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

INTRODUCTION: The following analyses are conducted for Uracil, Bio Pharma Grade product code UC4202, in accordance with the Uracil Testing Methods DCN: 16-001314 v.4.0 and Certificate of Analysis DCN: 19-002944 v.3.0. Specific details for the procedures were also obtained from Spectrum Two UATR SOP DCN: 16-001330 v.3.0.

1. APPEARANCE & COLOR — White to Slightly Yellow Powder:

1.1. Place a suitable amount of sample in a clean and dry glass beaker.
1.2. In an area with sufficient lighting, view the sample from all sides.
1.3. The sample should be white to slightly yellow and characteristic of a powder.

2. ASSAY — 97.0-102.0%:

2.1. Diluent and Mobile Phase: Prepare a 0.68% potassium phosphate solution to be used as diluent and mobile phase.
2.2. Assay Standard Solution: Prepare a 45-55µg/mL uracil CRS standard solution by weighing 90-110mg of uracil CRS and quantitatively transferring to a 100.0mL volumetric flask. Dissolve and dilute to 100.0mL with diluent. Quantitatively transfer 5.0mL of the solution to a 100.0mL volumetric flask, dilute to volume with diluent and mix thoroughly.
2.3. Sample Solution: Prepare a 45-55 µg/mL uracil sample solution by weighing 90-110mg of uracil sample and quantitatively transferring to a 100.0mL volumetric flask. Dissolve and dilute to 100.0mL with diluent. Quantitatively transfer 5.0mL of the solution to a 100.0mL volumetric flask, dilute to volume with diluent and mix thoroughly.
2.4. Set up qualified HPLC with the following method parameters:

Table 1: Instrument Method Parameters

Parameter	Setting
Flow Type	Isocratic
Mobile Phase	0.68% KH_2PO_4
Flow Rate	1 mL/min
Injection Volume	10 µL
Detector	UV at 266 nm λ
Detector Temperature	Ambient
Column Temperature	Ambient
Run Time	5 minutes

2.5. Column: C18(2)-3.9x150mm or equivalent
2.6. Analyze the Uracil Standard solution 5 times for system suitability.
2.6.1. % RSD must be NMT 0.73% to proceed with analysis.
2.7. Analyze the Uracil Sample Solution once.
2.8. Include a start blank (diluent) and an end blank (diluent) and a single QC check (Standard Solution) at the end of each run. The QC check is used to monitor drift. % Recovery of the QC Check Should be within 97.0-102.0% of the system suitability area average. If this criterion is

Printed On:	12-Oct-2020 09:05:44 AM	Ledergerber, Alissa	: Printed By
Authored By:	Hamelburg, Crystal	09-Oct-2020 10:00:11 AM	
Approval:	Baun, Cassie	09-Oct-2020 02:32:30 PM	
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		

not met, the analysis is not valid since the system was not stable. Contact a supervisor to review data immediately.

2.9. Integrate with area threshold at 1000, noise threshold at 10, and bunching factor at 1.

2.10. Calculate assay using the following equation:

$$\text{Result} = (r_u/r_s)/(C_s/C_u)(100)$$

2.10.1. Where:

2.10.1.1. r_u= peak response from Sample Solution

2.10.1.2. r_s= peak response from Standard Solution

2.10.1.3. C_u= peak response from Sample Solution (mg/mL) * Purity of CRS

2.10.1.4. C_s= peak response from Standard Solution (mg/mL)

3. IDENTIFICATION (IR) Passes Test:

3.1. Follow Spectrum Two UATR SOP utilizing the Loss on Drying sample.

3.2. For UATR analysis, follow Spectrum Two UATR SOP for Instrument Set-Up and Use.

3.2.1. Perform a background scan prior to use each day and after every ten samples.

3.2.2. Each analyst must run a Reference Standard prior to analyzing a product. A Reference Standard may be compared to multiple lots of the corresponding product on that day.

3.2.3. Enter the Lot Number, Expiration Date, Date of Analysis, and Analyst Initials in the Sample ID.

3.2.4. Place the Sample on the UATR crystal using a static free scoop.

3.2.5. Align the swinging arm with the crystal and apply force by turning the green arm clockwise.

3.2.6. Press “Scan” on the top Toolbar. The program will preview the sample. Turn the green arm until the Force Gauge is approximately 125, or until the noise has subsided.

3.2.7. Once the Force Gauge is adjusted, press “Scan”.

3.2.8. Once the scan is complete, release the swinging arm by turning it counterclockwise.

3.2.9. Clean the UATR crystal and the swinging arm with methanol and a Kim Wipe.

3.2.10. If the correlation is above 0.95. the comparison will be reported with Pass as the result.

4. LOSS ON DRYING Monitor:

4.1. Dry a Loss on Drying (LOD) vial in the oven at 105 ± 2˚C for 30 minutes.

4.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.

4.3. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2mm by quickly crushing.

4.4. Transfer approximately 1- 2g of the sample to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.

4.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.

4.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.

4.7. Reweigh the LOD vial and sample and retain the dried sample to perform the IR.

4.8. Calculate the %LOD as follows:

$$\%LOD = \frac{[\text{initial sample weight (g)} - \text{final sample weight (g)}]}{\text{initial sample weight (g)}} x\ 100$$

Printed On:	12-Oct-2020 09:05:44 AM	Ledergerber, Alissa	: Printed By
Authored By:	Hamelburg, Crystal	09-Oct-2020 10:00:11 AM	
Approval:	Baun, Cassie	09-Oct-2020 02:32:30 PM	
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		

5. REACTION **Passes Test:**

5.1. The 1% solution prepared for solubility must be neutral to faintly basic (light blue) when tested with red litmus paper.

5.2. If an ambiguous or neutral result is obtained, the pH of the solution may be taken in comparison to the purified water used for analysis. If the sample is more acidic than the water, an investigation must be initiated.

6. SOLUBILITY **Passes Test:**

6.1. Weigh 1 g of sample and dissolve in 99 mL of boiling water.

6.2. In an area with sufficient lighting, view the sample from all sides.

6.3. The solution must be clear or faintly hazy with no more than a light yellow color in order to pass test.

7. TAMC **Refer to Certificate of Analysis:**

7.1. Microbial analysis will be performed by an approved Outside Testing Laboratory.

7.1.1. Total Aerobic Microbial Count (TAMC)

Printed On:	12-Oct-2020 09:05:44 AM	Ledergerber, Alissa	: Printed By
Authored By:	Hamelburg, Crystal	09-Oct-2020 10:00:11 AM	
Approval:	Baun, Cassie	09-Oct-2020 02:32:30 PM	
Notice:	CONFIDENTIAL: The information contained herein is the property of BioSpectra. The recipient is responsible for its safe-keeping and the prevention of unauthorized appropriation, use, disclosure and copying.		