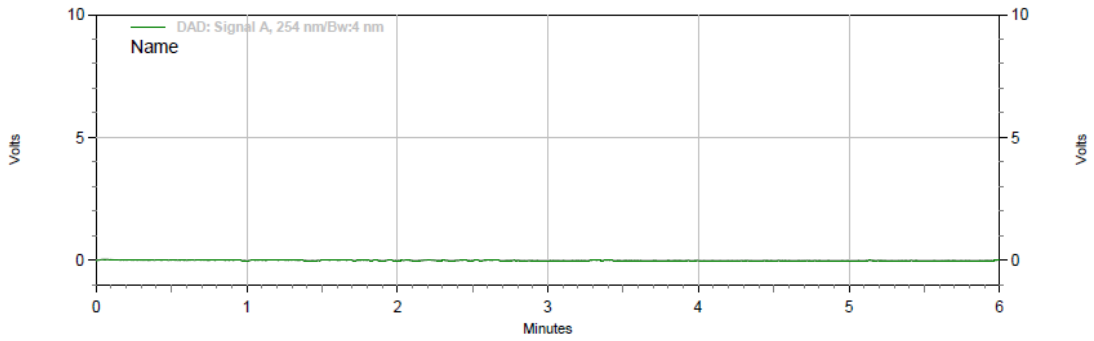
P = Potency of methyl paraben standard in % w/w.

LC = Label claim of methyl paraben in mg/ml.

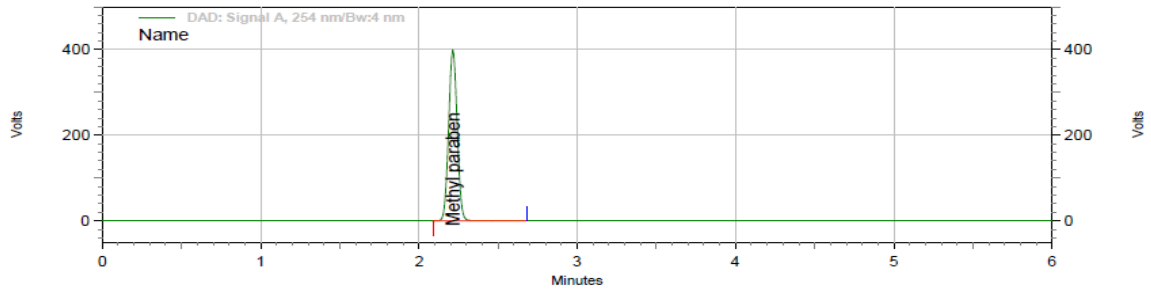
## 7.0 Reference Chromatograms:

| S.No | Chromatogram | Page No. |
|---|---|---|
| 1.0 | Blank | 12 |
| 2.0 | Standard solution | 12 |
| 3.0 | Test solution | 12 |

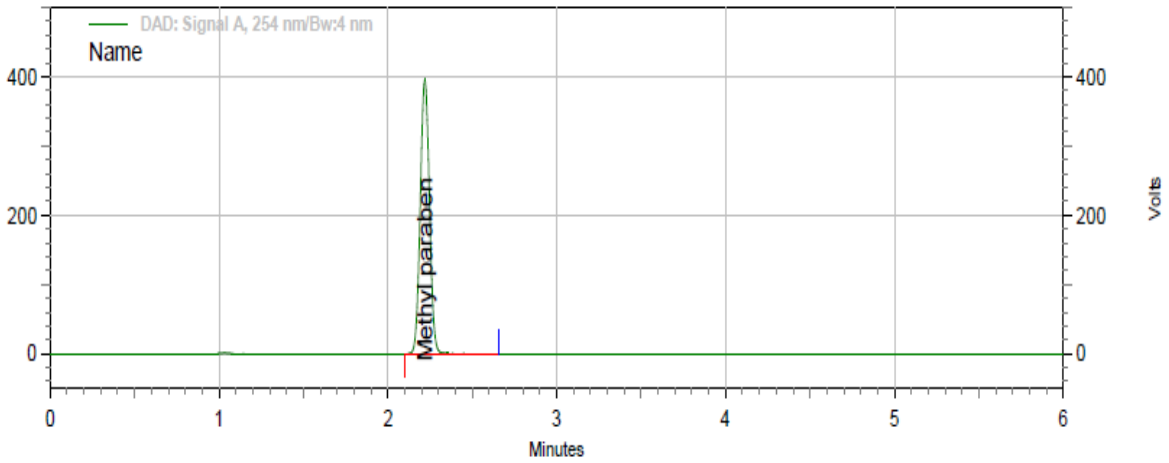
### 1.0 Blank Chromatogram:



**2.0 Standard solution Chromatogram:**




**3.0 Test solution Chromatogram:**




## CONCLUSION

From the study and data it is concluded that, the method is found to be specific. The method is also Stability indicating as evidenced by forced degradation studies. The method is found to be Precise. The method is found to be Linear in the specified range. The method is robust w.r.t. flow and temperature variations. System suitability is established and recorded. Hence, this method can be used for routine analysis.

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