BIOSPECTRA
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ANALYTICAL METHOD VALIDATION REPORT: URIDINE ASSAY BY UV/VIS SPECTROSCOPY

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1. PURPOSE:

1.1. The purpose of this Report is to:
  1.1.1. Ensure that the Uridine Assay by UV/Vis Spectroscopy procedure was adequately evaluated and validated.
  1.1.2. Verify that the Uridine Assay by UV/Vis Spectroscopy procedure met requirements for Accuracy, Precision, Specificity, Linearity, Range, and Intermediate Precision.
  1.1.3. Ensure that the proper reagents and testing materials were used and the correct documentation is provided for evaluation.

2. SCOPE:

2.1. This Analytical Method Validation Report applies to Uridine Assay by UV/Vis Spectroscopy.
2.2. This method has been validated as a Category I (Quantitative) Method.
2.3. This method validation was performed at BSI (BioSpectra Inc., located at 100 Majestic Way, Bangor, PA 18013). Two different analysts performed a portion of the validation protocol. Data must align with method performance requirements defined in the procedure herein.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing is responsible for the control, implementation, training, and maintenance of this report.
3.2. The Laboratory Analysts, and/or qualified designee were responsible for performing the testing stated in this protocol.
3.3. The Laboratory Analysts and/or qualified designee performing the test, with help from the Director of Laboratory Testing, if necessary, were responsible for completing the Method Validation Report using conclusions made from the results obtained from testing.
3.4. Safety: Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

4. REFERENCES:

4.1. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
4.2. BSI-SOP-0098, Balance SOP
4.3. BSI-SOP-0126, Laboratory Notebooks
4.4. BSI-SOP-0134, Pipette SOP
4.5. BSI-SOP-0436, Analytical Methods Validation Master Plan

5. PRE-VALIDATION REQUIREMENTS:

5.1. Equipment
  5.1.1. All equipment used in this validation were in proper working order and under current calibrations. This is documented in the Materials and Equipment portion of this Analytical Method Validation Report.
    5.1.1.1. Analytical Balance
    5.1.1.2. UV/Vis Spectrometer; Capable of measuring at 262 nm wavelength with 1 cm cell holder.
5.2. Personnel
  5.2.1. All personnel who performed this Validation were properly trained in accordance with the Analytical Methods Validation Master Plan.

5.3. Supplies

5.3.1. Any supplies used in this Validation was clean and appropriate for the intendeduse. A list of supplies is listed in the Materials and Equipment section of this Analytical Method Validation Report.

5.3.1.1. Micropipette Tips
5.3.1.2. Micropipettes
5.3.1.3. Volumetric Flasks
5.3.1.4. Weigh Boats/Transfer Funnels
5.3.1.5. Transfer Pipettes
5.3.1.6. Quartz Cuvette 1cm Pathlength

5.4. Reagents

5.4.1. All reagents were current, met required specifications and be suitable for the intended use. The list of reagents used is included in this Analytical Method Validation Report and laboratory documentation.

5.4.1.1. Purified water
5.4.1.2. Uridine

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment utilized in this validation are outlined in this section.

6.2. Equipment:

Table 1: Equipment

Equipment	Model / Part Number	Manufacturer	Serial Number	Calibration Due Date
Analytical Balance	MSE224S	Sartorius	24801744	4/24
Calibrated Pipette (100µL - 1000µL)	3123000063	Eppendorf Research Plus	M27701G	12/31/23
UV/Vis Spectrophotometer	Lambda 25	Perkin Elmer	501S13110518	12/31/23

6.3. Reagents and Standards:

Table 2: Reagents and Standards

Reagent / Standard	Lot Number	Manufacturer	CAS Number	Expiration Date
Uridine	URID-0122-00006-PV	BioSpectra	58-96-8	Not Applicable
Purified Water	D10DI01-112123	BioSpectra	7732-18-15	Not Applicable
Purified Water	D10DI01-112223	BioSpectra	7732-18-15	Not Applicable

6.4. Supplies:

Table 3: Equipment

Equipment	Model / Part Number	Manufacturer
Eppendorf tips (1000 µL)	022491555	Eppendorf
UV/Vis Spectrophotometer Cuvettes	B0631012	Perkin Elmer
Volumetric Flask (1000 mL)	56401000	Pyrex
Volumetric Flask (100 mL)	5635100	Pyrex
Volumetric Flask (50 mL)	563550	Pyrex
Weigh Boat / Paper	10770	VWR

7. PROCEDURE:

7.1. **Note:** All sample preparations may be scaled as needed.

7.2. Sample Preparation:

7.2.1. Uridine Stock Sample Solution (2400 mg/L Uridine): Dissolve 2.4 g of Uridine in purified water, dilute to 1000 mL with purified water, and mix well.

Table 4: Uridine Stock Sample Solution

Analyst	Uridine Weight (g)	Final Volume (mL)	Final Concentration (mg/L)
Analyst I	2.4088	1000	2408.8
Analyst II	2.4051	1000	2405.1

7.2.2. Uridine Sample Test Solution (24 mg/L Uridine): Dilute the 1.0 mL of *Uridine Stock Sample Solution* to 100 mL with purified water and mix well.

7.3. Analysis:

7.3.1. Verify the UV/Vis Spectrophotometer is within calibration and record due date and serial number of the instrument.

7.3.2. Select the method "Uridine UV Assay 262 nm" from the Perkin Elmer UV WinLab Software.

7.3.3. Refer to the Lambda 25 UV/Vis Operation and Calibration SOP, to measure the absorbance of the Uridine Sample Test Solution using a 1 cm pathlength cuvette.

7.3.4. Calculate the Final Sample Concentration using the following equation:

$$Sample\ Concentration\ (\frac{mg}{L}) = \frac{Sample\ Weight\ (g)}{0.001\frac{g}{mg}\ x\ 1L} \times \frac{1\ (mL)}{100\ (mL)}$$

7.3.5. Calculate the % Assay for Uridine, using the following equation:

$$Uridine\ Assay\ (\%) = \frac{Abs\ @\ 262\ nm\ (a.u.)}{1.000\ a.u.} \times \frac{24.18\ (\frac{mg}{L})}{Sample\ Concentration\ (\frac{mg}{L})} \times 100$$

7.3.5.1. Where:

7.3.5.1.1. **Abs @ 262 nm** = Absorbance Measured of Uridine Sample Test Solution at 262 nm; 1 cm pathlength.

7.3.5.1.2. **Sample Concentration (mg/L)** = Concentration of the Uridine Sample Test Solution (mg/L).

7.3.5.1.3. **24.18 mg/L** = Concentration of 1.00 a.u. absorbance Uridine Solution based on Molar absorptivity coefficient of 10100 a.u. per mole.

7.3.5.1.4. **1.00 a.u.** = Absorbance of 24.18 mg/L of Uridine based on 10100 molar absorptivity coefficient and 1cm pathlength.

7.4. Result Reporting:

7.4.1. Report the calculated Uridine value in %.

8. PERFORMANCE PARAMETERS:

8.1. **Accuracy:**

8.1.1. Accuracy was assessed using eighteen (18) determinations over five (5) concentration levels. Accuracy is assessed as percent recovery:

$$Percent\ Recovery\ (\%) = \frac{Calculated\ Uridine\ Concentration}{Theoretical\ Uridine\ Concentration} \times 100$$

8.1.2. Acceptance Criteria:

8.1.2.1. Percent Recovery of 98% to 102%.

8.2. **Precision:**

8.2.1. The precision of the analytical procedure was determined by using eighteen (18) determinations over five (5) concentration levels with six (6) determinations at the 100% concentration level and calculating valid estimates of standard deviation or relative standard deviation (%RSD).

$$Standard\ Deviation\ (s) = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n-1}}$$

$$\%RSD = \frac{Standard\ Deviation}{Average} \times 100$$

8.2.2. Acceptance Criteria:

8.2.2.1. Report the Standard Deviation (s) for each level.

8.2.2.2. A Relative Standard Deviation (%RSD) of NMT 1% at each level.

8.3. **Specificity:**

8.3.1. Specificity was demonstrated by performing a wavelength scan of the *Blank* and a preparation of the 100% Concentration Level *Uridine Sample Test Solution* and overlaying the spectra.

8.3.2. Acceptance Criteria:

8.3.2.1. No interference should be detected at the 262 nm wavelength.

8.3.2.2. Requirements for accuracy and precision were met.

8.4. **Linearity:**

8.4.1. Linearity was assessed across five (5) analysis levels. The average response (a.u.) was plotted against the concentration level (ppm), a linear regression was performed, and the Coefficient of Determination (r^2), Slope, and Y-Intercept were reported.

8.4.2. Acceptance Criteria:

8.4.2.1. Report the Slope and Y-Intercept.

8.4.2.2. The Coefficient of Determination (r^2) should be NLT 0.99.

8.5. **Range:**

8.5.1. The range was established by showing an acceptable degree of Accuracy, Precision, and Linearity.

8.5.2. Acceptance Criteria:

8.5.2.1. A minimum range of 80% to 120% of the 100% Concentration Level.

8.6. **Intermediate Precision:**

8.6.1. Intermediate Precision was assessed by having a second analyst (Analyst II) on a separate day, assay six (6) replicates of the 100% concentration level and calculate standard valid estimates of standard deviation or relative standard deviation.

8.6.2. Acceptance Criteria:

8.6.2.1. Report the individual and combined Standard Deviations.

8.6.2.2. The relative Standard Deviation (%RSD) of the individual results is 1% and combined results is NMT 2%.

9. VALIDATION SUMMARY:

Table 5: Validation Sample Preparation

Sample ID	Concentration Level (%)	Replicate	Uridine Stock Solution Amount (mL)	Final Volume (mL)	Uridine Concentration (mg/L)
Analyst I Validation Samples Preparation Table					
Blank	0	1	0.00	100	0
Linearity 1 Accuracy Precision	80	1	0.80	100	19.3
		2			
		3			
Linearity 2 Accuracy Precision	98	1	0.98	100	23.6
		2			
		3			
Linearity 3 Accuracy Precision	100	1	1.00	100	24.1
		2			
		3			
		4			
		5			
		6			
Linearity 4 Accuracy Precision	102	1	0.51	50	24.6
		2			
		3			
Linearity 5 Accuracy Precision	120	1	0.60	50	28.9
		2			
		3			
Analyst II Validation Samples Preparation Table					
Intermediate Precision	100	1	1.0	100	24.1
		2			
		3			
		4			
		5			
		6			

Table 6: Validation Summary		
Performance Parameters	**Acceptance Criteria**	**Results**
Accuracy	• All samples must have a percent recovery of 98% to 102%.	80% Level • Replicate 1 = 100% • Replicate 2 = 100% • Replicate 3 = 100% 98% Level • Replicate 1 = 100% • Replicate 2 = 100% • Replicate 3 = 100% 100% Level • Replicate 1 = 100% • Replicate 2 = 100% • Replicate 3 = 100% • Replicate 4 = 100% • Replicate 5 = 100% • Replicate 6 = 100% 102% Level • Replicate 1 = 100% • Replicate 2 = 99% • Replicate 3 = 100% 120% Level • Replicate 1 = 100% • Replicate 2 = 100% • Replicate 3 = 100%
Precision	• Each analysis level must have a %RSD of NMT 1%. • Standard Deviation: Report	80% Level • Standard Deviation = 0.1% • %RSD = 0.2% 98% Level • Standard Deviation = 0.2% • %RSD = 0.2% 100% Level • Standard Deviation = 0.1% • %RSD = 0.1% 102% Level • Standard Deviation = 0.3% • %RSD = 0.3% 120% Level • Standard Deviation = 0.2% • %RSD = 0.2%

Performance Parameters	Acceptance Criteria	Results
Specificity	• Meets the requirements for Accuracy and Precision. • Perform an absorbance scan of the Blank and a preparation of the 100% Concentration Level *Uridine Sample Test Solution* and overlay the spectra. • No interference should be detected at the 262 nm wavelength.	• Meets the requirements for Accuracy and Precision. • No interference was detected at a wavelength of 262nm.
Range	• Minimum range of 80% to 120% of the 100% Concentration Level must be 80% - 120%	• Range: 80% - 120%
Linearity	• Report the Slope, and Y-Intercept • The Coefficient of Determination (r^2) must be NLT 0.99.	• Coefficient of Determination (r^2) = 1 • Slope = 0.0099 • Y-Intercept = 0.003
Intermediate Precision	• Report the individual and combined (Analyst 1 and 2) Standard Deviation, %RSD, and 95% Confidence Interval • The Relative Standard Deviation (%RSD) of the individual results must be NMT 1% • The Relative Standard Deviation (%RSD) of the combined (Analyst I and Analyst II) results must be NMT 2%	Individual (Analyst I) • Standard Deviation = 0.1% • %RSD = 0.1% Individual (Analyst 2) • Standard Deviation = 0.1% • %RSD = 0.1% Combined (Analyst 1 and 2) • Standard Deviation = 0.1% • %RSD = 0.1%

10. VALIDATION RESULTS:

10.1. **Accuracy:**

10.1.1. Accuracy was assessed using fifteen (15) determinations over five (5) concentration levels. Accuracy was assessed as percent recovery:

$$Percent\ Recovery\ (\%) = \frac{Calculated\ Uridine\ Concentration}{Theoretical\ Uridine\ Concentration} \times 100$$

10.1.2. Acceptance Criteria:

10.1.2.1. Percent Recovery of 98% to 102%.

Table 13: Accuracy Results

Sample ID	Theoretical Uridine Concentration (%)	Replicate	Calculated Uridine Concentration (%)	Percent Recovery (%)
Uridine Sample Test Solution 1	80	1	79.9	100
		2	79.8	100
		3	80.1	100
Uridine Sample Test Solution 2	98	1	97.7	100
		2	98.0	100
		3	97.9	100
Uridine Sample Test Solution 3	100	1	99.6	100
		2	99.8	100
		3	99.8	100
		4	99.8	100
		5	99.7	100
		6	99.9	100
Uridine Sample Test Solution 4	102	1	101.8	100
		2	101.4	99
		3	101.8	100
Uridine Sample Test Solution 5	120	1	119.4	100
		2	119.9	100
		3	120.0	100

10.1.3. Accuracy Disposition: **Pass**

10.2. **Precision:**

10.2.1. The precision of the analytical procedure was determined by using eighteen (18) determinations over five (5) concentration levels with six (6) determinations at the 100% concentration level and calculating valid estimates of standard deviation or relative standard deviation (%RSD).

$$Standard\ Deviation\ (s) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$$

$$\%RSD = \frac{Standard\ Deviation}{Average} \times 100$$

10.2.2. Acceptance Criteria:

10.2.2.1. Report the Standard Deviation (s) for each level.

10.2.2.2. A Relative Standard Deviation (%RSD) of NMT 1% at each level.

Table 14: Precision Results

Uridine Sample	Replicate	Calculated Uridine Concentration (%)	Standard Deviation (%)	%RSD (%)
Test Solution 1	1	79.9	0.1	0.2
	2	79.8		
	3	80.1		
Test Solution 2	1	97.7	0.2	0.2
	2	98.0		
	3	97.9		
Test Solution 3	1	99.6	0.1	0.1
	2	99.8		
	3	99.8		
	4	99.8		
	5	99.7		
	6	99.9		
Test Solution 4	1	101.8	0.3	0.3
	2	101.4		
	3	101.8		
Test Solution 5	1	119.4	0.3	0.2
	2	119.9		
	3	120.0		

10.2.3. Precision Disposition: **Pass**

10.3. **Specificity:**

10.3.1. Specificity was demonstrated by performing a wavelength scan of the *Blank* and a preparation of the 100% Concentration Level *Uridine Sample Test Solution* and overlaying the spectra.

10.3.2. Acceptance Criteria:

10.3.2.1. No interference should be detected at the 262 nm wavelength.

10.3.2.2. Requirements for accuracy and precision were met.

Table 15: Specificity Results

Acceptance Criteria	Result
No interference detected at 262 nm in spectra overlay.	Pass
Meets requirements for Accuracy.	Pass
Meets requirements for Precision.	Pass

10.3.3. Specificity Disposition: **Pass**

10.4. **Range:**

10.4.1. The range was established by showing an acceptable degree of Accuracy, Precision, and Linearity.

10.4.2. Acceptance Criteria:

10.4.2.1. A minimum range of 80% to 120% of the 100% Concentration Level.

Range: 80% -120%

10.4.3. Range Disposition: **Pass**

10.5. **Linearity:**

10.5.1. Linearity was assessed across five (5) analysis levels. The average response (a.u.) vs. the theoretical spike level (ppm) was plotted, a linear regression was performed, and the Coefficient of Determination (r^2) was reported, Slope, and Y-Intercept.

10.5.2. Acceptance Criteria:

10.5.2.1. Report the Slope and Y-Intercept.

10.5.2.2. The Coefficient of Determination (r^2) should be NLT 0.99.

Table 16: Linearity Results

Uridine Sample	Concentration Level (%)	Replicate	Response (a.u.)	Average Response (a.u.)	Slope	Y-Intercept	Calibration Coefficient (r^2)
Test Solution 1	80	1	0.7962	0.7965	0.0099	0.003	1
		2	0.7953				
		3	0.7981				
Test Solution 2	98	1	0.9729	0.9748			
		2	0.9758				
		3	0.9756				
Test Solution 3	100	1	0.9923	0.9941			
		2	0.9944				
		3	0.9940				
		4	0.9943				
		5	0.9937				
		6	0.9956				
Test Solution 4	102	1	1.0141	1.0128			
		2	1.0098				
		3	1.0144				
Test Solution 5	120	1	1.1898	1.1932			
		2	1.1948				
		3	1.1950				

10.5.3. Linearity Disposition: **Pass**

10.6. **Intermediate Precision:**

10.6.1. Intermediate Precision was assessed by having a second analyst (Analyst II) on a separate day, assay six (6) replicates of the 100% concentration level and calculate standard valid estimates of standard deviation or relative standard deviation.

10.6.2. Acceptance Criteria:

10.6.2.1. Report the individual and combined Standard Deviations.

10.6.2.2. The relative Standard Deviation (%RSD) of the individual results was 1% and combined results was NMT 2%.

10.6.3. Intermediate Precision Results:

Table 19: Intermediate Precision Results

Uridine Sample	Analyst	Replicate	Calculated Uridine Concentration (%)	Standard Deviation (%)	%RSD (%)
Test Solution 3	Analyst I	1	99.6	0.1	0.1
		2	99.8		
		3	99.8		
		4	99.8		
		5	99.7		
		6	99.9		
	Analyst II	1	99.9	0.1	0.1
		2	99.9		
		3	100.0		
		4	99.8		
		5	99.8		
		6	99.8		
			Combined:	0.1	0.1

10.6.4. Intermediate Precision Disposition: **Pass**

11. CONCLUSION:

11.1. **Performance Summary:**

Table 20: Performance Summary	
Method Performance Parameter	**Result**
Accuracy	Pass
Precision	Pass
Specificity	Pass
Range	Pass
Linearity	Pass
Intermediate Precision	Pass

11.2. **Statement of Validation:** The method of analysis of Uridine Assay by UV/Vis Spectroscopy is considered a validated method of analysis at all BioSpectra facilities and is approved for use.

11.3. **Critical Changes, Deviations or Failures:** None