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Changes from previous version:

Section	Summary of Changes	Change Control Number
ALL	1. New document	

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1. PURPOSE

This document describes a UV-Vis method for the determination of oligonucleotide content.

2. SCOPE

The method described herein enables the quantification by % Assay of oligonucleotides. This is performed by using the various calculations outlined in this document.

In brief, the purity, molar extinction coefficient and water content of the test compound is compared against a reference to understand the APIs proportion of total composition on a percentage basis.

3. INTERNAL REFERENCES

Document ID	Title
QA001	Quality Policy

4. EXTERNAL REFERENCES

Document ID	Title

5. RESPONSIBILITIES

Job Function and/or Department	Responsibility
All Personnel	

6. DEFINITION

Term	Definition
Assay	Fundamental test for determining the active ingredient content of a sample
AEX	Anion exchange
FLP	Full length product
IP-RP	Ion-pairing reverse phase
MW	Molecular weight
NLT	No less than
SEC	Size exclusion chromatography
UV-Vis	Ultraviolet-visible

7. PROCEDURE

7.1. Instruments

7.1.1. Thermo Fisher evolution 200 series with a single or linear cell changer or equivalent.

7.1.2. Mettler Toledo analytical balance or equivalent.

7.2. Materials

7.2.1. Glassware and pipettes

7.2.2. Quartz cuvettes

7.3. Standards and Reagents

7.3.1. PBS pH 7.4 (10X), or equivalent.

7.3.2. Water, LCMS grade or equivalent.

7.4. Diluent Solution

7.4.1. To prepare 1X PBS (pH 7.4) first transfer 100 mL of 10X PBS to a 1L graduated cylinder. Q.S. to 1L with

water. Store at ambient temperature.

7.5. Blank, diluent

7.5.1. The blank for each sample should match the diluent used for the sample preparation.

7.6. Sample preparation

7.6.1. Weigh out NLT 5mg of oligonucleotide into a 100mL volumetric flask and record the weight. Fill the volumetric flask to volume with the desired diluent. Dilute samples down to a concentration that yields an absorbance within the linear range of the detector, 0.1-2 AU.

7.7. Experimental conditions

7.7.1. The starting conditions for the UV-Vis spectrophotometer are outlined in the table below. These may be adjusted to achieve an appropriate response when necessary.

Cuvette holder temperature	Ambient
Bandwidth	1nm
Absorbance range	0.1-2 au
Integration time	0.5 second
Detection	260nm

7.8. Data handling

7.8.1. Data acquisition will be performed using Thermos Fisher insight 2 software or equivalent.

7.9. Assay calculation

7.9.1. Select the calculation to be used from the equations below. This will be used to calculate the % Assay of an oligonucleotide based on a given basis.

7.9.2. Purity values can be generated by SEC, AEX or IPRP depending on the compound's stability indicating method. The purity for this calculation should be verified prior to the % Assay experiment being performed.

7.9.3. Absorbance values generated from the spectrophotometer should be within the linear range.

7.9.4. The calculations below are generated using an example sequence for guidance purposes.

Example Sequence

5'-TCG TCG TTT TGT CGT TTT GTC GTT-3'

All linkages are phosphorothioate

Molecular Weight Basis	Molecular Weight (Da)
As is	7698.2
Anhydrous Sodium Salt	8204.0
Hydrated Salt	8636.0

Sodium Salt weight is based on a stoichiometric sodium content.

Hydrated weight is assuming a hydration level of three H_20 molecules per base pair. (~5%).

Equation 1 Sodium Salt Anhydrous Basis

$$Assay_{Sodium\ Salt\ Anhydrous} = \frac{Purity\ by\ HPLC \times Absorbance\ by\ Spec \times Volume \times Dilution \times MW\ anhydous\ Na\ Salt}{Molar\ Extinction\ Coef. \times Weight \times (1 - H20)}$$

$$Assay_{Sodium\ Salt\ Anhydrous} = \frac{97.3 \times 1.2 \times 100 \times 1 \times 8204.0}{187000 \times 5.67 \times (1 - 0.051)} = 95.2$$

Equation 2 Sodium Salt As is Basis

$$Assay_{Sodium\ Salt\ as\ is} = \frac{Purity\ by\ HPLC \times Absorbance\ by\ Spec \times Volume \times Dilution \times MW\ anhydous\ Na\ Salt}{Molar\ Extinction\ Coef. \times Weight \times (1)}$$

$$Assay_{Sodium\ Salt\ as\ is} = \frac{97.3 \times 1.2 \times 100 \times 1 \times 8204.0}{187000 \times 5.67 \times (1)} = 90.0$$

Equation 3 Free Acid Anhydrous Basis

$$Assay_{free\ acid\ anhydrous} = \frac{Purity\ by\ HPLC \times Absorbance\ by\ Spec \times Volume \times Dilution \times MW\ free\ acid}{Molar\ Extinction\ Coef. \times Weight \times (1 - H20)}$$

$$Assay_{free\ acid\ anhydrous} = \frac{97.3 \times 1.2 \times 100 \times 1 \times 7698.2}{187000 \times 5.67 \times (1 - 0.051)} = 89.3$$

Equation 4 Free Acid As Is Basis

$$Assay_{free\ acid\ as\ is} = \frac{Purity\ by\ HPLC \times Absorbance\ by\ Spec \times Volume \times Dilution \times MW\ free\ acid}{Molar\ Extinction\ Coef. \times Weight \times (1)}$$

$$Assay_{free\ acid\ as\ is} = \frac{97.3 \times 1.2 \times 100 \times 1 \times 7698.2}{187000 \times 5.67 \times (1)} = 84.8$$

Equation 5 Hydrated Salt As Is Basis

$$Assay_{Hydrated\ Salt\ as\ is} = \frac{Purity\ by\ HPLC \times Absorbance\ by\ Spec \times Volume \times Dilution \times MW\ Hydrated\ Salt}{Molar\ Extinction\ Coef. \times Weight \times (1)}$$

$$Assay_{Hydrated\ Salt\ as\ is} = \frac{97.3 \times 1.2 \times 100 \times 1 \times 8636.0}{187000 \times 5.67 \times (1)} = 95.1$$