

## 1.2 Tecnologie per l'analisi dell'umidità

Esistono quattro tipi di metodi per l'analisi dell'umidità:

- l'analisi termogravimetrica (essiccamento in forno, alogeno/IR, a microonde, ecc.)
- analisi chimica (titolazione Karl Fischer, verifica a carburo di calcio)
- analisi spettroscopica (spettroscopia IR, a microonde, H-NMR)
- alte tipologie di analisi (gascromatografia, determinazione della densità, rifrattometria, ecc.)

Nel selezionare un metodo di analisi è necessario valutare una serie di fattori, come il volume di campione disponibile, il numero di campioni, la velocità di misura, la praticabilità dell'automazione, l'accuratezza o persino le proprietà chimico-fisiche del campione: tutti svolgono un ruolo fondamentale nel processo decisionale.

Campioni e sostanze liquidi o sensibili alla temperatura, che comprendono molti alimenti, devono essere analizzati con attenzione impiegando metodi termogravimetrici. Nel caso della maggior parte delle vitamine, degli enzimi o degli agenti aromatizzanti, i metodi termogravimetrici non possono essere utilizzati.

I solidi insolubili con acqua chimicamente combinata o sistemi di pori distinti, tuttavia, possono essere titolati con il metodo Karl Fischer laddove l'acqua venga resa accessibile, ad esempio utilizzando un omogeneizzatore o un forno per l'evaporazione dell'acqua stessa dal campione.

METTLER TOLEDO offre diverse soluzioni per l'analisi dell'umidità. Sono qui descritti in dettaglio due metodi termogravimetrici e il metodo di titolazione Karl Fischer.

## Determinazione WATER CONTENT

Il contenuto di umidità e quello di acqua figurano tra i parametri più importanti che vengono misurati negli alimenti. Il contenuto di umidità è inversamente correlato alla sostanza secca di un alimento: per questo motivo vi sono effetti economici diretti su consumatori e aziende di lavorazione. Ma soprattutto, il contenuto di umidità influenza anche la stabilità e la qualità dei prodotti. Non stupisce quindi che quello dell'umidità sia un argomento trattato profusamente da leggi e normative.

### Moisture Determination using Regulated Methods

- Many Pharmacopeia materials contain water as hydrates or in adsorbed form.
- The determination of the water content is important to maintaining compliance with the Pharmacopela guidelines.
- The USP may direct you to one of several chapters.
  - USP<731> Method for loss on drying
  - USP<561> articles of Botanical Origin
  - USP <921> offers several alternatives for moisture determination

Metodo analitico convenzionalmente usato nella determinazione della proprietà di interesse (es. titolazione di Karl Fisher nella determinazione della water content)

## Moisture Determination of Regulated Compounds

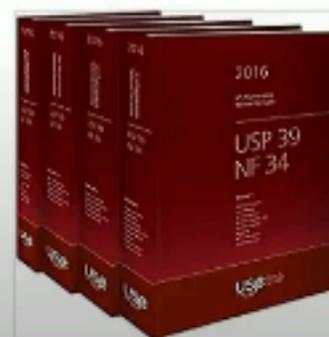
When searching for guidance, go to the USP Monograph(s) for individual item.  
Water determination if required will be listed under the heading Specific Tests

### SPECIFIC TESTS

- WATER DETERMINATION, Method <921>; NMT 2.0% for the anhydrous form; 6.0%–10.0% for the hydrate form.  
[NOTE—"Hydrate" refers to a mixture of dihydrate and trihydrate forms of **fexofenadine** hydrochloride.]

This example is from the **Fexofenadine Hydrochloride** monograph, extracted from the current USP online edition (USP40-NF35).

The information refers to chapter <921> followed by the allowed concentration ranges  
<921> may appear as a link to the specific regulated test method.



## USP <921> Water Determination

Chapter <921> provides the choice of three techniques/methods:

- Method I (Titrimetric)
- Method II (Azeotropic)
- Method III (Gravimetric)

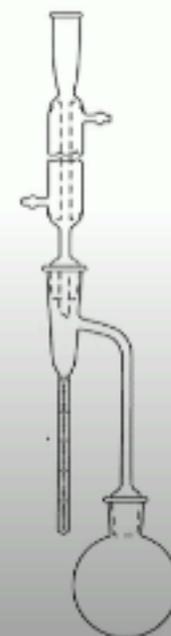
Chapter <921> also mentions loss on drying and refers to USP chapter <731>

This presentation will focus at titrimetry- Method I,  
but first lets briefly look at the other options.

## USP <921> Method II

Method II is the azeotropic distillation of water by toluene

- The Dean and Stark receiver 5 mL capacity and graduated 0.1 mL subdivisions
- Error is approximately 0.05 mL, dependent on glassware
- Test substance is weighed such that 2-4 mL of water will be yielded
- This is a % level determination
- Error is 1-2%



## USP <921> Method III

Method III is a Gravimetric

- Typically performed on a moisture balance
- For Chemicals see individual Monograph and Loss on Drying <731>  
For Biologics see the individual monograph
- Botanicals See Articles of Botanical Origin <561>
- Dry to constant weight
- Readability 1 mg
- Weight loss may not be entirely water if volatile components are present



## USP <921> Method I

Method I means titrimetric determination of water

- Karl Fischer is a titration method.
- The chemistry is specific for water, other volatile components are not measured.



## USP <921> Method I

- Method I titrimetric has 3 sub headings
  - Method Ia - Direct Titration
    - Test specimen is added to the titrator and moisture is directly titrated.
  - Method Ib - Residual Titration (back titration)
    - Test specimen is added to KF reagent unconsumed reagent is titrated with a standard solution of water in solvent.
  - Method Ic - Coulometric Titration
    - Iodine is generated in solution.



## Who and What is a Karl Fischer?

- **Karl Fischer** (24 March 1901 – 16 April 1958)
  - German chemist who published a method to determine trace amounts of water (1935).
- **The method is now called Karl Fischer titration**
  - It was originally performed manually, but today it is automated through instrumentation.



## Sample Requirements

### **Samples must be soluble in the solution**

- May need a co-solvent to increase solubility.
- Or a specialty solvent like CombiSolvent oils or CombiSolvent fats.

### **Sample should not react with the components of the reagent**

- May need a special buffer for strongly acidic or basic samples.

### **You may need to use an evaporator oven in conjunction with the titrator**

- For solids and insoluble samples
- We have reagents suitable for these applications as well.

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## Chemistry of the Karl Fischer Titration

**The KF reaction needs to the following:**

- Alcohol
  - Originally used: Methanol
  - Now used: Methanol, Ethanol, 2-MEO, DEGME, DEGEE, etc.

## Chemistry of the Karl Fischer Titration



Reaction 1 occurs when the reagents are made.



Reaction 2 occurs during the titration.

- Water and iodine are consumed in equimolar amounts in the reaction.
- Potentiometric titration measures the change in electrical conductivity of the solution to determine end point.

So, if you know the amount of  $\text{I}_2$  consumed, you can calculate the amount of water that was present in the sample.

## USP <921> Method Ia

### Direct Volumetric Karl Fischer titration

For samples with water > 1 %

- Common method for analysis
- Dosage of iodine containing titrant
- Potentiometric endpoint indication
- Calculation of water content from titrant consumption



## USP <921> Method 1b

### Indirect Volumetric Karl Fischer titration

- This method less commonly used in KF titration
- Dosage of an XS of iodine containing reagent
- Titrant is a water solution in a solvent for example methanol
- Excess KF reagent is titrated with the water solution
- Potentiometric endpoint indication



## USP <921> Method 1b

### Indirect Volumetric Karl Fischer titration

- This method less commonly used in KF titration
- Dosage of an XS of iodine containing reagent
- Titrant is a water solution in a solvent for example methanol
- Excess KF reagent is titrated with the water solution
- Potentiometric endpoint indication

The residual titration procedure avoids difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly



## USP <921> Method 1b - **disadvantages**

- Indirect method requires additional steps to perform
- KF titrators are not normally operated in this manner
- This method is not usually supported by common KF titration instruments
- Manual calculations may be required leading to possible errors
- Because of this result tracability may be impacted

## USP <921> Method Ic

### Coulometric Karl Fischer Titration

[For samples with water < 1 %](#)

- Iodine generation by anodic oxidation at generator electrode
- Potentiometric endpoint indication
- Water content is calculated from the amount of current used to generate iodine
- Low ppm levels easily and accurately determined



### Karl Fischer Titration – Coulometric Titration <1% H<sub>2</sub>O



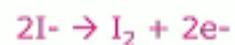
The reaction is the same, the difference is the iodine source.

## Karl Fischer Titration – Coulometric Titration <1% H<sub>2</sub>O



The reaction is the same, the difference is the iodine source.

In coulometric KF, I<sub>2</sub> is generated from the reagent at the anode



The cathode reaction produces H<sub>2</sub> from imidazole



## Karl Fischer (KF) Titration – Coulometric

### Two types of Coulometric KF

#### Cell with Diaphragm (Fritted Cell)

- The cell with the diaphragm uses two solutions, one in the cathode chamber the other in the anode chamber.
- A diaphragm (or frit) separates the anode from the cathode that form the electrolytic cell known as the generator electrode.
- The purpose of the frit is to prevent the iodine generated at the anode from being reduced back to iodide at the cathode instead of reacting with water.

#### Cell without Diaphragm (Fritless Cell)

- Uses one solution that has all the components needed for the reaction

## Example 1 Esomeprazole

Esomeprazole is a proton pump inhibitor and it is the S-enantiomer of omeprazole. It is used in the treatment of dyspepsia, peptic ulcer disease, etc. Common commercial brand names: Nexium, Essocam, Esoomezol

The USP-NF Monograph for Esomeprazole indicates Method <921> I

- **WATER DETERMINATION (921), Method I:** 6.0%–8.0% •for the trihydrate form•(RB 1-Apr-2016)
  - If labeled as dihydrate:** 4.5%–7.0%
  - If labeled as amorphous:** 7.0%–10.0%•(RB 1-Apr-2016)

Method 1 is the volumetric Karl Fischer titration

We have determined water in Esomeprazol using Karl Fischer (KF) titration, and Aquastar® volumetric titrants

## Example 2 - Olmesartan Medoxomil

Olmesartan Medoxomil is an angiotensin II receptor antagonist.  
It is an ester prodrug that is hydrolyzed to the active acid form, Olmesartan.  
It is used to treat high blood pressure.  
Common commercial brand name: Benicar, Olmetec, WinBP, Olsar, Golmeetc.

The USP-NF Monograph for Olmesartan indicates Method <921> Ic

- **WATER DETERMINATION, Method Ic(921):** NMT 0.5%

Method 1c is the coulometric Karl Fischer titration

We have determined water in Olmesartan using Aquastar® coulometric titrants 1.09257 and 1.09255

## Example 2 – Olmesartan Medoxomil

Note that in the monograph for Olmesartan there is a limit for acetone

- **LIMIT OF ACETONE (IF PRESENT)**

**Internal standard solution:** 1% solution of 1-butanol in dimethyl sulfoxide. [NOTE—This solution is stable for 1 month at room temperature.]

**Standard solution:** 0.37  $\mu\text{L}/\text{mL}$  of acetone and 2  $\mu\text{L}/\text{mL}$  of 1-butanol from the *Internal standard solution* in dimethylsulfoxide. [NOTE—This solution is stable for 8 h at room temperature.]

**Sample solution:** 25 mg/mL of **Olmesartan** Medoxomil and 2  $\mu\text{L}/\text{mL}$  of 1-butanol from the *Internal standard solution* in dimethylsulfoxide. [NOTE—This solution is stable for 8 h at room temperature.]

If present, acetone will interfere with KF titrations and lead to erroneously high results

When acetone is present consider using a Coulometric instrument with a frit (diaphragm) and use Aquastar<sup>®</sup> Coulomat AK, AX1699E in the anode and Aquastar<sup>®</sup> Coulomat AC AX1699F in the cathode for best results.

