SUITABILITY OF USE PROTOCOL

Sucralose, NF

USP COMPENDIAL PROCEDURE – ASSAY METHOD BY HPLC

DOCUMENT #: PRO-02616-1.0

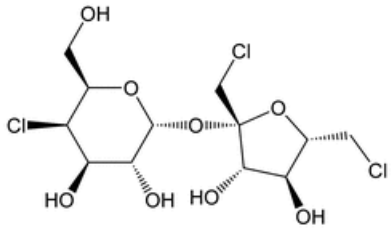
Analytical Research and Development

Frontida BioPharm, LLC.
1100 Orthodox Street | Philadelphia, PA 19124

TABLE OF CONTENTS

1 INTRODUCTION

Sucralose (Chemical Formula: ($C_{12}H_{19}Cl_3O_8S$); Molecular Weight: 397.63 g/mol) is for Sucralose. The structural formula of Sucralose is represented below:



This protocol pertains to the suitability use evaluation of the USP Compendial *Assay* procedure found in the Sucralose monograph (USP43-NF38). Appropriate studies will be performed in order to demonstrate that the Compendial procedure is suitable for use.

A modification to the *Assay* procedure is proposed within the scope of USP-NF <621> *Chromatography* as follows:

Parameter	**USP-NF Monograph**	**Proposed**	**USP <621>**
Column/Packing/ Dimension	Resolve C18 Radial Pak (totally porous particle, non-endcapped) 8-mm x 10-cm, 5 µm; packing L1	Zorbax SB C18 (totally porous particle, non-endcapped) 4.6 mm x 15-cm, 5 µm packing L1	- No change of identity of substituent; similar surface modification. - Ratio of the column length (L) to the particle size (dp) remains constant or in the range between −25% to +50% of the prescribed L/dp ratio
Flow Rate	1.5 mL/min	0.6 mL/min	- Internal diameter change; maintain linearity velocity. - Additional change in flow rate within ±50%
Injection Volume	20 µL	10 µL	- Injection volume may be varied provided System Suitability criteria remain within their established acceptability limits

This protocol describes the methodology for the execution of the suitability of use evaluation and defines the criteria to assess the results.

The following studies will be performed for the suitability of use evaluation:

- System Suitability
- Specificity
- Linearity
- Precision
- Solution stability for the standard, sample and mobile phase

The analytical procedure will be evaluated in accordance with SOP MPC QC/RD-017 (current version) and this protocol.

If during the suitability of use evaluation any chances or deviations are deemed necessary, appropriate additional validation mat be performed. The analytical report and method will reflect any changes.

2 ANALYTICAL METHOD

2.1 Chromatographic Parameters

Table 2-1. HPLC Parameters

Instrument	HPLC with RI detector and equipped with a suitable data acquisition system.
Column	Zorbax SB C18, 150 mm × 4.6 mm; 5 µm Part. No: 883975-902
Mobile Phase	Acetonitrile: Water, 3:17
Needle Wash	Water : Acetonitrile (50:50)
Needle Wash Setting	Extended
Flow Rate	0.6 mL/minute
Injection volume	10 µL
Column Temperature	35 °C ± 3°C
Run Time	15 min

2.2 Reagents and Materials

- Purified Water, Millipore
- Acetonitrile, HPLC Grade
- Sucralose, USP Reference Standard

2.3 Mobile Phase A Solution Preparation

Prepare a mixture of Acetonitrile and Water at a ratio of 3:17. Degas.

2.4 Diluent Preparation

Use the mobile phase solution as diluent.

2.5 Standard Solution Preparation

Accurately weigh and quantitatively transfer about 50 mg of *Sucralose* into a 50-mL volumetric flask. Fill with diluent to about half the flask volume. Sonicate to dissolve if necessary. Dilute to volume with diluent and mix well.

The concentration of Sucralose is 1 mg/mL.

Prepare a check standard solution in a similar manner.

2.6 Sample Solution Preparation

Accurately weigh and quantitatively transfer about 50 mg of *Sucralose* into a 50-mL volumetric flask. Fill with diluent to about half the flask volume. Sonicate to dissolve if necessary. Dilute to volume with diluent and mix well.

The concentration of Sucralose is 1 mg/mL.

2.7 Procedure

Inject equal volumes (10 µL) of the Diluent, , Standard/Check Standard, and Sample Solutions into the chromatograph. Record the chromatograms and measure peak area responses.

Table 2-2 Injection Sequence

Solutions	Number of Injections
Diluent	≥1
Working Standard Solution	5
Working Check Standard Solution	1
Procedural Control Standard (PCS)	1
Sample Solution	≤6
Procedural Control Standard (PCS)	1

2.8 System Suitability Requirements

- The RSD of the Sucralose peak area responses for the five (5) consecutive injections of working standard solution is NMT 2.0%.
- The percent recovery of Sucralose in the check standard solution is within 98.0% - 102.0%.

2.9 Calculations

Calculate the % Label Claim as follows:

$$\%\text{Assay (as-is basis)} = \frac{R_u}{R_s} \times \frac{W_s \times P}{50\ (mL)} \times \frac{50\ (mL)}{W_{Spl}} \times 100\%$$

$$\%\text{Assay (on dried basis)} = \frac{R_u}{R_s} \times \frac{W_s \times P}{50\ (mL)} \times \frac{50\ (mL)}{W_{Spl}} \times \frac{100\%}{100\%\text{-Water}} \times 100\%$$

Where,

R_u	:	The area response of Sucralose in the sample solution
R_s	:	The area response of Sucralose in the standard solution
W_s	:	Weight of Sucralose in the standard solution, in mg
W_{Spl}	:	Weight of Sucralose in the sample solution, in mg
P	:	Purity of standard expressed as % Purity/100%
Water	:	Water of sample in %

3 SYSTEM SUITABILITY/SYSTEM PRECISION

The System Suitability and System Precision of the test method will be performed and demonstrated as part of establishing system suitability for the subsequent validation studies. The successful establishment of the system suitability requirements (as described in **Section 2.8**) will be considered fulfillment of this study.

4 SPECIFICITY STUDY (INTERFERENCE)

Specificity studies will be performed in order to determine peak identities as well as evaluate whether there are any significantly interfering peaks arising from the diluent that may affect the quantitation of the intended analyte.

4.1 Diluent Interference Solution Preparation

Use diluent as the diluent interference solution.

4.2 Procedure

- Establish system suitability per **Section 2.8**. (Note—The check standard recovery requirement is not need as this study is non-quantitative.)
- Inject once the diluent.
- Compare all chromatograms and evaluate for peak interference.

4.3 Validity Criteria

- Meets the system suitability requirements in **Section 2.8**.

4.4 Acceptance criteria

- The diluent solution does not show any significantly interfering peaks near the retention time of the Sucralose peak.

5 LINEARITY

Linearity of Sucralose will be evaluated from a concentration of 0.8 mg/mL to 1.2 mg/mL, which corresponds to 80% and 120%, respectively, of the nominal Sucralose concentration in the standard and sample solutions.

5.1 Stock Sucralose Solution Preparation

Accurately weigh and quantitatively transfer about 200 mg of Sucralose into a 50 mL volumetric flask. Fill with diluent to about half the flask volume. Sonicate to dissolve if necessary. Dilute to volume with diluent and mix well.

The concentration of Sucralose is 4 mg/mL.

5.2 Working Sucralose Solution Preparation

Prepare the working linearity solutions for the L1 to L5 levels as directed in **Table 5-1**. Dilute each to volume with the diluent and mix well.

Table 5-1. Preparation of working Sucralose linearity solutions

Linearity Level	Nominal Conc. (%)	Volume of Stock Sucralose Solution (mL)	Flask Volume (mL)	Approx. Conc. of Sucralose (mg/mL)
L1	80	4.0	20	0.8
L2	90	4.5	20	0.9
L3	100	5.0	20	1.0
L4	110	5.5	20	1.1
L5	120	6.0	20	1.2

5.3 Procedure

- Establish system suitability per **Section 2.8**.
- Inject each linearity level solution once.
- For each linearity injection, calculate the response factor and relative response factor relative to the mean response factor of the 100% level as follows:
- $\text{Linearity Response Factor} = \frac{\text{Peak Area Response}}{\text{Concentration}}$

- Linearity Response Factor to 100% level= $\frac{\text{Response Factor}}{\text{Mean Response Factor of the 100\% Level}}$
- Construct a plot of the peak area responses vs. concentration.
- Perform a linear regression analysis and determine the correlation coefficient (r), slope, and y-intercept.

5.4 Validity Criteria

- Meet system suitability as per **Section 2.8**.

5.5 Acceptance Criteria

- Meet the linearity range of a minimum of five consecutive levels.
- The correlation coefficient, r, is NLT 0.999.
- The linearity relative response factor (RRF) at each level is within 98.0% to 102.0%.
- The percent y-intercept relative to nominal 100% level is NMT 2%.

6 PRECISION

6.1 Precision

Perform the Precision study by preparing six (6) sample solutions using Sucralose as directed in **Section 2.6**.

6.2 Procedure

- Establish system suitability per **Section 2.8**.
- Inject each solution once.
- Determine the percent Assay.

6.3 Validity Criteria

- Meet the system suitability requirements in **Section 2.8**.

6.4 Acceptance Criteria

- The percent RSD of the results from the six preparations is NMT 2.0%.

7 STABILITY STUDY

The stability of the standard and sample solutions will be evaluated at normal laboratory environmental condition (NLEC) to determine whether they are stable for use within the set time frame and the storage condition.

The stability of the standard and sample solutions will be determined by periodically evaluating the recovery of Sucralose in the solutions against freshly prepared standard solutions.

The stability of the mobile phase will be evaluated concomitantly with that of the standard and sample solutions.

7.1 Procedure

- Prepare a standard solution as per **Section 2.5**. Record the time at which the preparation of the solution was completed. (Note—The check standard solution prepared to establish system suitability may be used.) Store solution at evaluation condition.
- Prepare the sample solutions as per **Section 2.6**. Record the time at which the preparation of the solution was completed. (Note—Sample solution stability may be determined from a sample solution prepared for the precision.) Store solution at evaluation condition.
- Periodically evaluate the standard and sample solutions against a freshly prepared standard solution. Establish system suitability as per **Section 2.8** for each evaluation.
- Inject each solution once per stability timepoint evaluation.
- Determine the percent relative recoveries of the standard and sample solutions at each time interval.
- Evaluate the retention time of the Sucralose peak obtained from the injections of the system suitability working standard solution.

7.2 Validity Criteria

- Meet the system suitability requirements in **Section 2.8**.

7.3 Acceptance Criteria

- The standard and sample solutions are considered stable if the relative recovery obtained at the evaluated time interval is within 98.0%-102.0% of the original results (t_0).
- The mobile phase is considered stable if the mean of retention times of the standards in the system suitability is within 10% of that obtained from the initial run (t_0).

Document Approvals
Approved Date: 8/11/2023

Approval Task Verdict: Approve	Marjorie Cordero, (Marjorie.Cordero@adareps.com) Approval 10-Aug-2023 17:45:51 GMT+0000

Approval Task Verdict: Approve	Timothy Kim, (Timothy.Kim@adareps.com) Approval 11-Aug-2023 14:00:16 GMT+0000

Approval Task Verdict: Approve	Shiying Tian, (Shiying.Tian@adareps.com) Approval 11-Aug-2023 14:32:28 GMT+0000

Task: QA Approval Verdict: Approve	Nicole Flood, (Nicole.Allonardo@adareps.com) Quality Assurance Approval 11-Aug-2023 17:22:17 GMT+0000