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 were 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 include a specification for any limits on the impurities found when applicable.

2. RESPONSIBILITIES:

2.1. The Associate Director of Product Life Cycle is responsible for control, implementation, training, and maintenance of this procedure.
2.2. Quality Assurance personnel or designees are responsible for the review and approval of degradation and impurity profiles and associated reports.
2.3. The QC Analyst (or qualified designees) are responsible for performing the testing stated in the protocol and recording all results.
2.4. The Associate Director of Product Life Cycle, or designee is responsible for completing the degradation and impurity testing report.
2.5. 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-0092, Uridine Assay and Related Substances by UPLC with UV Detection.
3.2. BSI-RPT-1015, Analytical Method Validation Report: Residual Solvents by Head Space GC FID (Uridine).
3.3. BSI-SOP-0069, Preparation of Samples for Outside Testing.
3.4. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration.
3.5. BSI-SOP-0094, Muffle Furnace SOP and Calibration.
3.6. BSI-SOP-0098, Balance SOP.
3.7. BSI-SOP-0126, Laboratory Notebooks.
3.8. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP.
3.9. BSI-SOP-0134, Pipette SOP.
3.10. BSI-SOP-0135, Laboratory Chemicals.
3.11. BSI-SOP-0140, Standardization of Titrants.
3.12. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP.
3.13. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration.
3.14. BSI-SOP-0242, Bangor Portable Turbidimeter and Calibration
3.15. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration.
3.16. BSI-SOP-0254, Spectrum Two UATR SOP.
3.17. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP.
3.18. BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP.
3.19. BSI-SOP-0303, NexION 350X ICP-MS SOP.
3.20. BSI-SOP-0348, Waters Acquity UPLC H-Class Plus SOP.
3.21. BSI-SOP-0345, Endosafe Nexgen-PTS Endotoxin Reader SOP.
3.22. BSI-SOP-0420, Elemental Impurities via ICP-MS in Cytidine, Uridine, L-Arginine HCL, and L-Glutamine.
3.23. BSI-SOP-0422, Empower 3 General Procedure.
3.24. *ACS, Reagent Chemicals,* current edition
3.25. *Current EP/BP*
3.26. *Current USP*
3.27. *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-0322-0012	Raw Material	Report	Almost White Powder
RMAT-0322-0013			Almost White Powder
URID-0122-00005-PV Beginning	Finished Goods		White Powder
URID-0122-00006-PV Middle			Almost White Powder
URID-0122-00007-PV End			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
RMAT-0322-0012	Raw Material	Report	99.5%
RMAT-0322-0013			99.8%
URID-0122-00005-PV ML	Mother Liquor		18.6%
PMAT-0622-00726			17.5%
PMAT-0622-00728			20.7%
URID-0122-00005-PV WC Top	Wet Crystal		74.0%
URID-0122-00005-PV WC Bottom			77.9%
URID-0122-00006-PV WC Top			74.4%
URID-0122-00006-PV WC Bottom			72.5%
URID-0122-00007-PV WC Top			77.6%
URID-0122-00007-PV WC Bottom			84.5%
URID-0122-00005-PV Beginning	Finished Goods		100.2%
URID-0122-00006-PV Middle			100.2%
URID-0122-00007-PV End			100.4%

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) outside testing are detailed in the table below.

TABLE 3: BIOBURDEN (TAMC/TYMC)

Lot Number	Stage	Specification	Result	
			TAMC	TYMC
RMAT-0322-0012	Raw Material	Report	<100 CFU/g	<100 CFU/g
RMAT-0322-0013			<100 CFU/g	<100 CFU/g
URID-0122-00005-PV	Finished Goods		<100 CFU/g	<100 CFU/g
URID-0122-00006-PV			<100 CFU/g	<100 CFU/g
URID-0122-00007-PV			<100 CFU/g	<100 CFU/g

Lot Number	MPL Lab #
RMAT-0322-0012	22K513
RMAT-0322-0013	22L3009
URID-0122-00005-PV	22K514
URID-0122-00006-PV	22K1882
URID-0122-00007-PV	22K3008

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 IMPURITY

Lot Number	Stage	Specification	Result
RMAT-0322-0012	Raw Material	Report	Refer to BSI-RPT-1085 for Elemental Impurity Assessment for Uridine
RMAT-0322-0013			
URID-0122-00005-PV ML	Mother Liquor		
PMAT-0622-00726			
PMAT-0622-00728			
URID-0122-00005-PV WC Top	Wet Crystal		
URID-0122-00005-PV WC Bottom			
URID-0122-00006-PV WC Top			
URID-0122-00006-PV WC Bottom			
URID-0122-00007-PV WC Top			
URID-0122-00007-PV WC Bottom			
URID-0122-00005-PV Beginning	Finished Goods		
URID-0122-00006-PV Middle			
URID-0122-00007-PV End			

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
RMAT-0322-0012	Raw Material	Report	<0.5 EU/mg
RMAT-0322-0013			<0.5 EU/mg
URID-0122-00005-PV Beginning	Finished Goods		<0.5 EU/mg
URID-0122-00006-PV Middle			<0.5 EU/mg
URID-0122-00007-PV End			<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
RMAT-0322-0012	Raw Material	Report	Passes Test; 0.99149
RMAT-0322-0013			Passes Test; 0.992818
URID-0122-00005-PV ML	Mother Liquor		0.207486
PMAT-0622-00726			0.217212
PMAT-0622-00728			0.316699
URID-0122-00005-PV WC Top	Wet Crystal		0.944011
URID-0122-00005-PV WC Bottom			0.955738
URID-0122-00006-PV WC Top			0.948623
URID-0122-00006-PV WC Bottom			0.946919
URID-0122-00007-PV WC Top			0.94837
URID-0122-00007-PV WC Bottom			0.947439
URID-0122-00005-PV Beginning	Finished Goods		Passes Test; 0.997513
URID-0122-00006-PV Middle			Passes Test; 0.993491
URID-0122-00007-PV End			Passes Test; 0.997079

4.7. KARLFISCHER:

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
RMAT-0322-0012	Raw Material	Report	0.21%
RMAT-0322-0013			0.18%
URID-0122-00005-PV ML	Mother Liquor		44.53%
PMAT-0622-00726			39.11%
PMAT-0622-00728			43.87%
URID-0122-00005-PV WC Top	Wet Crystal		14.50%
URID-0122-00005-PV WC Bottom			16.04%
URID-0122-00006-PV WC Top			15.45%
URID-0122-00006-PV WC Bottom			14.70%
URID-0122-00007-PV WC Top			6.43%
URID-0122-00007-PV WC Bottom			27.61%
URID-0122-00005-PV Beginning	Finished Goods		0.20%
URID-0122-00006-PV Middle			0.15%
URID-0122-00007-PV End			0.17%

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 (%)
RMAT-0322-0012	Raw Material	Report	0.0819
RMAT-0322-0013			0.0758
URID-0122-00005-PV ML	Mother Liquor		80.8629
PMAT-0622-00726			82.2452
PMAT-0622-00728			80.1890
URID-0122-00005-PV WC Top	Wet Crystal		38.4072
URID-0122-00005-PV WC Bottom			29.5223
URID-0122-00006-PV WC Top			28.8118
URID-0122-00006-PV WC Bottom			37.6482
URID-0122-00007-PV WC Top			34.2902
URID-0122-00007-PV WC Bottom			43.5876
URID-0122-00005-PV Beginning	Finished Goods		0.0915
URID-0122-00006-PV Middle			0.0454
URID-0122-00007-PV End			0.1015

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-0322-0012	Raw Material	Report	167.3-168.3°C
RMAT-0322-0013			167.3-168.3°C
URID-0122-00005-PV Beginning	Finished Goods		167.1-168.5°C
URID-0122-00006-PV Middle			167.1-168.5°C
URID-0122-00007-PV End			167.1-168.5°C

4.10. RELATED SUBSTANCES:

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

TABLE 10: RELATED SUBSTANCES

Lot Number	Stage	Specification	Result
RMAT-0322-0012	Raw Material	Report	0.22%
RMAT-0322-0013			0.19%
URID-0122-00005-PV ML	Mother Liquor		0.36%
PMAT-0622-00726			0.39%
PMAT-0622-00728			0.40%
URID-0122-00005-PV WC Top	Wet Crystal		0.06%
URID-0122-00005-PV WC Bottom			0.05%
URID-0122-00006-PV WC Top			0.06%
URID-0122-00006-PV WC Bottom			0.06%
URID-0122-00007-PV WC Top			0.05%
URID-0122-00007-PV WC Bottom			0.08%
URID-0122-00005-PV Beginning	Finished Goods		0.06%
URID-0122-00006-PV Middle			0.06%
URID-0122-00007-PV End			0.08%

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 11: RESIDUAL SOLVENTS

Lot Number	Stage	Specification	Result		
			Ethanol	2-Propanol	Methanol
RMAT-0322-0012	Raw Material	Report	<2520 ppm	ND	ND
RMAT-0322-0013			<2520 ppm	<2690 ppm	ND
URID-0122-00005-PV	Finished Goods		ND	<2630 ppm	ND
URID-0122-00006-PV			ND	<2630 ppm	ND
URID-0122-00007-PV			ND	<2630 ppm	ND

ND = None Detected

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 12: RESIDUE ON IGNITION

Lot Number	Stage	Specification	Result
RMAT-0322-0012	Raw Material	Report	<0.0862%
RMAT-0322-0013			<0.0193%
URID-0122-00005-PV Beginning	Finished Goods		<0.0200%
URID-0122-00006-PV Middle			<0.0200%
URID-0122-00007-PV End			<0.0197%

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 13: SOLUBILITY

Lot Number	Stage	Specification	Result
RMAT-0322-0012	Raw Material	Report	Clear/Colorless Liquid
RMAT-0322-0013			Clear/Colorless Liquid
URID-0122-00005-PV ML	Mother Liquor		Clear/Colorless Liquid
PMAT-0622-00726			Clear/Colorless Liquid
PMAT-0622-00728			Clear/Colorless Liquid
URID-0122-00005-PV WC Top	Wet Crystal		Clear/Colorless Liquid
URID-0122-00005-PV WC Bottom			Clear/Colorless Liquid
URID-0122-00006-PV WC Top			Clear/Colorless Liquid
URID-0122-00006-PV WC Bottom			Clear/Colorless Liquid
URID-0122-00007-PV WC Top			Clear/Colorless Liquid
URID-0122-00007-PV WC Bottom			Clear/Colorless Liquid
URID-0122-00005-PV Beginning	Finished Goods		Clear/Colorless Liquid
URID-0122-00006-PV Middle			Clear/Colorless Liquid
URID-0122-00007-PV End			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 Solution 5% testing are detailed in the table below.

TABLE 14: TRANSMITTANCE OF SOLUTION 5%

Lot Number	Stage	Specification	Result (%)
RMAT-0322-0012	Raw Material	Report	99.2089
RMAT-0322-0013			99.4931
URID-0122-00005-PV ML	Mother Liquor		99.9736
PMAT-0622-00726			99.8868
PMAT-0622-00728			99.9311
URID-0122-00005-PV WC Top	Wet Crystal		99.5497
URID-0122-00005-PV WC Bottom			99.3798
URID-0122-00006-PV WC Top			99.5337
URID-0122-00006-PV WC Bottom			99.8367
URID-0122-00007-PV WC Top			99.1957
URID-0122-00007-PV WC Bottom			99.5828
URID-0122-00005-PV Beginning	Finished Goods		98.7756
URID-0122-00006-PV Middle			98.8955
URID-0122-00007-PV End			97.6456

5. RESULTS AND CONCLUSION:

5.1. Results:

5.1.1. Appearance and Color

5.1.1.1. Appearance and color was found to be improved through processing qualitatively from white to off/white powder to white/powder through processing.

5.1.2. Assay (HPLC)

5.1.2.1. Assay via liquid chromatography was monitored throughout processing. Assay values fluctuated due to solvent additions and removal. The finished product produced a superior assay to the raw material used for manufacturing, this is primarily attributed to organic impurity reduction through recrystallization.

5.1.3. Bioburden

5.1.3.1. BioBurden was not detected in any amount at any stage of the process. The use of alcohol in the mother liquor, filtration, WFI water and bioburden free raw material are all contributing factors to this finding.

5.1.4. Elemental Impurities

5.1.4.1. Magnesium was detected in the raw material up to 11ppm, and was lowered through processing. The highest detected level of Magnesium in the finished product was 2.8ppm (Lot URID-0122-00007 End Sample). The mother liquor PMAT-0722-00728 retained 1.7ppm Magnesium demonstrating recrystallization is the primary mechanism of Magnesium reduction in the process. Magnesium was reduced by 75%.

5.1.5. Endotoxin

5.1.5.1. Endotoxin was detected in small amounts ranging from 0.0038EU/mg to 0.0355EU/mg in the raw materials. The finished product endotoxin results range from <0.0010EU/g to 0.0128EU/g. The results demonstrated a reduction in endotoxin from raw material to finished goods through processing.

5.1.6. Identification (IR)

5.1.6.1. Uridine maintained spectral conformity from starting material to finished processed material. Solvent peaks due to water and alcohol from the process negatively affected correlation for in-process stages.

5.1.7. Karl Fischer

5.1.7.1. Water content during processing was found to be as high as 44.5%, water was successfully removed from the resulting finished product and documented to be 0.20% w/w water maximum.

5.1.8. Loss on Drying

5.1.8.1. Solvents including water and alcohols were successfully removed through processing to acceptable levels.

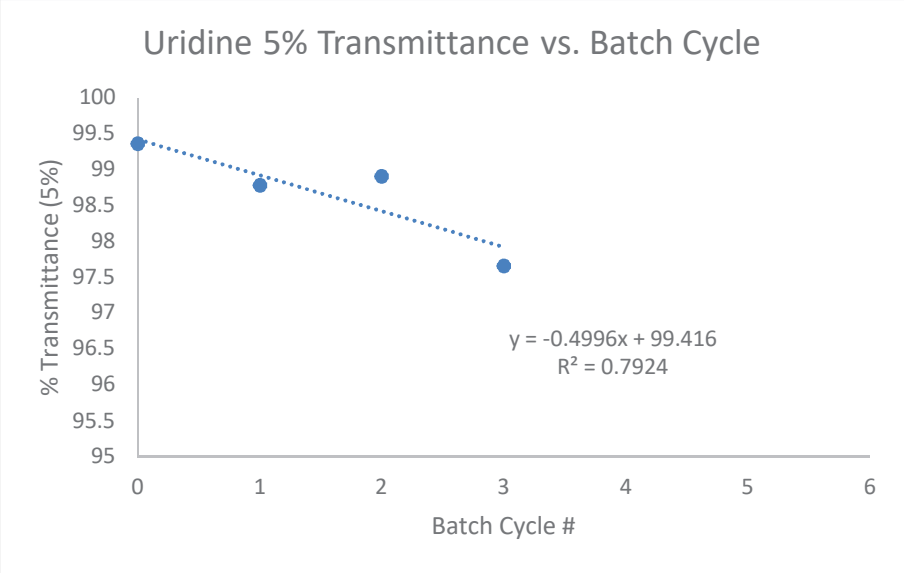
5.1.9. Melting Range

5.1.9.1. Melting range met specification from the raw material to the finished product. The melting range was concluded to be unaffected through processing.

5.1.10. Related Substances: Organic Impurities

5.1.10.1. Organic impurities were found in the raw materials up to 0.12% w/w at RRT 1.6 and 0.07% w/w at RRT 0.6. The 0.6 RRT impurity was removed through processing to undetectable levels, however, the RRT 1.6 impurity was still detected in the finished goods at levels up to 0.08%. The mother liquor organic impurity content at both RRT 0.6 and 1.6 increased with each batch cycle indicating that recrystallization was the principle reduction mechanism and the impurity is retained in the mother liquor.

5.1.11. Residue on Ignition
5.1.11.1. No residue on ignition residues were detected during all testing.
5.1.12. Residual Solvents: 2-Propanol/Methanol/Ethanol
5.1.12.1. 2-Propanol was introduced in to the process and controlled to levels <LoQ (<2,690ppm)
5.1.12.2. Ethanol was detected <LoQ in the raw material but removed during processing.
5.1.12.3. Methanol was not detected in either raw material or the finished product.
5.1.13. Solubility
5.1.13.1. Solubility remained clear and colorless throughout processing.
5.1.14. Transmittance of Solution 5%
5.1.14.1. Transmittance was the only negatively impacted CQA from batch processing. The raw material 5% transmittance measured at 99.35%, which the finished goods were measured as low as 97.65%. Transmittance was measured on a 5% solution to monitor changes throughout batch cycles. The slope of the batch cycle vs. % Transmittance 5% demonstrated an average decrease in transmittance about 0.5% per batch at 5% test concentration.



5.2. Conclusion:
5.2.1. In conclusion, no impurities in RM, ML, WC, and FG samples were found to be present based on the results reported herein. No limit adjustments or specification changes will occur as a result of the study. % Transmittance should be monitored over time to determine if the trend found during the degradation and impurity study continues during routine manufacturing. Action limits should be established if the trend continues.