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Water Determination by Karl Fischer

Karl Fischer titration has become one of the most widely used techniques for the determination of water content in a variety of substances. Many pharmaceutical products contain water in adsorbed form, and as a result, the determination of the water content is important in demonstrating compliance with the regulatory standards.

Eurofins BioPharma Product Testing offers significant capacity using the latest Karl Fischer technology, which offers analytical advantages. In particular, the Karl Fischer Oven Determination has become our platform approach for new moisture testing methods.

Why Choose Eurofins BioPharma Product Testing?

- We have more than 15 years of experience developing and executing methods for water determination by Karl Fischer analyses.
- We have completed a significant amount of water determination testing using multiple types of instrumentation.
- We offer quick turnaround time on method development and validation for Karl Fischer testing (in as little as two weeks).
- All Testing is performed in humidity controlled laboratories, eliminating moisture contaminations from the ambient environment.

Key Factors

A critical step in determining the most appropriate analytical approach is to address the following key factors:

- Expected water content of the material
- The amount of material available for analysis
- Thermodynamic properties of the material
- Material solubility information
- Chemical structure of the compound/material

Approaches

Karl Fischer Oven Determination

- Material is required to be thermodynamically stable.
- Requires about 15 to 50 mg of material.
- Avoids contamination of the oven and titration cell: no carry-over effects.
- Significantly reduces sample preparation steps.
- Significantly enhances sample throughput, accuracy and repeatability of results.

Volumetric Karl Fischer Determination

- Best suited for materials with at least 1% water and the test article should contain 2 to 250 mg of water.
- Karl Fischer reagents contain the sulfur dioxide, iodine, base and solvent necessary for USP <921> direct titration (Method 1a). The apparatus is designed for accurate determination of moisture content.
- The endpoint is determined potentiometrically, and the titration burette is controlled by a solenoid-operated valve, which closes when the endpoint is reached.

Coulometric Karl Fischer Determination

- Best suited for materials with less than 1% water or projects with limited sample availability.
- The test article should contain 0.5 to 5 mg of water.
- A sample extraction procedure is preferred.
- The same reaction used for volumetric KF analysis is used for coulometric KF analysis; however, rather than adding a volumetric solution, iodine for the reaction is produced by anodic oxidation in an iodide-containing solution within the titration vessel.
- When all the water in the test article has been consumed, an excess of iodine occurs, which is detected electrochemically, thus indicating the endpoint.



Development and Validation Approach

The following table provides our general development and validation approach for all three types of water determination instrumentation.

Instrument Type	Determined During Method Development	Method Validation
Karl Fischer Oven	· Optimal Temperature (Using Temperature Gradient) · Appropriate Sample Amount	· Precision (n=6) · Intermediate Precision (n=6) · Robustness (vary sample amount) · Accuracy and specificity (performing an accuracy check of water reference standard to verify instrument is running properly and that sample does not interfere with KF reagents)
Volumetric Karl Fischer or Coulometric Karl Fischer	· Appropriate Sample Amount · Extraction Solvent · Extraction Time · Instrument Parameters	· Precision (n=6) · Intermediate Precision (n=6) · Robustness (vary sample amount) · Accuracy and specificity (performing an accuracy check of water reference standard to verify instrument is running properly and that sample does not interfere with KF reagents)

Other Considerations for Method Validation

Specificity

- Spiked accuracy experiments not recommended due to limitations of accuracy/technique with spiking.

Quantitation Limit (QL)

- A reporting limit (not a validated QL) is typically set at 0.1%. Any established QL would be dependent upon the actual moisture content of the material available for the method validation, and the experiment would be subject to changes to the preparation that are not truly representative of the method (e.g., minimized amount of sample analyzed). For these reasons, we omit experimental determination of the quantitation limit from the method validation.

Linearity and Range

- For volumetric Karl Fischer titration, linear titration is a function of the instrument performance, rather than the sample analysis method, and therefore linearity experiments are deemed un-necessary. For coulometric Karl Fischer titration, the qualitative relationship between the current and iodine allows for the absolute dosing of iodine, and therefore linearity experiments are deemed unnecessary. A semi-annual instrument performance qualification is performed, which includes instrument accuracy, precision and linearity.
- A robustness study may replace a method linearity and range experiment, which challenges sample amount or extraction and injection volumes. The robustness study is generally designed such that 50%-150% of the nominal sample amount is titrated.