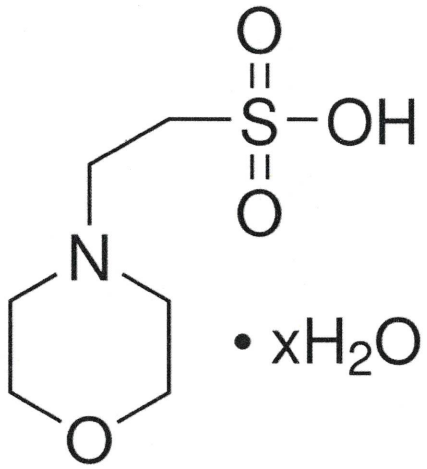
BIOSPECTRA

100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

DEGRADATION AND IMPURITY PROFILE: MES HYDRATE



MANUFACTURING SITE:

BioSpectra Inc.
100 Majestic Way
Bangor, PA 18013
Process Room E02, E03, and E05

COMPLIANT WITH STANDARDS OF:

ICH Q7 Good Manufacturing Practice Guide

COVERING PRODUCT GRADE(S):

Product Grade	Product Code(s)
Bio Excipient Grade	MESH-3201 MESH-3250

Made in the USA

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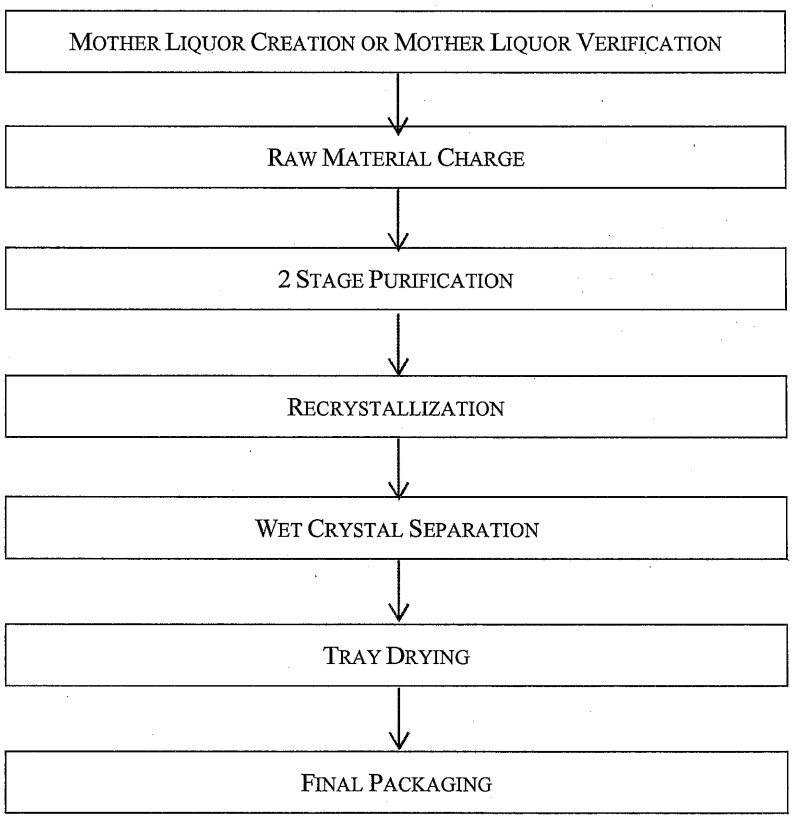
1. PURPOSE AND SCOPE:

1.1. The impurity profiling of MES Hydrate was performed to identify and potentially quantify impurities found in MES Hydrate (CAS 1266615-59-1) product manufactured and purified at BioSpectra Inc.

BioSpectra Excipient Manufacturing Process



1.1.1. The two stages of MES Hydrate that were tested are listed below:
1.1.1.1. Raw Material (Approved Supplier A and B)
1.1.1.2. Finished Good

1.1.2. Analytical Testing Plan:

Table 1:Analytical Testing Plan			
Analytical Test	**Process Stage Testing Plan ☒ Testing Required**		
	Purpose	**Raw Material (Supplier A and B)**	**Finished Good**
Appearance	Visually detect non-conforming impurities.	☒	☒
Infrared Spectrum (UATR)	Verify structure conforms to standard.	☒	☒
Water by Karl Fischer	Ensure hydrate form of MES / Residual Solvents	☒	☒
UV Absorbance (0.1M)	Screen for UV absorbing compounds (260 nm, 280 nm, and 290 nm)	☒	☒
Elemental Impurity Profile	ICHQ3 Screening	☒	☒
pH (1% Solution)	Critical Quality Attribute	☒	☒
Titration with NaOH (Assay)	Purity Assessment (Assay)	☒	☒
PVS	Specified Impurity: Polyvinyl Sulfonate	☒	☒
SVS	Specified Impurity: Sodium Vinylsulfonate as determined by ICP (Na)	☒	☒
Endotoxin	Bioburden residue screening	☒	☒
Enzymes (DNase (Exonuclease), NICKase, RNase, and Protease)	Enzymatically active impurity screening.	☒	☒
TAMC/TYMC	Viable bioburden screening.	☒	☒
Purity (TLC)	Unspecified organic impurity screening	☒	☒
Insoluble Matter	Gravimetric insoluble matter testing, detection of exchange resin and filter degradation	☒	☒
Solubility	Observation of color of solution is the visible wavelength region.	☒	☒
Morpholine	Specified Impurity: Morpholine	☒	☒
Chloride	Specified Impurity: Chloride	☒	☒
Formaldehyde	Specified Impurity: Formaldehyde	☒	☒

1.1.3. Analytical Sampling Plan:

Table 2: Analytical Sampling Plan		
Lot Selected	**Process Stage Testing Plan**	
	Purpose	**Material Type**
MESM-0123-00147	Lot 1 of 3 Purified MES	Finished Good
MESM-0123-00151	Lot 2 of 3 Purified MES	Finished Good
MESM-0123-00152	Lot 3 of 3 Purified MES	Finished Good
RMAT-0123-0172	Lot 1 of 3 MES Raw Material Supplier A	Raw Material
RMAT-0123-0173	Lot 2 of 3 MES Raw Material Supplier A	Raw Material
RMAT-0123-0174	Lot 3 of 3 MES Raw Material Supplier A	Raw Material
DMAT-0424-0093	Lot 1 of 3 MES Raw Material Supplier B	Raw Material
DMAT-0424-0094	Lot 2 of 3 MES Raw Material Supplier B	Raw Material
DMAT-0424-0095	Lot 3 of 3 MES Raw Material Supplier B	Raw Material

2. RESPONSIBILITIES:

2.1. The Senior Manager of Product Life Cycle is responsible for control, implementation, training, and maintenance of this procedure.
2.2. The Analysts (or qualified designees) were responsible for performing the testing stated in the protocol and recording all results.
2.3. The Senior Manager of Product Life Cycle, or qualified designee, is responsible for completing the degradation and impurity testing report.

3. REFERENCES:

3.1. BSI-ATM-0065, Analytical Method: Elemental Impurities via ICP-MS in MES Monohydrate
3.2. BSI-ATM-0123, MES Hydrate Testing Methods
3.3. BSI-PRL-0839, MES Hydrate Impurity Protocol
3.4. BSI-SOP-0069, Preparation of Samples for Outside Testing
3.5. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
3.6. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration
3.7. BSI-SOP-0094, Muffle Furnace SOP and Calibration
3.8. BSI-SOP-0098, Balance SOP
3.9. BSI-SOP-0126, Laboratory Notebooks
3.10. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
3.11. BSI-SOP-0134, Pipette SOP
3.12. BSI-SOP-0135, Laboratory Chemicals
3.13. BSI-SOP-0140, Standardization of Titrants
3.14. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
3.15. BSI-SOP-0242, Bangor Portable Turbidimeter and Calibration
3.16. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration
3.17. BSI-SOP-0254, Spectrum Two UATR SOP
3.18. BSI-SOP-0303, NexION 350X ICP-MS SOP
3.19. BSI-SOP-0345, Endosafe Nexgen-PTS Endotoxin Reader SOP
3.20. BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP
3.21. *ACS, Reagent Chemicals,* current edition
3.22. *Current EP/BP*
3.23. *Current USP*

4. ANALYTICAL RESULTS:

4.1. ABSORBANCE (0.1M) :

4.1.1. Weigh 0.53 grams of sample and accurately transfer the weighed sample to a graduated cylinder and Q.S. to 25mL with Purified Water.
4.1.2. Swirl to dissolve completely.
4.1.3. Refer to the Lambda 25 UV/Vis Spectrophotometer to determine the Absorbance of the sample at 260nm, 280nm, and 290nm.

Table 3: Absorbance (0.1M) Results

Lot Selected	Process Stage Testing			
	Material Type	Absorbance @ 260nm	Absorbance @ 280nm	Absorbance @ 290nm
MESM-0123-00147	Finished Good	0.0021 a.u.	0.0010 a.u.	0.0005 a.u.
MESM-0123-00151	Finished Good	0.0026 a.u.	0.0023 a.u.	0.0021 a.u.
MESM-0123-00152	Finished Good	0.0021 a.u.	0.0016 a.u.	0.0013 a.u.
RMAT-0123-0172	Raw Material	0.0063 a.u.	0.0057 a.u.	0.0056 a.u.
RMAT-0123-0173	Raw Material	0.0102 a.u.	0.0095 a.u.	0.0092 a.u.
RMAT-0123-0174	Raw Material	0.0147 a.u.	0.0131 a.u.	0.0119 a.u.
DMAT-0424-0093	Raw Material	0.0071 a.u.	0.0069 a.u.	0.0062 a.u.
DMAT-0424-0094	Raw Material	0.0079 a.u.	0.0074 a.u.	0.0072 a.u.
DMAT-0424-0095	Raw Material	0.0125 a.u.	0.0121 a.u.	0.0117 a.u.

4.2. APPEARANCE :

4.2.1. Weigh a suitable amount of sample into a clean, dry glass beaker.
4.2.2. In an area with sufficient lighting, view the sample from all sides.
4.2.3. The sample should be white in color and characteristic of crystals.

Table 4: Appearance Results

Lot Selected	Process Stage Testing	
	Material Type	Result
MESM-0123-00147	Finished Good	White / Crystals
MESM-0123-00151	Finished Good	White / Crystals
MESM-0123-00152	Finished Good	White / Crystals
RMAT-0123-0172	Raw Material	White / Crystals
RMAT-0123-0173	Raw Material	White / Crystals
RMAT-0123-0174	Raw Material	White / Crystals
DMAT-0424-0093	Raw Material	White / Crystals
DMAT-0424-0094	Raw Material	White / Crystals
DMAT-0424-0095	Raw Material	White / Crystals

4.3. ASSAY (ANHYDROUS BASIS) :

4.3.1. Standardize 0.1N Sodium Hydroxide in accordance with the Standardization of Titrants SOP utilizing the Metrohm Auto-Titrator.
4.3.2. Accurately weigh 0.8 grams of sample (measured as-is) and transfer to a suitable beaker.
4.3.3. Add 50mL of Purified Water and stir to dissolve.
4.3.4. Titrate to a potentiometric endpoint with 0.1N Sodium Hydroxide.
4.3.5. Submerge the probe in storage solution after analysis is completed to condition the glass electrode. To calculate assay on the anhydrous basis, use the below equation:

$$\%\text{MES Hydrate (As-Is, Anhydrous Basis)} = \frac{(mL \times N\ of\ NaOH)(19.524)}{Sample\ Weight\ (g)}$$

$$\%\text{MES, Hydrate (Anhydrous)} = \frac{\text{As-Is Assay (\%)}}{(100 - KF\ Value)} \times 100$$

Table 5: Assay (Anhydrous Basis) Results		
Lot Selected	**Process Stage Testing**	
	Material Type	**Result (%)**
MESM-0123-00147	Finished Good	100.79%
MESM-0123-00151	Finished Good	100.16%
MESM-0123-00152	Finished Good	100.58%
RMAT-0123-0172	Raw Material	100.34%
RMAT-0123-0173	Raw Material	99.36%
RMAT-0123-0174	Raw Material	100.39%
DMAT-0424-0093	Raw Material	100.59%
DMAT-0424-0094	Raw Material	100.63%
DMAT-0424-0095	Raw Material	101.01%

4.4. **CHLORIDES** :

4.4.1. Sample Preparation:

4.4.1.1. Weigh 2.0 grams of sample and dissolve in approximately 40mL of Purified Water. If necessary, neutralize the solution with Nitric Acid to litmus.

4.4.2. 0.03% Standard Preparation:

4.4.2.1. Pipette 0.82mL of 0.02N HCl into a Nessler Color Comparison Tube and dilute to approximately 40mL with Purified Water.

4.4.3. Procedure:

4.4.3.1. Add to each solution, 1mL of Concentrated Nitric Acid and 1mL of 0.1N Silver Nitrate.

4.4.3.2. Q.S. to 50mL with Purified Water. Cover with parafilm and mix by inversion.

4.4.3.3. After 5 minutes, the turbidity of the Sample Preparation does not exceed that produced by the 0.03% Standard Preparation when viewed against a dark background.

Table 6: Chlorides Results		
Lot Selected	**Process Stage Testing**	
	Material Type	**Result (%)**
MESM-0123-00147	Finished Good	<0.03%
MESM-0123-00151	Finished Good	<0.03%
MESM-0123-00152	Finished Good	<0.03%
RMAT-0123-0172	Raw Material	<0.03%
RMAT-0123-0173	Raw Material	<0.03%
RMAT-0123-0174	Raw Material	<0.03%
DMAT-0424-0093	Raw Material	<0.03%
DMAT-0424-0094	Raw Material	<0.03%
DMAT-0424-0095	Raw Material	<0.03%

4.5. **CHROMATOGRAPHIC PURITY** :

4.5.1. Solution Preparation:

4.5.1.1. Note: All solutions may be scaled as needed.

4.5.1.2. Mobile Phase (10:15:75 Methanol: Purified Water: Acetonitrile): Pipette 15mL of Purified Water into a 100mL volumetric flask, add 10mL of Methanol, fill to volume with Acetonitrile, and mix well.

4.5.1.3. Detection Solution (Iodine Vapor): Place a suitable amount of Iodine Flakes into a covered TLC chamber heated to 30°C to 40°C.

4.5.1.4. Sample Solution (200mg/mL MES Hydrate Sample): Accurately weigh 2.00 grams of sample and transfer to a 10mL volumetric flask. Dissolve and dilute to volume with Purified Water. Mix well.

4.5.1.5. Reference Solution (200mg/mL MES Hydrate Standard): Accurately weigh 2.00 grams of standard and transfer to a 10mL volumetric flask. Dissolve and dilute to volume with Purified Water. Mix well.

4.5.1.6. System Suitability Solution (2mg/mL MES Hydrate Standard): Pipette 0.50mL of *Reference Solution* into a 50mL volumetric flask, fill to volume with Purified Water, and mix well.

Table 7: Chromatographic System:

Parameter	Setting
Mode	Thin-Layer Chromatography (TLC)
Adsorbent	TLC Silica Gel 60 or equivalent
Application Volume	0.5µL
Mobile Phase	10:15:75 Methanol: Purified Water: Acetonitrile
Detection Solution	Iodine Vapor

4.5.2. Analysis:

4.5.2.1. Spot 0.5µL of *Sample Solution*, *Reference Solution*, and *System Suitability Solution* onto a TLC plate.

4.5.2.2. Place the plate into an enclosed TLC chamber containing *Mobile Phase,* ensuring the spots are above the surface of the *Mobile Phase*.

4.5.2.3. Allow the mobile phase to ascend the plate until the solvent front has traveled two-thirds of the length of the plate.

4.5.2.4. Remove the plate and mark the solvent front with a pencil.

4.5.2.5. Place the plate into the TLC chamber containing the Iodine Vapor and allow to sit for at least 4 minutes.

4.5.3. Acceptance Criteria:

4.5.3.1. System Suitability: The *System Suitability Solution* shows a clearly visible spot.

4.5.3.2. Result Reporting: Any secondary spot on the *Sample Solution* chromatogram must be less intense than the corresponding spot in the *System Suitability Solution* to report as <1.0%.

Table 8: Chromatographic Purity Results

Lot Selected	Process Stage Testing	
	Material Type	Result (%)
MESM-0123-00147	Finished Good	<1.0%
MESM-0123-00151	Finished Good	<1.0%
MESM-0123-00152	Finished Good	<1.0%
RMAT-0123-0172	Raw Material	<1.0%
RMAT-0123-0173	Raw Material	<1.0%
RMAT-0123-0174	Raw Material	<1.0%
DMAT-0424-0093	Raw Material	<1.0%
DMAT-0424-0094	Raw Material	<1.0%
DMAT-0424-0095	Raw Material	<1.0%

4.6. ENDOTOXIN

4.6.1. Accurately weigh 20mg of sample into a sterile tube.
4.6.2. Add 70µL of 1N NaOH.
4.6.3. Dilute to 10mL with LAL Reagent Water.
4.6.4. To 1mL of this solution, add 4mL of LAL Reagent Water. Mix thoroughly for a final concentration of 0.0004 g/mL.
4.6.5. Refer to the Endosafe NexGen-PTS Endotoxin Reader SOP for instrument analysis.

Table 9: Endotoxin Results

Lot Selected	Process Stage Testing	
	Material Type	Result (EU/g)
MESM-0123-00147	Finished Good	<23.9 EU/g
MESM-0123-00151	Finished Good	<25.0 EU/g
MESM-0123-00152	Finished Good	<25.0 EU/g
RMAT-0123-0172	Raw Material	<24.9 EU/g
RMAT-0123-0173	Raw Material	<24.8 EU/g
RMAT-0123-0174	Raw Material	<24.8 EU/g
DMAT-0424-0093	Raw Material	<24.8 EU/g
DMAT-0424-0094	Raw Material	<24.6 EU/g
DMAT-0424-0095	Raw Material	<25.0 EU/g

4.7. ENZYME ACTIVITY

4.7.1. DNase: Refer to DNase (Exonuclease) Assay (BSI-SOP-0138) for sample preparation and analysis.
4.7.2. NICKase: Refer to DNase (NICKase) Assay (BSI-SOP-0595) for sample preparation and analysis.
4.7.3. RNase: Refer to RNase (Ribonuclease) Assay (BSI-SOP-0096) for sample preparation and analysis.
4.7.4. Protease: Refer to Protease Assay (BSI-SOP-0139) for sample preparation and analysis.

Table 10: Enzyme Activity Results					
Lot Selected	Process Stage Testing				
	Material Type	DNase (Exonuclease) Assay Results	DNase (NICKase) Assay Results	RNase (Ribonuclease) Assay Results	Protease Assay Results
MESM-0123-00147	Finished Good	None Detected	None Detected	None Detected	None Detected
MESM-0123-00151	Finished Good	None Detected	None Detected	None Detected	None Detected
MESM-0123-00152	Finished Good	None Detected	None Detected	None Detected	None Detected
RMAT-0123-0172	Raw Material	None Detected	None Detected	None Detected	None Detected
RMAT-0123-0173	Raw Material	None Detected	None Detected	None Detected	None Detected
RMAT-0123-0174	Raw Material	None Detected	None Detected	None Detected	None Detected
DMAT-0424-0093	Raw Material	None Detected	None Detected	None Detected	None Detected
DMAT-0424-0094	Raw Material	None Detected	None Detected	None Detected	None Detected
DMAT-0424-0095	Raw Material	None Detected	None Detected	None Detected	None Detected

4.8. **FORMALDEHYDE** :

4.8.1. Refer to Analytical Method: Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine By GC-MS (BSI-ATM-0050) for sample preparation and analysis.

Table 11: Formaldehyde Results		
Lot Selected	Process Stage Testing	
	Material Type	Result (ppm)
MESM-0123-00147	Finished Good	0.11
MESM-0123-00151	Finished Good	0.10
MESM-0123-00152	Finished Good	0.12
RMAT-0123-0172	Raw Material	0.17
RMAT-0123-0173	Raw Material	0.10
RMAT-0123-0174	Raw Material	0.12
DMAT-0424-0093	Raw Material	0.03
DMAT-0424-0094	Raw Material	0.03
DMAT-0424-0095	Raw Material	0.03

4.9. **IDENTIFICATION (IR) (As-is)** :

4.9.1. Follow the Spectrum Two UATR SOP.

4.9.2. Analyze sample as-is.

Table 12: Identification (IR) (as-is) Results		
Lot Selected	**Process Stage Testing**	
	Material Type	**Result**
MESM-0123-00147	Finished Good	Passes Test
MESM-0123-00151	Finished Good	Passes Test
MESM-0123-00152	Finished Good	Passes Test
RMAT-0123-0172	Raw Material	Passes Test
RMAT-0123-0173	Raw Material	Passes Test
RMAT-0123-0174	Raw Material	Passes Test
DMAT-0424-0093	Raw Material	Passes Test
DMAT-0424-0094	Raw Material	Passes Test
DMAT-0424-0095	Raw Material	Passes Test

4.10. **INSOLUBLE MATTER** :

4.10.1. Accurately weigh 20.0 grams of sample and transfer to a 600mL beaker.

4.10.2. Add 200mL of Purified Water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve the sample.

4.10.3. Heat to boiling and digest on a hot plate in a covered beaker for 1 hour.

4.10.4. Prepare a Gooch filtering crucible and 6 – 15-micron filter by drying at 105°C ± 2°C for 1 hour. Allow to cool in ambient air for 15 minutes and weigh.

4.10.5. Filter sample solution through conditioned filtering crucible and 6 – 15-micron filter. Rinse thoroughly with at least 3 crucible volumes of hot purified water.

4.10.6. Dry the crucible at 105°C ± 2°C for 1 hour.

4.10.7. Cool in ambient air for 15 minutes and reweigh.

4.10.8. Calculate the % Insoluble Matter as follows:

$$\%\ Insoluble\ Matter = \frac{Residue\ Weight\ (g)}{Sample\ Weight\ (g)} \times 100$$

Table 13: Insoluble Matter Results		
Lot Selected	**Process Stage Testing**	
	Material Type	**Result (%)**
MESM-0123-00147	Finished Good	0.0045%
MESM-0123-00151	Finished Good	0.0130%
MESM-0123-00152	Finished Good	0.0035%
RMAT-0123-0172	Raw Material	0.0065%
RMAT-0123-0173	Raw Material	0.0045%
RMAT-0123-0174	Raw Material	0.0045%
DMAT-0424-0093	Raw Material	<0.0005%
DMAT-0424-0094	Raw Material	0.0130%
DMAT-0424-0095	Raw Material	0.0035%

4.11. **MICROBIAL** :

4.11.1. Microbial analysis will be performed by an outside testing laboratory.

4.11.1.1. Package and send NLT 35 grams of sample to an Approved Contract Laboratory.

4.11.2. Analyses:

4.11.2.1. Total Aerobic Microbial Count (TAMC).

4.11.2.2. Total Yeast Microbial Count (TYMC).

Table 14: Microbial Results			
Lot Selection	**Process Stage Testing**		
	Material Type	**Total Aerobic Microbial Count (TAMC) (CFU/g)**	**Total Yeast Microbial Count (TYMC) (CFU/g)**
MESM-0123-00147	Finished Good	<10 CFU/g	<10 CFU/g
MESM-0123-00151	Finished Good	<10 CFU/g	<10 CFU/g
MESM-0123-00152	Finished Good	<10 CFU/g	<10 CFU/g
RMAT-0123-0172	Raw Material	<10 CFU/g	<10 CFU/g
RMAT-0123-0173	Raw Material	<10 CFU/g	<10 CFU/g
RMAT-0123-0174	Raw Material	<10 CFU/g	<10 CFU/g
DMAT-0424-0093	Raw Material	<10 CFU/g	<10 CFU/g
DMAT-0424-0094	Raw Material	<10 CFU/g	<10 CFU/g
DMAT-0424-0095	Raw Material	<10 CFU/g	<10 CFU/g

4.12. **MORPHOLINE BY GC** :

4.12.1. GC Method Parameters:

Table 15: GC Method Parameters	
Parameter	**Setting**
Column	Rtx-5 Amine GC Capillary Column, 30m, 0.53mm ID, 1.00µm or equivalent
Injection Port Temperature	200°C
Detection Type	Flame Ionization
Detector Temperature	250°C
Flow Rate	Total: 52.6 mL/min; Column: 4.32 mL/min
Carrier Gas	Helium
Injection Volume	1µL
Autosampler	AOC-20i
Split Ratio	10.0
Temperature Gradient	Temperature Gradient Program

4.12.2. Morpholine Calibration Standards:

4.12.2.1. Add the amount of Morpholine specified in the Morpholine Calibration Standards Table into a 25mL volumetric flask containing 2.5mL of Purified Water and 0.40mL of 10N Sodium Hydroxide.

4.12.2.2. Fill to volume with Methanol and mix well.

4.12.2.3. Calculate the actual concentrations of the calibration standards using the following equation:

$$\text{Morpholine Concentration } (ppm) = \frac{\text{Morpholine Volume } (\mu L) \times \text{ Morpholine Density } \left(\frac{g}{mL}\right)}{\text{Final Volume } (L)} \times \text{ CoA Purity}$$

Table 16: Morpholine Calibration Standards	
Morpholine Calibration Standard	**Amount of Morpholine (µL)**
0ppm	0
250ppm	6.3
400ppm	10.1
500ppm	12.6
600ppm	15.1
750ppm	18.9

4.12.3. Sample Preparation:
- 4.12.3.1. Weigh 1.0 gram of sample and transfer to a 25mL volumetric flask.
- 4.12.3.2. Add 2.5mL of Purified Water and dissolve completely.
- 4.12.3.3. Add 0.40mL of 10N Sodium Hydroxide.
- 4.12.3.4. Dilute to volume with Methanol and mix well.

4.12.4. System Suitability:
- 4.12.4.1. Inject each standard once and build a calibration curve.
- 4.12.4.2. Result Reporting:
  - 4.12.4.2.1. Theoretical LOQ of method listed above is the lowest standard concentration multiplied by the dilution factor for the sample. Any results less than LOQ will be reported as <LOQ and any results above LOQ will be reported as-is. Method can be adjusted accordingly to hit desired amount of morpholine.

Table 17: Morpholine Results		
Lot Selected	**Process Stage Testing**	
	Material Type	**Result (ppm)**
MESM-0123-00147	Finished Good	<6250
MESM-0123-00151	Finished Good	<6250
MESM-0123-00152	Finished Good	<6250
RMAT-0123-0172	Raw Material	<6250
RMAT-0123-0173	Raw Material	<6250
RMAT-0123-0174	Raw Material	<6250
DMAT-0424-0093	Raw Material	<6275
DMAT-0424-0094	Raw Material	<6275
DMAT-0424-0095	Raw Material	<6275

4.13. **pH of a 1% SOLUTION** :

4.13.1. Weigh 1.0 grams of sample. Transfer to a suitable beaker.

4.13.2. Add 100mL of Purified Water and stir to mix.

4.13.3. Follow the appropriate SOP for calibration and pH measurement.

Table 18: pH of a 1% Solution Results

Lot Selected	Process Stage Testing	
	Material Type	Result
MESM-0123-00147	Finished Good	3.77 @ 24.5°C
MESM-0123-00151	Finished Good	3.77 @ 25.3°C
MESM-0123-00152	Finished Good	3.76 @ 24.6°C
RMAT-0123-0172	Raw Material	3.72 @ 23.0°C
RMAT-0123-0173	Raw Material	3.70 @ 23.1°C
RMAT-0123-0174	Raw Material	3.72 @ 23.1°C
DMAT-0424-0093	Raw Material	3.76 @ 23.7°C
DMAT-0424-0094	Raw Material	3.73 @ 23.9°C
DMAT-0424-0095	Raw Material	3.76 @ 24.0°C

4.14. **PVS CONTENT** :

4.14.1. Solution Preparation:

4.14.1.1. Sample Preparation: Dissolve 1.066 grams of sample in approximately 80mL of Purified Water. Adjust the pH to 5.9 – 6.1 with 50% Sodium Hydroxide and dilute to 100.0mL with Purified Water.

4.14.1.2. Blank: Dissolve 5.33 grams of previously approved MES or Ultra-Pure MES in approximately 400mL of Purified Water. Adjust the pH to 5.9 – 6.1 with 50% Sodium Hydroxide and dilute to 500.0mL with Purified Water.

4.14.1.3. 5mg/mL IgG Antibody Solution: Dissolve 15mg of IgG from Human Serum in 3mL of PBS Buffer. Vortex gently to dissolve. Scale as required.

4.14.1.4. 1000ppm PVS Stock Solution: Dilute 1.0mL of 25% Poly(VinylSulfonic Acid), Sodium Salt to 250.0mL with Purified Water.

4.14.1.5. 50ppm PVS Standard Solution: Dilute 5.0mL of *1000ppm PVS Stock Solution* to 100.0mL with *Blank* and mix well.

4.14.1.6. 10ppm PVS Standard Solution: Dilute 10.0mL of *50ppm PVS Stock Solution* to 50.0mL with *Blank* and mix well.

4.14.1.7. 1ppm PVS Standard Solution: Dilute 5.0mL of *10ppm PVS Stock Solution* to 50.0mL with *Blank* and mix well.

4.14.2. Analysis:

4.14.2.1. In a clean test tube or other suitable vessel, pipette 6mL of test aliquot solution and 0.4mL of the 5mg/mL IgG Antibody Solution into each tube. Cap or parafilm and mix gently by inversion ensuring no air bubbles are formed. Start a timer for 30 minutes.

4.14.2.2. Let test mixtures stand for at least 5 minutes.

4.14.2.3. Using the Portable Turbidimeter, analyze the Blank, 1ppm PVS Standard Solution, and samples within 30 minutes after IgG is added. (Before the timer goes off).

4.14.2.4. Measure each sample in triplicate and average the results.

4.14.2.5. The turbidity of the sample solution should not exceed the 1ppm PVS Standard Solution to report as <1ppm PVS.

Table 19: PVS Content Results		
Lot Selected	Process Stage Testing	
	Material Type	Result (ppm)
MESM-0123-00147	Finished Good	<1ppm
MESM-0123-00151	Finished Good	<1ppm
MESM-0123-00152	Finished Good	<1ppm
RMAT-0123-0172	Raw Material	<1ppm
RMAT-0123-0173	Raw Material	<1ppm
RMAT-0123-0174	Raw Material	<1ppm
DMAT-0424-0093	Raw Material	<1ppm
DMAT-0424-0094	Raw Material	<1ppm
DMAT-0424-0095	Raw Material	<1ppm

4.15. **SOLUBILITY (0.1M)** :

4.15.1. Accurately weigh 0.53 grams of sample.

4.15.2. Transfer accurately weighed sample to a 25mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

4.15.3. View sample from all sides under sufficient lighting noting any apparent color or undissolved particulate. Solution should be clear (complete) and colorless to pass test.

Table 20: Solubility (0.1M) Results		
Lot Selected	Process Stage Testing	
	Material Type	Result
MESM-0123-00147	Finished Good	Passes Test
MESM-0123-00151	Finished Good	Passes Test
MESM-0123-00152	Finished Good	Passes Test
RMAT-0123-0172	Raw Material	Passes Test
RMAT-0123-0173	Raw Material	Passes Test
RMAT-0123-0174	Raw Material	Passes Test
DMAT-0424-0093	Raw Material	Passes Test
DMAT-0424-0094	Raw Material	Passes Test
DMAT-0424-0095	Raw Material	Passes Test

4.16. **SVS CONTENT** :

4.16.1. Refer to Analytical Method: Determination of Elemental Impurities in MES Hydrate, DCN: BSI-ATM-0115 and NexION 350X ICP-MS SOP, DCN: BSI-SOP-0303.

4.16.2. Calculate potential SVS (Sodium Vinyl Sulfonate) content from the sodium result using the following equation:

$$SVS\ (ppm) = Na\ (ppm) \times 5.66$$

Table 21: SVS Content Results		
Lot Selected	Process Stage Testing	
	Material Type	Result
MESM-0123-00147	Finished Good	<33.96ppm
MESM-0123-00151	Finished Good	<33.96ppm
MESM-0123-00152	Finished Good	<33.96ppm
RMAT-0123-0172	Raw Material	<33.96ppm
RMAT-0123-0173	Raw Material	<33.96ppm
RMAT-0123-0174	Raw Material	<33.96ppm
DMAT-0424-0093	Raw Material	<33.96ppm
DMAT-0424-0094	Raw Material	<33.96ppm
DMAT-0424-0095	Raw Material	<33.96ppm

4.17. **TRACE ELEMENTS** :

4.17.1. Refer to Analytical Method: Determination of Elemental Impurities in MES Hydrate, DCN: BSI-ATM-0115 and NexION 350X ICP-MS SOP, DCN: BSI-SOP-0303.

Table 22: Elemental Impurities and Trace Metals Results

Element	Process Stage Testing and Selected Lots								
	Finished Good			Raw Material					
	MESM-0123-00147 (ppm)	MESM-0123-00151 (ppm)	MESM-0123-00152 (ppm)	RMAT-0123-0172 (ppm)	RMAT-0123-0173 (ppm)	RMAT-0123-0174 (ppm)	DMAT-0424-0093 (ppm)	DMAT-0424-0094 (ppm)	DMAT-0424-0095 (ppm)
Ag	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Al	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
As	<0.45	<0.45	<0.45	<0.45	<0.45	<0.45	<0.45	<0.45	<0.45
Au	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0	<3.0
Ba	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Bi	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Ca	<15	<15	<15	<15	<15	<15	<15	<15	<15
Cd	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Co	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Cr	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Cu	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Fe	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	2.5	1.6	1.7
Hg	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09
Ir	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Li	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Na	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
Mg	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Mn	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Mo	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Ni	<0.60	<0.60	<0.60	<0.60	<0.60	<0.60	<0.60	<0.60	<0.60
Os	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Pb	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Pd	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Pt	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Rh	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Ru	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Sb	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Se	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
Sn	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Ti	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24
V	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Zn	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5

4.18. **WATER BY KARL FISCHER** :

4.18.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

4.18.2. Immediately weigh grams of as-is sample into the glass weighing spoon and tare it.

4.18.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.

4.18.3.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

4.18.4. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, transfer the sample weight to the auto-titrator software.

4.18.5. Check to make sure there is no residual sample stuck to the sides of the titration vessel.

4.18.6. Ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

4.18.7. The moisture content will be determined by the Metrohm Auto Titrando 907, using the following equation:

$$\%\ Moisture = \frac{(mL\ of\ Composite\ 5)\left(\frac{mg}{mL}\ of\ Composite\ 5\right)(0.1)}{Sample\ Weight\ (g)}$$

Table 23: Water by Karl Fischer Results

Lot Selected	Process Stage Testing	
	Material Type	Results (%)
MESM-0123-00147	Finished Good	8.84%
MESM-0123-00151	Finished Good	8.69%
MESM-0123-00152	Finished Good	8.81%
RMAT-0123-0172	Raw Material	8.68%
RMAT-0123-0173	Raw Material	7.67%
RMAT-0123-0174	Raw Material	8.67%
DMAT-0424-0093	Raw Material	8.70%
DMAT-0424-0094	Raw Material	8.73%
DMAT-0424-0095	Raw Material	8.93%

5. IMPURITY PROFILE SUMMARY AND CONCLUSION:

5.1. Impurity Profile Summary:

5.1.1. Organic Impurities: Impurities were reduced through processing as indicated by improvement in the chromatographic purity from the raw materials to finished good testing and improvement of UV absorbance from raw material to finished good.

5.1.2. Inorganic Impurities: Iron was detected in the raw material and improved to below detection limits of the method of analysis through processing.

5.1.3. Solvents: Water is an intentionally introduced solvent and critical to the formation of MES hydrate crystals. No other solvents were or are introduced in the process and all Karl Fischer testing performed was within specifications. Although the hydration factor is undefined, Karl Fischer results suggest a monohydrate hydrate variation of MES hydrate.

5.1.4. Bioburden: No bioburden was detected in the raw materials or finished product. Enzymatic testing indicated no enzymatic activity was detected in both the raw materials and the finished good.

5.2. Conclusion: The process of manufacture and purification of MES Hydrate at BioSpectra Inc. successfully lowers organic and inorganic impurities in MES Hydrate (MESH).