Water Testing in Pharmaceutical Products

Margaret McClure, Ph.D. and Raymond Steffen, Ph.D
BASi, 2701 Kent Avenue, West Lafayette, Indiana 47906 USA

Thousands have lived without love, not one without water. – W.H. Auden

Water is a part of so many things and is a substance needed for life. Water is also an important compound when dealing with drug substances and drug products. It can impact the manufacturing process in either a positive or negative way, depending on the drug substance. Water can enhance the production of a formulated product or impede the formulation process. It can promote degradation of products, which is certainly undesirable from a product stability standpoint. The amount of water a product absorbs over time can lead to product failure. Consequently, testing for water content in drug substances and drug products becomes critical.

Techniques for Evaluating Water Content

There are many techniques available for assessing water content of pharmaceutical products. These include thermogravimetric analysis (TGA), loss on drying (LOD), azeotropic-toluene distillation, or Karl Fischer titration (KF). TGA and LOD methods are not limited to only water, but measure the total of all volatile substances in the product, including water. In many cases, the assumption is made that water is the only volatile substance. The distillation method is a cumbersome technique and not readily amenable to work in a production environment. One of the best techniques is Karl Fischer titration. This article will briefly discuss detection of water in pharmaceutical products using Karl Fischer techniques.

Karl Fischer Reagents and Reactions

The method, developed in the 1930s by Karl Fischer (1), involves the reaction between an alcohol and an organic base and is given by the following reactions:

$$(1)\ ROH + SO_2 + R'N \rightarrow [R'NH]SO_3R$$
$$(2)\ H_2O + I_2 + [R'NH]SO_3R + 2R'N \rightarrow [R'NH]SO_4R + 2[R'NH]I$$

In the first, alcohol reacts with sulfur dioxide and a base (R′N) to form a reactive complex. The complex subsequently reacts with iodine and water in number 2, consuming any moisture present in the solution. Iodine reacts quantitatively with water, which is the basis of the water determination by KF titration. Detection is usually performed electrometrically. Once all the water in the vessel is consumed, the presence of minimal quantities of excess iodine in the titration media causes a large change in voltage across the double platinum electrode, signaling the titration endpoint.

Fischer originally used pyridine in ethanol for a titrant. Since then many combinations of organic bases and alcohol have been tried. In modern KF titration, patented, pre-mixed reagents are available from most chemical suppliers, such as Sigma-Aldrich and Fischer. Most scientists use methanol as the alcohol of choice, although ethanol is gaining in popularity since it is non-toxic. The reagents usually come as one-component or two-component types. For one-component reagents, the solution contains all of the reactants (except water) needed to carry out the titration. In a typical two-component titration the base, sulfur dioxide and methanol, form one part, and the iodine and methanol comprise the second. One-component reagents are less expensive and more convenient than their two-component counterparts; however, they tend to have shorter shelf lives and lower buffer capacities. Methods based on two-component reagents are likely to work better for the determination of low masses of water since the titrant (iodine) mass is easy to control in small quantities.

KF titrations are generally performed in one of two ways, depending on the mechanism by which iodine is introduced into the system. In volumetric KF titration, the iodine is a component of the titrant and is added mechanically to the solvent containing the sample. Alternatively, in coulometric KF titration, the iodine needed is generated *in situ* by electrochemical means. The coulometric titration is often used for samples with low water contents (1 ppm to 5%), whereas samples with higher water content (100 ppm to 100%) are best analyzed by volumetric KF titration. With both techniques, instruments that control the reaction, detect the endpoint, and print out a result are commercially available.

Volumetric Karl Fischer Titration

Methods based on volumetric KF titration may use either one- or two-component reagents. In volumetric KF titration, the solvent in the titration vessel is usually methanol for one component titrations or sulfur dioxide in methanol for a two-component method. Imidazole is used as a buffer to maintain optimal pH for the reaction. The buret is filled with titrant containing the iodine. The methanol solvent is pre-titrated to a stable endpoint to remove any residual moisture. A known amount of analyte is added to the titration vessel and dissolved, if possible. The titration is then continued until a second stable endpoint is reached. The volume of titrant needed to reach the second endpoint is multiplied by the water equivalence of the titrant (i.e., titer) to yield the amount of water in the sample.

For work in a regulated environment, the titer is often determined daily using a certified standard containing a known amount of water. A measured quantity of standard material is added to the titration vessel and the volume of titrant needed to reach the endpoint is determined. The resulting water equivalence is reported in units such as mg of water per buret or per mL of titrant. Multiple standard runs are performed, and the average water equivalence and %RSD are calculated. The %RSD is used as a measure of system suitability and must be within a predetermined acceptance limit before sample analysis can begin. Periodic checks of the titrator performance during the course of sample analysis are recommended, and must also be within predetermined limits before bracketed results are considered acceptable.

Coulometric Karl Fischer Titration

Unlike volumetric KF titration where iodine is an existing component of the titrant, the iodine in coulometry is generated electrochemically by anodic oxidation of iodide. Iodine generation occurs at a generator electrode incorporated in the cell next to the measuring electrode (platinum pin electrode) in

the titration vessel. Reduction of hydrogen ion to hydrogen occurs concurrently at the cathode. The amount of iodine produced, and therefore the amount of water that has reacted with the iodine, can be calculated from the current and time, provided current is only used for iodine production. Since current and time can be accurately measured, no titer determination is required. Consequently, coulometric KF titration is considered an absolute method. It is used when measuring low water content levels or when very accurate water determinations are required.

Two different types of cells can be used for coulometric KF titration: cells where the anode and cathode compartments are separated by a diaphragm, and cells where the anode and cathode compartments are not separated. Originally, the diaphragm was part of the generator electrode and was used to prevent the iodine from being reduced back to iodide at the cathode. However, design changes have eliminated the need for a diaphragm except in instances where extremely accurate or low (< 50 µg water/sample) water content measurements are needed, or when the test substance contains components that are easily reduced. In a production setting, cells without diaphragms are preferable since they are easier to clean, do not suffer blockage or contamination of the diaphragm and demonstrate a lower drift (i.e., atmospheric moisture penetration as a function of time) than cells with a diaphragm.

References

(1) K. Fischer, Angew. Chem. 48 (1935) 394.

Solid Dose Calibrator for Karl Fischer Titrations

W. A. Young, Facet Analytical Services and Technology, LLC
www.facetllc.com

This article discusses the advantages of using a tablet as a standard for Karl Fischer titrations, and compares the results obtained using a tablet standard with those from two other commercial standards based on other delivery systems.

Purpose

Moisture content determination is an important measurement for agricultural, petroleum, pharmaceutical, plastics, cosmetics, and foodstuff industries. The Karl Fischer titration is a widely-used method for this determination (1). Volumetric Karl Fischer titrations typically use certified water samples for calibration prior to analyses. However, these calibrations can be cumbersome, requiring a syringe or a weigh boat to dispense the standard. Depending upon the skill of the analyst, this can lead to assay variability due to uncontrolled ingress of external moisture or residual standard unaccounted for during transfer to the vessel. Automation of these processes would also be costly and require extensive administrative control.

However, a water standard in tablet form (Fastrate™ *volumetric*) is now available as an alternative for titrator calibration. The moisture content of these tablets can be varied according to the application (**F1**), and is determined for each lot of tablets by in-process testing, the results of which are reported on a certificate of analysis. Since delivery of a tablet is simpler than delivery of a bulk powder or delivery using a syringe, use of a tablet should eliminate the possibility of residual standard and should also shorten the transfer time, thereby reducing ingress of external moisture. Therefore, the assay variability should be reduced. Tablets can also be used as a check standard throughout a run, and could also facilitate complete automation of the measurement, since the delivery mechanism is the same for both standard and sample.

In this study, moisture content determination was performed on Fastrate *volumetric* 10 mg tablets, and the results compared with those obtained using two other standards, one requiring a bulk powder, the other a syringe. The times required for each of the sample preparation and transfer steps were also compared.

Method

Apparatus

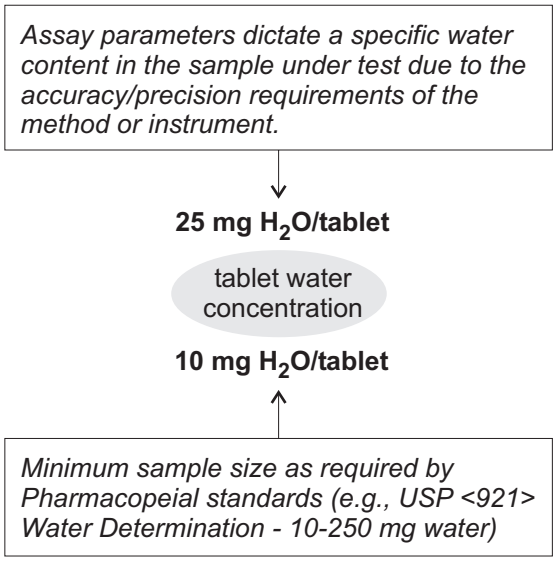
Titrations were performed using commercial analytical instrumentation and a one-component reagent system. Instrument calibration was performed per Standard Operating Procedure (SOP) using purchased standards of known water content.

Procedure

Approximately 80 mL of solvent is required to completely submerge the electrode. The working medium is added to the titration vessel and conditioned to equilibrium (end point) with Karl Fischer reagent. The weighed standard/sample is transferred to the vessel and titrated to the same end point. The volume of reagent required to reach the end point is determined by the moisture content of the sample. This volume is influenced by titer stability, analyst technique, and ambient conditions (e.g., humidity) and determines the reagent strength factor of the titer during instrument calibration. Standards of theoretical water contact are titrated to the end point as described above until a mean value of < 1.0% Coefficient of Variation (CV) for a predetermined number of replicates (n = 3) has been achieved.

Moisture content determination using volumetric Karl Fischer titration was performed on Fastrate *volumetric* and two leading conventional standards after instrument calibration was complete (**T1**). One standard (C1) required injection using a syringe, and the other (C2) used a bulk powder. Each replicate of Fastrate *volumetric* was bracketed by replicates of C1 and C2 in a group (**F2** and **F3**). Six groups were analyzed and a single instrument was used to perform all

F1.



analyses on the same day. During analysis, sample preparation, transfer and exposure times were recorded for each replicate. The working medium was replaced and reconditioned to equilibrium following completion of three consecutive titrations. All analyses were completed under the same instrument calibration.

Results

The times required for sample preparation, transfer and exposure for Fastrate™ *volumetric* and the two comparators are shown in **F2**. The times for each step are consistently shorter for Fastrate *volumetric*, and the variability between replicates is less. The reduced contamination by external moisture is shown by greater precision in the measured water content (**F3** and **T1**) for Fastrate *volumetric*, with an %RSD of 0.59% for the Fastrate *volumetric* compared with %RSDs of 4.76% and 4.61% for the two comparators.

Conclusions

Fastrate *volumetric* as a solid dose calibration standard for Karl Fischer instrumentation simplifies transfer to the vessel and minimizes differences in analyst technique. More precise and quantitative standard addition is achieved because the cumbersome use of a syringe and injection and bulk powder delivery to the vessel are replaced with a single hand transfer of the tablet through the sample port.

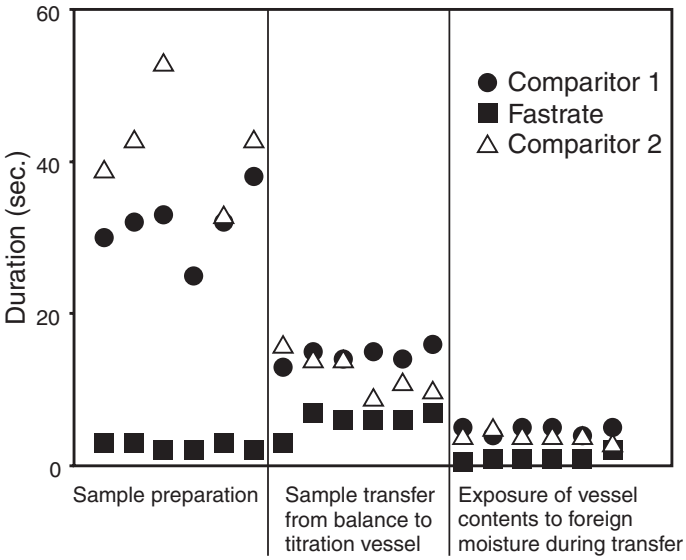
Since Fastrate *volumetric* can be formulated and pre-packaged to a specific moisture concentration, lengthy and variable sample preparations are eliminated.

Since the delivery mechanism is the same for both standard and sample, two different logistics platforms to support standards and samples are no longer required by the automated system. In addition, the tablet can be used as an independent calibration check throughout the run sequence.

T1.

Replicate	Sample Identification	Sample Weight (mg)	Statistics (Mean, STDEV, %RSD)	Water Content (mg)	Statistics (Mean, STDEV, %RSD)
1	Comparitor 1	1168.20		12.52	
2	Comparitor 1	1107.60		11.04	
3	Comparitor 1	1144.10		11.36	
4	Comparitor 1	1153.60	1137.52	11.55	11.49
5	Comparitor 1	1142.40	24.35	11.45	0.55
6	Comparitor 1	1109.20	2.14%	11.04	4.76%
1	Fastrate®	224.70		10.84	
2	Fastrate®	224.00		10.76	
3	Fastrate®	224.20		10.74	
4	Fastrate®	224.00	224.58	10.77	10.76
5	Fastrate®	225.00	0.64	10.80	0.06
6	Fastrate®	225.60	0.28%	10.65	0.59%
1	Comparitor 2	62.50		9.90	
2	Comparitor 2	64.57		10.15	
3	Comparitor 2	63.90		10.05	
4	Comparitor 2	59.70	63.75	9.26	10.00
5	Comparitor 2	68.30	2.81	10.69	0.46
6	Comparitor 2	63.50	4.41%	9.97	4.61%

F2.



F3.