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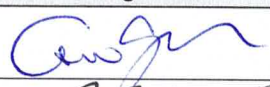
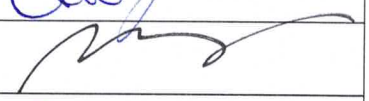
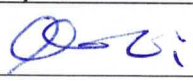
Owner	**Sijin Guo**	**20March2025**	
Operation Management	**Baozhong Zhao**	**20March2025**	
Quality Assurance	**Xibo Li**	**20March2025**	

Changes from previous version:

Section	Summary of Changes	Change Control Number
ALL	1. New document	

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1. PURPOSE

The purpose of this document is to describe the calibration, use and maintenance of the Metrohm Karl- Fisher Volumetric Titration System.

2. SCOPE

The Metrohm 874/852/803 coulometric oven Karl Fischer moisture determination apparatus is used for measuring water content in samples, including those unsuitable for conventional moisture analysis. An oven is used to extract the moisture from the sample, sealed in a gas-tight vial, prior to evacuation of the vial headspace via a heated transfer line into the reaction vessel of a coulometric Karl Fischer apparatus. In principle, 100% of the moisture contained in the sample vial is extracted and introduced into the reaction cell, therefore in theory samples with water content from extremely low levels (ppm) up to samples which would be more conventionally suited to e.g. titrimetric Karl Fischer (~5%) may be analyzed using this apparatus.

Certain types of samples are unsuitable for moisture determination using conventional Karl Fischer techniques e.g., titrimetric. These samples include, but are not limited to, reaction intermediates containing contaminating material which may interfere with the chemistry in the reaction vessel, or hygroscopic materials which would take up water during sample handling outside of the instrument e.g., lyophilized products. The Metrohm 874/852 apparatus can also be used to analyze conventional samples.

3. INTERNAL REFERENCES

Document ID	Title

4. EXTERNAL REFERENCES

Document ID	Title
ICH Q7 (API)	Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients
ICH Q9	Quality Risk Management
ICH 010	Pharmaceutical Quality System

5. RESPONSIBILITIES

Job Function and/or Department	Responsibility
Operational Employees	Analysts generating results from the Metrohm Karl Fisher Titration System are responsible for adhering to this procedure, as well as complete documentation in the associated lo book.
Quality Assurance	Responsible for the maintenance of the SOP template, processing and maintaining SOPs. Quality Assurance is also responsible for ensuring that current SOPs adhere to this procedure and are approved by the appropriate organizational unit(s} and reviewed and approved by Quality Assurance.

6. DEFINITION

Term	Definition
Coulometric	A technique in analytical chemistry that determines the amount of matter transformed during an electrolysis reaction by measuring the amount of electricity (in coulombs) consumed or produced.
Hygroscopic	A substance that readily attracts water from its surroundings, through either absorption or adsorption.
SOP	Abbreviation for Standard Operating Procedure, provides detailed high-level direction on performing a specific task.
Titration	Also known as titrimetric, it is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of an identified analyte.

7. PROCEDURE

7.1. Prior to use

7.1.1. Ensure the molecular sieve is dry.

7.1.2. Ensure the gas supply is on and supplying constant pressure as required in the instrument manual.

7.1.3. Ensure the reagent in the reaction cell is clean and clear.

7.1.4. If the material on test has a specific SOP, proceed according to the operating parameters defined e.g., recommended sample weights, oven temperature, extraction time etc.

7.1.5. In the case of samples for which no operating parameters have been supplied or determined it may be necessary to carry out analytical validation to establish the suitability of the technique to effectively measure the moisture content of the samples. It can be assumed that 100% of water in a sample vial is transferred into the reaction cell and the only limitation on sample size is a) the physical size of the vials being used and b) the time required to titrate very high levels of water. Therefore, if no sample weight is established, it may be possible to use the expected water content (e.g. from API/product specification) to deduce a sample amount to place in the vial: low water content will need more sample and vice versa.

7.2. Blank/Sample/Standard Preparation

An example sequence is outlined below. The number of samples placed between standard vials may vary from customer to customer and material to material. If no guidance is available, the following considerations should be applied.

7.2.1. Standards are used to determine the precision of the instrument i.e. they are performance checks as opposed to being used to set a parameter in the instrument, therefore if a standard fails, not within +/- 0.2% of CofA for water content, any samples analyzed in the bracket immediately before and after it must be discarded as the instrument cannot be said to have been operating suitably. It is recommended that no more than four samples are analyzed between standards, but this is a guideline only.

7.2.2. It may be possible for a standard in the middle of a sequence to fail and subsequent standards pass. If this is the case, any samples which are bracketed by passing standards determinations may be deemed to be suitable for use.

7.2.3. Since a failing standard is an indication of poor system performance, any samples bracketed by failing standards can be considered invalid and the data disregarded from any statistics. Such samples must be repeated.

7.2.4. If standards are observed to fail regularly it may be necessary to replace the reagent and/or dry the molecular sieve. This should be carried out by an instrument super user. See 7.5 for instructions on how to carry out this basic maintenance.

7.2.5. Vial 36 requires an empty, capped and crimped vial for system conditioning and is not used for data acquisition.

TABLE 1: EXAMPLE OVEN KF SEQUENCE

System Preparation Vial
Blank x 3
Standard x 3
Samples
Standard
Samples
Standard

7.3. Sample Analysis

7.3.1. Once standards, blanks and samples have been vialed, capped, crimped and placed into the sample carousel, use the Metrohm Tiamo software to generate an analytical sequence including vial designation (blank/standard, etc.), sample name, sample mass, oven temperature and instrument method etc.

7.3.2. The instrument and software will then automatically heat the vials, transfer the water to the reaction vessel and perform the titration to the end point before calculating the amount of water present in the standards and samples. The blank determination is used to set a value for any particular sequence and is stored along with the rest of the titration data on the software database.

7.3.3. It is recommended that samples are analyzed in duplicate where possible. Due to the nature of the analysis, variability between samples is expected. No criteria for data acceptability are defined but may be determined specifically for a material by validation.

7.3.4. Reports can be printed as required from the Tiamo software.

7.4. Post Run

7.4.1. Ensure that vials are removed from the carousel, the instrument components are powered down and the gas supply line is turned off.

7.4.2. Ensure that vials are removed from the carousel, the instrument components are powered down and the gas supply line is turned off.

7.5. Maintenance

This equipment is exempt from regular maintenance visits as performance is verified every time the instrument is used. The following activities are considered to be routine tasks which can be carried out in-house.

If necessary, the molecular sieve and reagent can be changed. The reagent need not be changed if the molecular sieve needs changing, but this is advisable since opening the vessel introduces water into the reagent. Follow the instructions below to complete the change of both reagent and molecular sieve. Omit any steps relating to reagent change to simply change molecular sieve.

7.5.1. Ensure the components are switched off prior to carrying out any maintenance.

7.5.2. Remove the central tube from the cathode chamber in the reaction vessel, remove the stopper and transfer the molecular sieve into a suitable dish for drying. The two jars containing molecular sieve can be removed and unscrewed to allow transfer of the molecular sieve within into a suitable vessel for drying. Molecular sieve should be dried at >120°C for at least one hour and allowed to cool (protected from moisture) before refilling and reassembling the apparatus.

7.5.3. If the reagent is also being changed, remove the detection electrode, transfer line and cathode chamber from the reaction vessel.

7.5.4. Care must be taken with each of the parts of the reaction vessel, especially the cathode chamber which has a platinum mesh electrode on the bottom and should always be stored on its side to prevent damage to the mesh.

7.5.5. Empty the oven KF reagent into a suitable waste container and clean all of the parts with methanol and tissue. Allow the parts to dry before reassembling.

7.5.6. Apply a small amount of silicone grease to any ground glass surfaces to ensure a good seal and prevent moisture ingress into the vessel.

7.5.7. Add oven KF reagent into the cathode chamber to a level sufficient to submerge the transfer line tip and all electrodes (approximately 3 to 4cm).

7.5.8. Once reagent has been added, replace the drying tube into the cathode chamber and ensure all joints are greased and airtight.