NCL Method PCC-24

Determination of Water Content using the Karl Fischer Coulometric Method

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1. Introduction

The Karl Fischer (KF) titration method utilizes volumetric or coulometric titration to quantify trace amounts of moisture in a sample [1]. The coulometric method is primarily used for determining small amounts of water in various samples such as drug substances, drug products, and organic liquids [2–3]; the Metrohm KF Coulometers work in a determination range of 10 µg–200 mg water [4].

In coulometric titration, iodine is generated from an electrochemical reaction. When an electric current is applied to the anode in the titration cell, iodine is produced *in situ* and immediately reacts with the water present in the sample. The amount of iodine generated is proportional to the charge passed through the solution, as described by Faraday's Law, and can be used to calculate the water content of the sample using Equation 1:

$$m = \frac{M \times Q}{Z \times F} \qquad \text{Eq. 1}$$

where,

m = mass of converted substance in grams

M = molar mass in g/mol (iodine, I_2, 253.81 g/mol)

Q = measured amount of charge in A*s (ampere-second)

Z = number of exchanged electrons (equivalence number, charge number)

F = Faraday constant, 1 F = 96,485 coulomb/mol

The chemical reactions for iodine are:

$$2I^- - 2e^- \rightarrow I_2 \qquad \text{Eq. 2}$$

$$H_2O + I_2 + 3C_5H_5N \rightarrow 2C_5C_5N\cdot HI + C_5H_5N \qquad \text{Eq. 3}$$

The current releases a stoichiometrically corresponding amount of iodine from the iodide-containing reagent (e.g.,126.9 g iodine is released by 96,485 A in 1 s). This process must take place with 100% current efficiency with no side reactions. Since the 917 KF coulometer instrument works according to the galvanostatic (with constant current) principle, these requirements are met with commercially available KF reagents [4].

2. Reagents, Materials, and Equipment

Note: The NCL does not endorse any of the suppliers listed below; these reagents were used in the development of the protocol, and their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be substituted. Please note that suppliers may undergo a name change due to a variety of factors. Brands and part numbers typically remain consistent but may also change over time.

2.1 Reagents
  2.1.1 Hydranal™ Water Reference Standard 1.0 (Honeywell, Fluka or equivalent)
  2.1.2 Hydranal™ Coulomat AG KF Reagent (Honeywell, Fluka or equivalent)
2.2 Equipment and Materials
  2.2.1 917 KF Coulometer with Q3X Printer
  2.2.2 Analytical balance with 0.000x g accuracy
  2.2.3 Gas tight syringes (Hamilton or similar)
  2.2.4 Vortex

3. 917 KF System Preparation

3.1 **Instrument setup:** Perform necessary checks to verify reagents' integrity and titration performance. Set up the titration vessel with appropriate accessories, electrodes, and stirring apparatus. Ensure all connections are secure and free from leaks to prevent moisture ingress during titration. Turn on the instrument, allow

10–15 minutes for equilibration, and finish instrument set up using parameters listed in Table 1.

Table 1.Method Parameter for a Coulometric KF Titration

Instrument Parameters	*Setting*
Titration Rate	optimal
Time Interval MP	2 s
Temperature	25°C
Start Drift	20 µg/min
Drift Correction	Auto
Generator Electrode	without diaphragm
Generator Current	400 mA
I(pol)	10 A

3.2 **Conditioning and Drift:** Conditioning removes water contained in the reagent and on the surfaces of the equipment. The water content determination of the sample should only be started once a low and stable drift is reached; a constant drift equal to, or lower than, 10 µg/min is acceptable. Important: the titration cell must be dried before the measurements can be started.

3.3 **Calibration of 917 KF Coulometer:** Coulometric titration does not require standardization because the titrant is generated electrochemically *in situ* during the titration process. The generation of titrant eliminates the need for a standardization process, as the titrant concentration is already known and does not need to be adjusted. However, the instrument's suitability as a fully integrated measuring system should be verified using certified commercial Hydranal™ water standard solutions with a water content of 1.00 ± 0.006 mg/g and/or 0.10 ± 0.005 mg/g (acceptance criteria: %RSD < 2.0%, n=3). The recommended amount for the 1.0 mg/g standard is 0.2–2.0 g and 0.5–5.0 g for the 0.1 mg/g standard.

The following steps are recommended for the calibration of the 917 KF coulometer.

3.3.1 Open the ampoule containing the standard as recommended by the manufacturer.

3.3.2 Aspirate approximately 0.5 mL of the standard into the syringe and then eject the standard into the waste.

3.3.3 Aspirate the remaining content of the ampoule into the needle (eject the air out of the syringe)

3.3.4 Remove excess liquid from the outside of the needle with Kimwipes.

3.3.5 Place the needle on a balance and tare the balance. Then start the determination and inject a suitable amount of standard through the septum into the titration vessel (**Note:** Do not inject the whole content of the syringe. The standard should be injected into the reagent not at the electrode or the inner wall of the titration vessel. This leads to unreproducible results).

3.3.6 After injecting the standard, place the syringe again on the balance and enter the injected sample weight in the instrument method software.

3.3.7 Repeat steps 3.3.4 to 3.3.6 at least three times. If the complete content of an ampoule has been injected, the needle can be filled with fresh standard (same batch/lot). In this case, the needle does not need to be rinsed again. Note that there are two possibilities to add liquid standard. It can be injected with the tip of the needle above the reagent level, in which case the last drop must be aspirated back into the syringe, otherwise, it is wiped off at the septum and might not be determined despite the weight taken into account. Alternatively, if the needle is long enough, it can be immersed in the reagent directly; in this case there is no last drop, and the needle can be pulled out of the titration vessel without aspirating back any liquid.

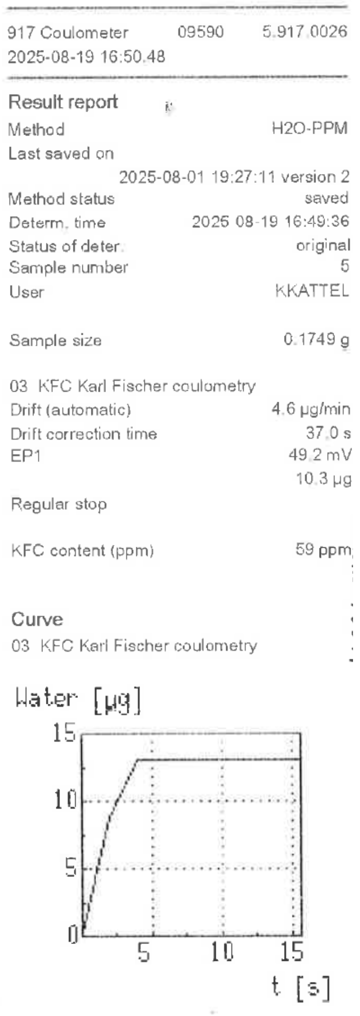
4. Sample Measurement

4.1 **Sample Addition and Sample Size:** Note, the coulometric titration cell should never be opened to add liquid test samples. The influence of humid air entering the titration cell would alter the results. Liquid samples are vortexed and added with the aid of a gas-tight syringe. Solid test samples, however, can be added directly into the titration vessel. A blank value correction should be made for the samples. Sample sizes can be estimated from Table 2.

Table 2. Appropriate sample sizes are provided for various estimated water contents.

Water Content of Sample	*Sample Size*	*Water* **Content** *Determined*
50,000 ppm = 5%	25 mg	2,500 µg
10,000 ppm = 1%	10–100 mg	100–1,000 µg
1,000 ppm = 0.1%	0.1–1 g	100–1,000 µg
100 ppm = 0.01%	1 g	100 µg
10 ppm = 0.001%	5 g	50 µg

4.2 **KF Titration**: Weigh the test sample. Once the drift reaches < 20 µg/min, press the enter key and add the test sample into the titration vessel. The KF reading will be displayed. Enter the exact sample weight (in grams) taken for analysis in the displayed box and press the ‘continue’ button to initiate the titration. The results will be saved and printed automatically once the end point is reached. Please see Figure 1 for an example read-out from the instrument.



```
917 Coulometer        09590      5.917.0026
2025-08-19 16:50.48

Result report
Method                             H2O-PPM
Last saved on
                2025-08-01 19:27:11 version 2
Method status                        saved
Determ. time           2025 08-19 16:49:36
Status of deter.                  original
Sample number                            5
User                                KKATTEL

Sample size                       0.1749 g

03  KFC Karl Fischer coulometry
Drift (automatic)               4.6 µg/min
Drift correction time               37.0 s
EP1                                49.2 mV
                                   10.3 µg
Regular stop

KFC content (ppm)                   59 ppm

Curve
03  KFC Karl Fischer coulometry
```

Figure 1. 917 KF coulometer output for water content analysis of in-use HPLC grade acetonitrile, presented in ppm.

5. Exchange of KF Reagent, Cleaning and Maintenance of Titration Vessel, and Troubleshooting

5.1 The electrolyte solutions should be exchanged and/or titration vessel cleaned under the following conditions:

- Contamination of KF reagent
- When the titration vessel is too full
- When the capacity of the reagent is exhausted
- If the drift is too high, and shaking the cell does not result in any improvement

- If a two-phase mixture is formed in the titration vessel. In this case, only the sample phase can be aspirated off.
- If, during the measurement, the error message "check generator electr." appears. For the generator electrode with diaphragm, the catholyte should be exchanged approximately once a week. Extended use may cause darkening of the catholyte and yellow precipitation in the cathode compartment. An unpleasant smell also indicates the need for catholyte exchange.

5.2 **Maintenance**: If moisture penetrates into the titration vessel, for example, due to exhausted molecular sieves (replace sieves), septum pierced too many times (replace septum) or seals are not properly done (replace seals)

5.3 **Troubleshooting**:

- If results are widely scattered, this could be due to unstable drift, inhomogeneity of the samples, or poor reproducibility of the sample addition.
- An unstable drift could be due to poor stirring; efficient stirring should be done without forming air bubbles.
- If depots containing water are in the titration vessel, shake the titration vessel to lower the drift.

6. Calculations

Water content in the sample can be reported in terms of ppm (Eq. 4) or % (w/w) (Eq. 5).

$$KFC\ content\ (ppm) = \frac{EP1}{Sample\ weight\ (g)} \quad \text{Eq. 4}$$

$$KFC\ content\ (\%) = \frac{EP1}{Sample\ weight\ (g) x\ 10{,}000} \quad \text{Eq. 5}$$

where, EP1 = water equivalence factor from experiment at end point (µg).

7. References

1. J. Mitchell, Jr. and D.M. Smith, *"Aquametry"- A Treatise on Methods for the Determination of Water*, Part III—The Karl Fischer Reagent. 2nd Ed., J. Wiley and Sons, Inc., New York, NY 1990.
2. USP General Chapter <921>—Water Determination, Method 1C, Coulometric Titration .
3. ASTM International E1064-05, Standard Test Method for Water in Organic Liquids by Coulometric Karl Fischer Titration.
4. Metrohm Application Bulletin 137/6 e, Coulometric Water Content Determination according to Karl Fischer.