BIOSPECTRA

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DEGRADATION AND IMPURITY PROFILE REPORT: URIDINE (EXCIPIENT)

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1. PURPOSE AND SCOPE:

1.1. The impurity profiling of Uridine was intended to identify and potentially quantify impurities found in Uridine (CAS 58-96-8) product manufactured and purified at BioSpectra.

1.1.1. In the case where an impurity was found, a limit was set to the maximum allowable without measurable compromise to predetermined critical quality attributes or toxicity. In the case where a limit could not be set, a procedure was written and followed, to identify if the possible impurity was present or not (i.e. an identity test, which is qualitative and not quantitative.)

1.1.2. The profiling results and data allowed BioSpectra to understand the purity and characteristics of Uridine through all stages of manufacturing.

1.1.3. The four stages of Uridine that were tested are Raw Material, Mother Liquor, Wet Crystal and the finished product.

1.1.4. Tables were generated to include all sample results in the Uridine Degradation and Impurity Profile Report.

1.1.5. The tests that were used to determine the presence of impurities and degradation products will be as follows:

1.1.5.1. Appearance and Color

1.1.5.1.1. Raw Material and Finished Product only.

1.1.5.2. Assay (HPLC)

1.1.5.2.1. All four stages.

1.1.5.3. Bioburden

1.1.5.3.1. Raw Material and Finished Product only.

1.1.5.4. Elemental Impurities

1.1.5.4.1. All four stages.

1.1.5.5. Endotoxin

1.1.5.5.1. Raw Material and Finished Product only.

1.1.5.6. Identification (IR)

1.1.5.6.1. All four stages.

1.1.5.6.2. ML and WC Identification (IR) contains water and alcohol contamination and is not representative of the finished product.

1.1.5.7. Karl Fischer

1.1.5.7.1. All four stages.

1.1.5.8. Loss on Drying

1.1.5.8.1. All four stages.

1.1.5.9. Melting Range

1.1.5.9.1. Raw Material and Finished Product

1.1.5.10. Related Substances: Organic Impurities

1.1.5.10.1. All four stages. (Run concurrently with assay at each stage)

1.1.5.11. Residue on Ignition

1.1.5.11.1. Raw Material and Finished Product only.

1.1.5.12. Residual Solvents: 2-Propanol/Methanol/Ethanol

1.1.5.12.1. Raw Material and Finished Product only.

1.1.5.13. Solubility

1.1.5.13.1. All four stages.

1.1.5.14. Transmittance of Solution 5%

1.1.5.14.1. All four stages.

1.2. All results were recorded in the appropriate laboratory documentation. The results were detailed and analyzed in the degradation and impurity profile report. This report includes all relevant data as well as references to the initial documented results. This report discusses any impurities found in the product and includes specification for any limits on the impurities found when applicable.

2. RESPONSIBILITIES:

2.1. The Laboratory Manager is responsible for control, implementation, training,and maintenance of this procedure.
2.2. The Analysts, or qualified designees, are responsible for performing the testing stated in the protocol and recording all results.
2.3. The Associate Director of Product Lifecycle, or designee, is responsible for completing the degradation and impurity testing report.
2.4. It is the responsibility of all personnel to read and understand the SDS and don the appropriate PPE for handling and disposing of chemicals in a safe manner.

3. REFERENCES:

3.1. BSI-ATM-0086, Uridine Testing Methods
3.2. BSI-ATM-0092, Uridine Assay and Related Substances Determination by UPLC with UV Detection
3.3. BSI-PRL-0543, Uridine Process Validation Protocol (N05)
3.4. BSI-PRL-0678, Uridine Bio Excipient Grade Validation Protocol- N02
3.5. BSI-RPT-1015, Analytical Method Validation Report: Residual Solvents by Head Space GC FID (Uridine)
3.6. BSI-RPT-1382, Elemental Impurity Assessment: Uridine N02 2023
3.7. BSI-SOP-0069, Preparation of Samples for Outside Testing
3.8. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
3.9. BSI-SOP-0094, Muffle Furnace SOP and Calibration
3.10. BSI-SOP-0098, Balance SOP
3.11. BSI-SOP-0126, Laboratory Notebooks
3.12. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
3.13. BSI-SOP-0134, Pipette SOP
3.14. BSI-SOP-0135, Laboratory Chemicals
3.15. BSI-SOP-0140, Standardization of Titrants
3.16. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
3.17. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration
3.18. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration
3.19. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration
3.20. BSI-SOP-0254, Spectrum Two UATR SOP
3.21. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
3.22. BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP
3.23. BSI-SOP-0303, NexION 350X ICP-MS SOP
3.24. BSI-SOP-0348, Waters Acquity UPLC H-Class Plus SOP.
3.25. BSI-SOP-0345, Endosafe Nexgen-PTS Endotoxin Reader SOP
3.26. BSI-SOP-0420, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) via Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Cytidine, Uridine, L-Arginine HCL, and L-Glutamine
3.27. BSI-SOP-0422, Empower 3 General Procedure
3.28. *ACS, Reagent Chemicals,* current edition
3.29. *Current EP/BP*
3.30. *Current USP*
3.31. *Current USP General Chapter <791> pH*

4. PROCEDURE:

4.1. APPEARANCE AND COLOR :

4.1.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the appearance and color testing are detailed in the table below.

TABLE 1: APPEARANCE AND COLOR

Lot Number	Stage	Specification	Result
RMAT-0322-0014	Raw Material	Report	Almost White Powder
RMAT-0523-0008	Raw Material		White to Almost White Powder
URID-0123-00005-PV Beginning	Finished Good	White to Almost White Powder	White to Almost White Powder

4.2. ASSAY (HPLC) :

4.2.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Assay (HPLC) testing are detailed in the table below.

TABLE 2: ASSAY (HPLC)

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	17.9%
RMAT-0322-0014	Raw Material		99.7%
RMAT-0523-0008	Raw Material		99.7%
URID-0123-00005-PV WC First Basket	Wet Crystal		98.4%
URID-0123-00005-PV Beginning	Finished Good	98.0 – 102.0%	100.0%

4.3. BIOBURDEN (TAMC/TYMC) :

4.3.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Bioburden (TAMC/TYMC) testing are detailed in the table below.

TABLE 3: BIOBURDEN (TAMC/TYMC)

Lot Number	Stage	Specification	Result	
			TAMC	TYMC
RMAT-0322-0014	Raw Material	Report	<100 CFU/g	<100 CFU/g
RMAT-0523-0008	Raw Material		<100 CFU/g	<100 CFU/g
URID-0123-00005-PV Beginning	Finished Good	TAMC: ≤100 CFU/g TYMC: ≤100 CFU/g	<100 CFU/g	<100 CFU/g

4.4. ELEMENTAL IMPURITY :

4.4.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Elemental Impurity testing are detailed in the table below.

TABLE 4: ELEMENTAL IMPURITIES

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	Refer to BSI-RPT-1382 for Elemental Impurity Assessment: Uridine N02 2023
RMAT-0322-0014	Raw Material		
RMAT-0523-0008	Raw Material		
URID-0123-00005-PV WC First Basket	Wet Crystal		
URID-0123-00005-PV Beginning	Finished Good		

4.5. ENDOTOXIN :

4.5.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the endotoxin testing are detailed in the table below.

TABLE 5: ENDOTOXIN

Lot Number	Stage	Specification	Result EU/mg	Result EU/g
RMAT-0322-0014	Raw Material	Report	0.0355 EU/mg	35.5 EU/g
RMAT-0523-0008	Raw Material		0.0268 EU/mg	26.8 EU/g
URID-0123-00005-PV Beginning	Finished Good	≤0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg

4.6. IDENTIFICATION TEST (IR) :

4.6.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Identification IR testing are detailed in the table below.

TABLE 6: IDENTIFICATION TEST (IR)

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	Passes Test; 0.999342
RMAT-0322-0014	Raw Material		Passes Test; 0.992631
RMAT-0523-0008	Raw Material		Passes Test; 0.997638
URID-0123-00005-PV WC First Basket	Wet Crystal		Passes Test; 0.998406
URID-0123-00005-PV Beginning	Finished Good	Conforms to Spectrum of Reference Standard	Passes Test; 0.999524

4.7. KARL FISCHER :

4.7.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Karl Fischer testing are detailed in the table below.

TABLE 7: KARL FISCHER

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	36.21%
RMAT-0322-0014	Raw Material		0.22%
RMAT-0523-0008	Raw Material		0.18%
URID-0123-00005-PV WC First Basket	Wet Crystal		0.67%
URID-0123-00005-PV Beginning	Finished Good		0.10%

4.8. LOSS ON DRYING :

4.8.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Loss on Drying testing are detailed in the table below.

TABLE 8: LOSS ON DRYING

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	84.6114%
RMAT-0322-0014	Raw Material		0.1230%
RMAT-0523-0008	Raw Material		0.1551%
URID-0123-00005-PV WC First Basket	Wet Crystal		6.9032%
URID-0123-00005-PV Beginning	Finished Good	≤0.5%	0.1%

4.9. MELTING RANGE :

4.9.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the melting range testing are detailed in the table below.

TABLE 9: MELTING RANGE

Lot Number	Stage	Specification	Result
RMAT-0322-0014	Raw Material	Report	166.3 – 168.0°C
RMAT-0523-0008	Raw Material		165.9 – 167.3°C
URID-0123-00005-PV Beginning	Finished Good		167.0 – 168.3°C

4.10. RELATED SUBSTANCES :

4.10.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Related Substances testing are detailed in the table below.

TABLE 10: RELATED SUBSTANCES

Lot Number	Stage	Specification	Result	
			Uracil (%)	Pseudouridine (%)
PMAT-0523-00701	Mother Liquor	Report	<0.05%	<0.05%
RMAT-0322-0014	Raw Material		<0.05%	<0.05%
RMAT-0523-0008	Raw Material		<0.05%	<0.05%
URID-0123-00005-PV WC First Basket	Wet Crystal		<0.05%	<0.05%
URID-0123-00005-PV Beginning	Finished Good		<0.05%	<0.05%

TABLE 11: RELATED SUBSTANCES CONTINUED

Lot Number	Stage	Specification	Result		
			RRT 0.62 (%)	RRT 1.64 (%)	Total Impurities (%)
PMAT-0523-00701	Mother Liquor	Report	0.16%	0.31%	0.47%
RMAT-0322-0014	Raw Material		0.08%	0.14%	0.23%
RMAT-0523-0008	Raw Material		0.05%	0.17%	0.22%
URID-0123-00005-PV WC First Basket	Wet Crystal		<0.05%	<0.05%	<0.05%
URID-0123-00005-PV Beginning	Finished Good		<0.05%	0.05%	0.05%

4.11. RESIDUAL SOLVENTS :

4.11.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Residual Solvents testing are detailed in the table below.

TABLE 12: RESIDUAL SOLVENTS

Lot Number	Stage	Specification	Result		
			Ethanol	Methanol	IPA
RMAT-0322-0014	Raw Material	Report	<2390 ppm	<500 ppm	<2640 ppm
RMAT-0523-0008	Raw Material				
URID-0123-00005-PV Beginning	Finished Good				

4.12. **RESIDUE ON IGNITION** :

4.12.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Residue on Ignition testing are detailed in the table below.

TABLE 13: RESIDUE ON IGNITION

Lot Number	Stage	Specification	Result
RMAT-0322-0014	Raw Material	Report	<0.0194%
RMAT-0523-0008	Raw Material		0.0199%
URID-0123-00005-PV Beginning	Finished Good	≤0.1%	<0.01%

4.13. **SOLUBILITY** :

4.13.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Solubility testing are detailed in the table below.

TABLE 14: SOLUBILITY

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	Clear/Colorless Liquid
RMAT-0322-0014	Raw Material		Clear/Colorless Liquid
RMAT-0523-0008	Raw Material		Clear/Colorless Liquid
URID-0123-00005-PV WC First Basket	Wet Crystal		Clear/Colorless Liquid
URID-0123-00005-PV Beginning	Finished Good		Clear/Colorless Liquid

4.14. **TRANSMITTANCE OF SOLUTION 5%** :

4.14.1. Refer to the Degradation and Impurity Profile Protocol: Uridine (Excipient) for testing methods and requirements. The results of the Transmittance of 5% Solution are detailed in the table below.

TABLE 15: TRANSMITTANCE OF SOLUTION 5%

Lot Number	Stage	Specification	Result
PMAT-0523-00701	Mother Liquor	Report	99.7738%
RMAT-0322-0014	Raw Material		99.1644%
RMAT-0523-0008	Raw Material		98.7518%
URID-0123-00005-PV WC First Basket	Wet Crystal		99.0979%
URID-0123-00005-PV Beginning	Finished Good	≥ 98.0%	99.3%

5. CONCLUSION

5.1. Water was identified as an intentionally introduced solvent due to the aqueous purification process, but was removed through drying and all finished material met moisture specifications.

5.2. Organic Impurities were removed from the process through the purification stages based on the decrease in related substances and improved transmittance of the post processing samples in relation to the raw materials. Improved purity was also indicated by a higher melting point onset in the finished good from the raw material.

5.3. In conclusion, all samples from all stages of the process met the required specifications as listed in the Degradation and Impurity Profile Protocol.