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ANALYTICAL METHOD VALIDATION REPORT: URACIL ASSAY VIA HPLC

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1. PURPOSE:

1.1. The purpose of this report is to:
- 1.1.1. Show that the Uracil Assay method on HPLC is adequately evaluated and validated.
- 1.1.2. Provide Validation that the Uracil assay procedure meets all requirements for:
  - 1.1.2.1. System Suitability
  - 1.1.2.2. Accuracy
  - 1.1.2.3. Precision
  - 1.1.2.4. Specificity
  - 1.1.2.5. Linearity
  - 1.1.2.6. Range

2. SCOPE:

2.1. This Analytical Method Validation report applies to the Uracil Assay procedure via HPLC.
2.2. The Assay method will be considered validated as a category I quantitative test.
- 2.2.1. The Analytical Method Validation Master Plan dictates that this report will include assessment and conclusive statements of validation on the following: System Suitability, Accuracy, Precision, Specificity, Linearity, and Range.

3. RESPONSIBILITIES:

3.1. The Executive Director of Quality Control (QC) is responsible for the draft and maintenance of this report.
3.2. The analysts who executed the validation protocol, with help and training from the Director of QC and/or the QC Manager, if necessary, are responsible for completing the Method Validation Report using conclusions made from the results obtained from testing.

4. REFERENCES:

4.1. Analytical Methods Validation Master Plan
4.2. Balance SOP
4.3. Perkin Elmer Flexar HPLC SOP
4.4. Pipette SOP
4.5. European Pharmacopeia Method 1215
4.6. Laboratory Notebooks
4.7. Analytical Method Validation Protocol: Uracil Assay via HPLC
4.8. USP <1225> Validation of Compendial Procedures
4.9. USP <1226> Validation of Compendial Procedures

5. EQUIPMENT:

5.1. Equipment
- 5.1.1. All equipment used in this validation was in proper working order and within calibration.

5.2. Personnel
- 5.2.1. All personnel were properly trained in accordance with the Analytical Methods Validation Master Plan.

5.3. Supplies
- 5.3.1. All supplies used in the validation were clean and appropriate for their intended use.

5.4. Reagents
- 5.4.1. All reagents were current and suitable for their intended use.

5.5. Reference Standards

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5.5.1. All standards that were used in this Validation were current and are listed in Section 6: Materials and Equipment.

5.6. Method

5.6.1. See Table 1 below for the instrument method on the HPLC.

TABLE 1: INSTRUMENT METHOD PARAMETERS

Parameter	Setting
Flow Type	Isocratic
Mobile Phase	0.68% KH_2PO_4
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Detector	UV at 266nm λ
Detector Temperature	Ambient
Column Temperature	Ambient
Run Time	5 minutes

6. MATERIALS AND EQUIPMENT:

6.1. Instrumentation and Equipment

6.1.1. Analytical Balance

6.1.1.1. Manufacturer: Secura

6.1.1.2. Model: 124-1S

6.1.1.3. Serial Number: 29212172

6.1.1.4. Last Serviced: 10/19

6.1.1.5. Next Service: 4/20

6.1.2. Micropipette

6.1.2.1. Manufacturer: Eppendorf

6.1.2.2. Model: Research Plus (0.5-5mL)

6.1.2.3. Serial Number: I45595H

6.1.2.4. Last Serviced: 1/20

6.1.2.5. Next Service: 7/20

6.1.3. Micropipette

6.1.3.1. Manufacturer: Eppendorf

6.1.3.2. Model: Research Plus (100-1000µL)

6.1.3.3. Serial Number: O39512B

6.1.3.4. Last Serviced: 1/20

6.1.3.5. Next Service: 7/20

6.1.4. HPLC

6.1.4.1. Manufacturer: Perkin Elmer

6.1.4.2. Model: Flexar

6.1.4.3. Serial Numbers:

6.1.4.3.1. Solvent Manager: 260S13111110F

6.1.4.3.2. Autosampler: 293H3080804A

6.1.4.3.3. Binary Pump: 291S13111109F

6.1.4.3.4. UV Detector: 292S14031703F

6.1.4.4. Last Serviced: 8/19

6.1.4.5. Next Service: 8/20

6.1.5. Analytical Column

6.1.5.1. Luna 5µ C18(2) 100A, 150 x 3.9mm

6.1.5.2. Manufacturer: Phenomenex
6.1.5.3. Part Number: 00F-4252-C0
6.1.5.4. Serial Number: 760356-1

6.2. Reagents
6.2.1. Purified Water
6.2.1.1. Supplier: BioSpectra, Inc.
6.2.1.2. Lot Number: D10DI01-04070
6.2.1.3. Expiration Date: Not applicable.
6.2.2. Uracil
6.2.2.1. Supplier: Acros (99.9% Purity)
6.2.2.2. Part Number: 157300250
6.2.2.3. Lot Number: A0385948
6.2.2.4. Expiration Date: 12/31/21
6.2.2.5. Open Date: 5/21/19
6.2.3. Potassium Phosphate, Monobasic
6.2.3.1. Supplier: J.T. Baker
6.2.3.2. Part Number: 3246-01
6.2.3.3. Lot Number: 0000096815
6.2.3.4. Expiration Date: 10/11/21
6.2.3.5. Open Date: 2/19/15

6.3. Reference Standards
6.3.1. Uracil Certified Reference Material (CRS)
6.3.1.1. Supplier: Sigma Aldrich (99.6% Purity)
6.3.1.2. Part Number: PHR1581-100MG
6.3.1.3. Lot Number: LRAC4849
6.3.1.4. Expiration Date: 1/31/24
6.3.1.5. Open Date: 4/7/20

6.4. Supplies
6.4.1. Micropipette Tips
6.4.2. Polypropylene weigh boats

7. PROCEDURE:

7.1. Solution Preparation:
7.1.1. Assay Standard Solution (~50µg/mL Uracil CRS)
7.1.1.1. Prepared a ~50µg/mL solution of uracil CRS in mobile phase.
7.1.1.2. Solution was scaled up as per section 7.1.1 in DCN: 20-003199.
7.1.2. Assay Sample Solution (~50µg/mL Uracil)
7.1.2.1. Prepared a ~50µg/mL solution of uracil sample in mobile phase.
7.1.2.2. Solution was scaled up as per section 7.1.1 in DCN: 20-003199.
7.1.3. System Suitability Solution
7.1.3.1. Refer to Assay Standard Solution
7.1.4. Specificity Solutions:
7.1.4.1. Refer to mobile phase.
7.1.5. Accuracy/Precision/Linearity/Range Samples: Prepared the following concentration of uracil samples for performance analysis.

TABLE 2: ASSAY PERFORMANCE SAMPLES

Concentration Level	Actual Prepared Concentration Level
5.0µg/mL	5.0 µg/mL
25µg/mL	25.2 µg/mL
40µg/mL	40.2 µg/mL
50µg/mL	50.0 µg/mL
60µg/mL	60.1 µg/mL

7.2. Setting up the instrument

7.2.1. Set up Perkin Elmer Flexar HPLC using method parameters specified in section 5.6.

7.3. Processing chromatograms:

7.3.1. Enable Peak Detection

7.3.2. Area Threshold = 1000

7.3.3. Noise Threshold = 10

7.3.4. Bunching Factor = 1[1]

[1]Refer to conclusion regarding optimized integration parameters.

7.4. Calculations:

7.4.1. Assay:

7.4.1.1. Result = $(r_u/r_s)(C_s/C_u)(100)$

7.4.1.2. Where:

7.4.1.2.1. r_u= peak response from the *Sample Solution*

7.4.1.2.2. r_s= Peak response from the *Standard Solution*

7.4.1.2.3. C_s= Concentration of Uracil RS in the standard solution (mg/mL prepared * Purity of CRS)

7.4.1.2.4. C_u= concentration of Uracil in the *Sample Solution* (mg/mL)

8. PERFORMANCE REPORT:

8.1. System Suitability: Assay

8.1.1. Injected the Assay Standard Solution five times.

8.1.1.1. Acceptance Criteria:

8.1.1.1.1. Relative Standard Deviation: NMT 0.73%.

8.1.1.1.2. Result: Pass

System Suitability Data					
Replicate	**Retention Time (min)**	**Area Count**	**Average**	**% RSD (NMT 0.73%)**	**Result**
1	1.483	1784448.40	1783680.72	0.23	Pass
2	1.482	1776594.40			
3	1.481	1786088.80			
4	1.482	1784404.80			
5	1.481	1786867.20			

8.2. Linearity: Assay

8.2.1. Injected the 10%, 50% Level, 80% Level, 100% Level, and 120% Level

8.2.1.1. Acceptance Criteria:

8.2.1.1.1. Report the y-intercept, slope, and residual sum of squares.

8.2.1.1.2. Correlation coefficient ≥ 0.995

8.2.1.1.3. Result: Pass

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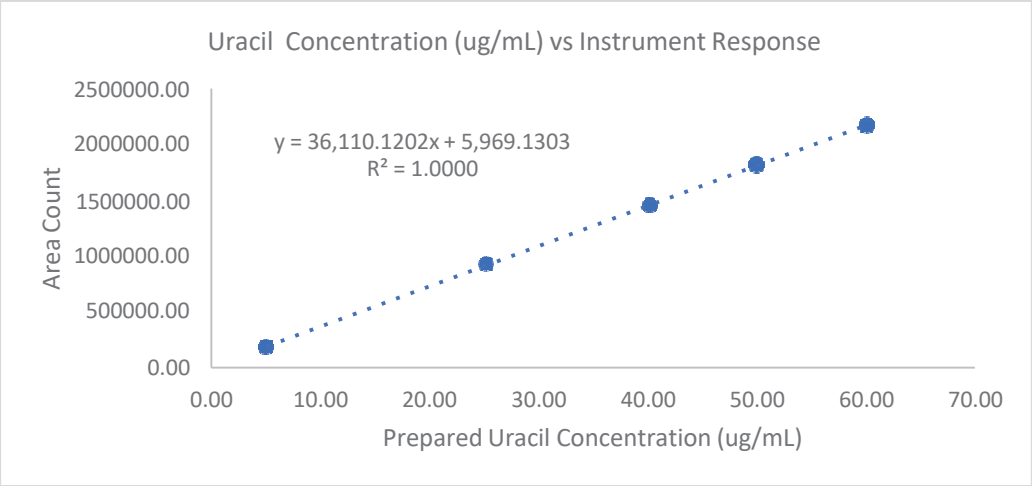
8.2.1.1.4. Slope: 36,110

8.2.1.1.5. Residual Sum of Squares: 353794115.3



8.3. Accuracy and Precision: Assay

8.3.1. Injected the 10%, 80% Level and 120% Level in triplicate. Injected the 100% Level six times.

8.3.1.1. Acceptance Criteria

8.3.1.1.1. Percent Recovery: 97.0-102.0% at each level

8.3.1.1.2. Relative Standard Deviation: NMT 0.73% at each level

8.3.1.1.3. Result: Pass

Sample ID	Prepared Concentration (µg/mL) (ppm)	% Recovery (97.0-102.0%)	% RSD (NMT 0.73%)
10% Replicate 1	5.02	100.8	0.51
10% Replicate 2	5.02	100.5	
10% Replicate 3	5.02	101.5	
80% Replicate 1	40.15	99.8	0.00
80% Replicate 2	40.15	99.8	
80% Replicate 3	40.15	99.8	
100% Replicate 1	50.00	99.7	0.18
100% Replicate 2	50.00	99.9	
100% Replicate 3	50.00	99.8	
100% Replicate 4	50.00	100.0	
100% Replicate 5	50.00	100.1	
100% Replicate 6	50.00	99.7	
120% Replicate 1	60.05	99.5	0.19
120% Replicate 2	60.05	99.6	
120% Replicate 3	60.05	99.3	

8.4. Range: Assay

8.4.1. The range of an analytical procedure is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with a suitable level of precision, accuracy, and linearity using the procedure as written.

8.4.2. The quantitative range of the method is 5µg/mL (ppm) to 60µg/mL (ppm) of uracil in mobile phase. Samples should be diluted to the working range of the instrumental method.

8.5. Specificity: Assay

8.5.1. A blank (mobile phase) was analyzed three times in order to demonstrate that the matrix does not interfere at the retention time of the analyte.

8.5.2. Acceptance Criteria: Diluents and reagents should not interfere with peaks of interest.

8.5.3. Result: Pass, diluents and reagents do not interfere with peaks of interest; no peaks detected.

8.6. Conclusion: Uracil Assay Method Validation

8.6.1. In conclusion, the Uracil Assay method via HPLC has been adequately evaluated and validated. This method meets all requirements for System suitability, Accuracy, Precision, Specificity, Linearity, and Range. This method is now considered a validated category I quantitative test. The method may be used as a limit-based test for trace detection uracil based on the fact that the method meets specificity requirements. Limit of detection of applicable limit standard must provide a signal to noise ration of at least 3:1 to meet ICH Q2 (R1) requirements for impurity testing.

8.6.2. Critical Changes to Method Validation Protocol:

8.6.2.1. In order to optimize integration of the uracil peak, the bunching factor was changed from 5 to 1 in the TotalChrom software. This update was documented in the audit log of the instrument software and the method file.

8.6.2.2. The standard preparation was scaled to match the 100% level dictated in the validation protocol. At the 80% level the method met all requirements so both standards and samples are validated to be analyzed from 40-60µg/mL after appropriate dilution.