Moisture Content in Tobacco Products by Volumetric Karl Fischer Titration

Scope and Application

This method is for the determination of moisture content in cigarettes, roll-your-own, pipe tobacco, snuff, and chewing tobacco. This method is validated for tobacco samples containing water in the range between 5 and 50 milligrams of water. Based on this range, the following table should be used as a guideline when preparing tobacco samples for analysis. This table reflects the sample weight ranges used to validate the method:

moisture content (% by weight)	sample weight	sample moisture content (mg)
3.34 %	150 mg	5 mg
5 %	100 – 150 mg	5 – 7.5 mg
10 %	50 – 150 mg	5 – 15 mg
25 %	50 – 150 mg	12.5 – 37.5 mg
50 %	30 – 100 mg	15 – 50 mg

This method will be used to calibrate instruments in the laboratory that require moisture content correction for analytical measurements. It will also be used to determine moisture content in samples where the measurement will be useful in the determination of product tax classification.

Regulatory Tolerances:

There are no regulatory limitations on moisture content in tobacco products.

Levels and Limitations

Analyte	Detection Limit (mg)	Quantitation Limit (mg)	Linear Range (mg)	Known Interferences
Water	5	0.05	5 - 50	fructose (above 105ºC)

Equipment

- Mitsubishi Chemical Analytech KF-200 Moisture Meter
- Mitsubishi Chemical Analytech VA 230 Water Vaporizer
- A&D GH-202 Analytical Balance
- Calibrated 20 µl adjustable pipettor (Eppendorf or equivalent)

Reagents and raw materials

- Aquamicron titrant SS-Z 5 mg (API Corporation, Osaka, Japan)
- Aquamicron solvent GEX (API Corporation, Osaka, Japan)
- Ultrapure water (18.0 MΩ) (Millipore Corporation, Billerica, MA)
- 10 ml glass headspace vials
- 20 mm open seal crimp caps with Teflon/silicone seal
- Type 4A Molecular Sieve Beads (Na_2O, MgO, Al_2O_3, SiO_2)
- Silica gel blue, 3-5 mm mesh
- 10 mg water standard for volumetric KF titration (HYDRANAL®-Water Standard 10.0 or equivalent)
- Lactose Monohydrate KF oven standard (HYDRANAL®-Water Standard KF-Oven, 140-160°C or equivalent)

Procedure

a. Measurement preparation

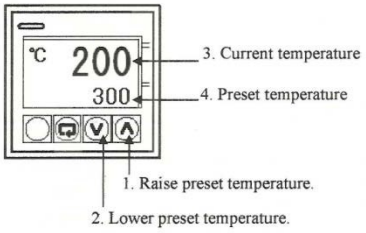
i. Check the silica gel dessicant in the titration vessel drying tube and titrant bottle vent line. If half the silica gel has turned pink or clear, replace it with fresh material.

ii. Turn on the KF-200 titrator by flipping the switch at the bottom of the right hand side of the front panel. This supplies power to the titrator and buret tower. The power for the KF oven is controlled by a toggle switch on the front panel of the VA-230.

iii. The instrument runs through the startup sequence and opens the main window/measurement screen. Make sure that channel 1 is selected for analysis. This is indicated by the orange "CH1" icon in the upper left-hand corner of the display panel. If it is not, press the **channel select** button in the lower right-hand corner of the titrator keypad.

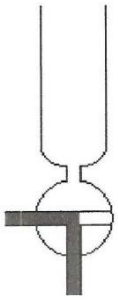
iv. Turn on the flow of nitrogen from the tank. The nitrogen should be very low in moisture content (ultra high purity nitrogen). The flow can be controlled at the instrument on the front panel of the VA-230 oven unit. The analog dial should be set to deliver approximately 75 ml/min. Because of the relative insensitivity to flow rate, it is not necessary to have exactly 75 ml/min flow.

v. Set the temperature of the VA-230 front panel thermometer. A diagram of the display is shown below:



vi. Allow the instrument approximately ½ hour to warm up.

vii. Open the stopcock on the drying tube that is positioned on top of the titration vessel. The open position is illustrated below:



viii. Once the instrument has warmed up, press the **titration** button on the titrator keypad. This allows the instrument to dehydrate the solvent in the titration vessel by titrating excess water to the endpoint.

ix. Once the vaporization unit oven temperature has stabilized, use it to prepare sample vials by placing them individually in the oven for 3 to 4 minutes to drive off moisture. After this step, loosely affix a crimp top cap to each vial until ready for sample preparation.

x. To check titrator performance, first select the appropriate method on the instrument by pressing the **parameter** key on the titrator keypad. This opens a list of methods written for instrument calibration, tobacco, and water analysis. Select the LIQ method by pressing the enter key. The parameters for this method are shown below:

Parameter File		
Parameter	**setting**	**comments**
file name:	liq	
delay:		leave blank
minimum titrant:		leave blank
titration stop:		leave blank
end sense:	30 seconds	stable potential reading
end potential (mV):	140 mV	
print form:	3	R + S + P (default)
calc form:	1	M/(W-w)
calc unit:	0	%, ppm
blank test:	0	default
minimum drop:	10 µl	choice of 2 – 100 µl
gain:	1	default
maximum vol:	20 ml	20 to 50 mL range
factor:	x.xxxx	determined experimentally
VA select:	0	no software control VA-230

Once this method is selected, press the **escape** key to return to the top level display.

xi. Titrator performance is checked by adding a water droplet of known weight to the titration vessel. The dropper bottle (a small glass vessel with the glass pipette top) is used to add the water. First, the total weight of the bottle is determined using the analytical balance that is connected to the titrator. Press the **sample** key on the titrator keypad to open the editable window that includes the weight inputs. Scroll down to the entry for "W" using the down arrow key. Place the sample on the analytical balance, close the door, and allow it to stabilize. Once the reading is stable, press the **print** key on the balance. This sends the weight value to the titrator electronically.

xii. Remove the dropper bottle from the scale and press the **stop/start** key on the keypad of the titrator. Remove the large teflon plug from the top of the titration vessel and add a drop of water. Quickly replace the teflon stopper into the vessel top.

xiii. The buret should have begun dispensing titrant into the titration vessel.

xiv. Place the dropper bottle back onto the analytical balance. Press the **sample** key on the keypad. Scroll down to the "w" entry. Once the balance has stabilized, press the **print** key to transfer the weight to the titrator.

xv. Press the **escape** key to return to the top level display. The display should be reading a steadily advancing percentage value as the water is titrated.

xvi. Once the endpoint is reached, the display value for water in the titration vessel should be in the range of 100 ± 3% over three runs. If any lie outside this range, the titer of the titrant should be re-evaluated (see the **titer determination** section).

xvii. Check the VA-230 system performance using ultrapure high resistivity water (Millipore). The performance is checked by adding a known volume of water to a headspace sample vial and placing it in the oven unit for testing. The instruction manual for the Mitsubishi KF-200 states that the instrument performance for a 15 µl injection should be 15 ± 0.75 mg with repeatability on the oven unit within 3% (for three runs).

xviii. To analyze samples, first select the appropriate method on the instrument by pressing the **parameter** key on the keypad and selecting the tobacco method. The parameters for this method follow:

Parameter File		
parameter	**Setting**	**comments**
file name:	Tobacco	
delay:	3 minutes	allows for vaporization
minimum titrant:		leave blank
titration stop:		leave blank
end sense:	30 seconds	stable potential reading
end potential (mV):	140 mV	
print form:	3	R + S + P (default)
calc form:	6	(M-B)/(W-w)
calc unit:	1	% only
blank test:	0	default
minimum drop:	10 µl	choice of 2 – 100 µl
gain:	1	default
maximum vol:	20 ml	20 to 50 mL range
factor:	x.xxxx	determined experimentally
VA select:	0	no software control VA-230

xix. Press the **escape** key on the titrator keypad to return to the main menu.

xx. Change the sample name by pressing the **sample** key on the titrator keypad. Use the **arrow** key to move the cursor to the sample name entry. Press the parameter key on the titrator keypad. This will open an alphanumeric softkey pad. Use the **arrow** keys to highlight appropriate characters and press the **enter** key to select them. Once the name of the sample has been entered, scroll to the **end** key on the softkey pad and press **enter**. This will bring the sample menu back. Press **enter** on the titrator keypad until you pass the "w" entry line. This should be accompanied by a long audible beep. Press the **escape** key to bring up the main window.

xxi. Tare the analytical balance that is attached to the titrator. Place one of the headspace vials prepared in step a.ix. on the analytical balance. Once the weight has stabilized, press the **sample** key on the titrator keypad to open the editable window that includes the weight inputs. Scroll down to the entry for "w" using the **arrow** key. Press the **print** key on the balance. This sends the weight value to the titrator electronically.

xxii. Remove the headspace vial from the analytical balance and, using a calibrated 20 µl adjustable pipettor, add 15 µl of ultrapure high resistivity water to the headspace vial, seal it with a crimping tool and place it on the tared analytical balance. Once the weight has stabilized, press the **sample** key on the titrator keypad to open the editable window that includes the weight inputs. Scroll down to the entry for "W" using the **arrow** key. Press the **print** key on the balance to send the weight value to the titrator.

xxiii. Press the **escape** key to return to the top level display. Press the **start/stop** button on the titrator keypad to begin the titration.

xxiv. As the delay on the titrator counts down from three minutes, place the headspace vial in the oven chamber, rotate the needle assembly into place over the vial and plunge the needle into the vial. Within a few minutes, the readout for the potential on the titrator should begin to change to positive values.

xxv. The endpoint is reached when a negative potential is maintained for 30 seconds. This is signaled by a sequence of three audible beeps. When the reading is complete, remove the vial and return to step b.iii. to repeat the process for at least two more samples.

xxvi. If the 15 µl check standard fails to meet the performance requirements described in step a.x.vii, go to the **titrator maintenance** section and follow the VA-230 maintenance steps.

b. Sample analysis

i. To analyze samples, first select the appropriate method on the instrument by pressing the **parameter** key on the keypad and selecting the tobacco method. The parameters for this method follow:

Parameter File		
parameter	**Setting**	**comments**
file name:	Tobacco	
delay:	3 minutes	allows for vaporization
minimum titrant:		leave blank
titration stop:		leave blank
end sense:	30 seconds	stable potential reading
end potential (mV):	140 mV	
print form:	3	R + S + P (default)
calc form:	6	(M-B)/(W-w)
calc unit:	1	% only
blank test:	0	default
minimum drop:	10 µl	choice of 2 – 100 µl
gain:	1	default
maximum vol:	20 ml	20 to 50 mL range
factor:	x.xxxx	determined experimentally
VA select:	0	no software control VA-230

ii. Press the **escape** key on the titrator keypad to return to the main menu.

iii. According to the instruction manual, the optimal measurement range for the model KF-200 is 0.5 to 50 mg of water. In practice, it was found that the error in the determination expanded significantly below 1 mg. For the tobacco products specified for this method, the nominal sample weight is set at 100 mg. Since tobacco products generally contain water in the 1 – 50 % range, this sample weight was chosen because it provides a wide dynamic range for moisture content. Consult the **Scope and Applications** section to determine the appropriate sample size for tobacco.

iii. Change the sample name according to the instructions in **section a.xix**.

iv. Tare the analytical balance that is attached to the titrator. Place one of the headspace vials prepared in step a.ix. on the analytical balance. Once the weight has stabilized, press the **sample** key on the titrator keypad to open the editable window that includes the weight inputs. Scroll down to the entry for "w" using the **arrow** key. Press the **print** key on the balance. This sends the weight value to the titrator electronically.

v. Unseal the packaging on the tobacco sample and remove enough of the sample for analysis. Seal the remaining sample in a plastic bag. Remove the headspace vial from the analytical balance and quickly place the sample in the vial, loosely affix the crimp-top seal, and place it on the tared analytical balance. Once the weight has stabilized, press the **sample** key on the titrator keypad to open the editable window that includes the weight inputs. Scroll down to

the entry for "W" using the **arrow** key. Press the **print** key on the balance to send the weight value to the titrator.

vi. Remove the headspace vial from the analytical balance and seal it with a crimping tool.

vii. Press the **escape** key to return to the top level display. Press the **start/stop** button on the titrator keypad to begin the titration.

viii. As the delay on the titrator counts down from three minutes, place the headspace vial in the oven chamber, rotate the needle assembly into place over the vial and plunge the needle into the vial. Within a few minutes, the readout for the potential on the titrator should begin to change to positive values.

ix. The endpoint is reached when the negative potential stabilizes for 30 seconds. This is signaled by a sequence of three audible beeps. When the reading is complete, remove the vial and return to step b.iv. to repeat the process for at least two more samples.

c. Titer determination

i. To determine titer, first select the appropriate method on the instrument by pressing the **parameter** key on the keypad and selecting the CALIB method. The parameters for this method follow:

Parameter File		
parameter	**Setting**	**comments**
file name:	CALIB	
delay:		leave blank
minimum titrant:		leave blank
titration stop:		leave blank
end sense:	30 seconds	stable potential reading
end potential (mV):	140 mV	
print form:	3	R + S + P (default)
calc form:	12	(W-w)*1000/KFmL
minimum drop:	20 µl	choice of 2 – 100 µl
gain:	1	default
maximum vol:	20 ml	20 to 50 mL range
factor:	x.xxxx	determined experimentally

ii. Press the **escape** key on the titrator keypad to return to the main menu.

iii. Titer determination is carried out by adding a water droplet of known weight to the titration vessel. The dropper bottle is used to add the water. First, the total weight of the bottle is determined using the analytical balance that is connected to the titrator. Press the **sample** key

on the titrator keypad to open the editable window that includes the weight inputs. Scroll down to the entry for "W" using the down arrow key. Place the sample on the analytical balance and allow it to stabilize. Once the reading is stable, press the **print** key on the balance. This sends the weight value to the titrator electronically.

iv. Remove the dropper bottle from the scale and press the **stop/start** key on the keypad of the titrator. Remove the large teflon plug from the top of the titration vessel and add a drop of water. Quickly replace the teflon stopper into the vessel top.

v. The buret should have begun dispensing titrant into the titration vessel.

vi. Place the dropper bottle back onto the analytical balance. Press the **sample** key on the keypad. Scroll down to the "w" entry. Once the balance has stabilized, press the **print** key to transfer the weight to the titrator.

vii. Press the **escape** key to return to the top level display. The display should be reading a steadily decreasing concentration value (mg/ml) as the water is titrated.

viii. The endpoint is reached when a negative potential is maintained for 30 seconds. This is signaled by a sequence of three audible beeps. When the reading is complete, remove the vial and return to step c.iii. to repeat the process for at least two more determinations.

ix. Once three determinations have been run, the software will automatically populate the ***Factor*** entry in each method. If more than three determinations are made, all runs will be used to determine the titer. To exclude runs based on operator/experimental error, the average value of the remaining runs must be calculated using the titrator software. This is done by pressing the **function** key on the titrator keypad. When the window pops up, select the **statistical calculation** entry. When the new window opens, select the **internal** calculation entry. The next window shows the data files. Select the appropriate runs using the arrow and enter keys. Deselecting an entry is done by scrolling over a file highlighted by an asterisk and pressing the **enter** key. Press the **print** key on the titrator keypad to print the average.

x. The average value of the titer can be manually entered for any one of the methods by opening the method in the **parameter** screen and scrolling down to the **Factor** entry. The number keypad on the titrator can be used to input the value. This should autopopulate for all methods.

Traceable standards

i. A number of calibrants are available on the open market for Karl Fischer titrators. Two that were chosen for use in this procedure are the 10 mg water standard and the lactose monohydrate KF oven standard (approximately 5% water by weight, 140-160°C). The 10 mg

water standard contains 1% water by weight and is the most commonly used standard for volumetric Karl Fischer titrations. It is introduced directly into the titration vessel and, as such, is not useful as a standard for the water vaporization component of the titrator. The lactose monohydrate KF oven standard is used specifically to test the performance of the entire titrator including the water vaporizer. The instruction manual for the Mitsubishi KF-200 states that the instrument performance for the Aquamicron water standard should be within 3% of the guaranteed value (over three measurements). The literature provided with the Hydranal KF oven standard states that the user should accept a variation of ± 0.2% (abs) from the water content indicated on the certificate of analysis. This converts to an RSD of 4% for the lactose monohydrate standard described above. Using the 10 mg water standard and the KF oven standard, the performance of the titrator with and without the oven can be studied.

ii. The KF oven standard is tested as any other sample and should be analyzed according to **section b**. The only adjustments being that the temperature range for the analysis is 140 – 160 ºC and the sample size should be in the 200 mg range. The oven standard should be run monthly or when the VA-230 system performance check outlined in **section a** fails and cannot be rectified using simple maintenance procedures.

Quality Control

1. Run three injections of water according to **section a** in the procedure. Once the endpoint is reached, the display value for water in the titration vessel should be in the range of 100 ± 3% over three runs. If any lie outside this range, the titer of the titrant should be re-evaluated.

2. The performance of the VA 230 oven is checked according to procedure **section a** by adding a known volume of water to a headspace sample vial and placing it in the oven unit for testing. The performance criteria for three runs of 15 µl injections should be 15 ± 0.75 mg with repeatability on the oven unit within 3%. If any lie outside this range, the **maintenance/troubleshooting** section should be consulted.

3. Run the LCS (Kentucky reference cigarette 3R4F) with every batch of samples. The result for the LCS should be in agreement with the 3R4F precision study shown in the validation package titled “Moisture Content in Tobacco Products by Volumetric Karl Fischer Titration”.

Maintenance/Troubleshooting

i. *Cleaning the electrode when it is contaminated* - A sign of contamination is the loss of precision in the determination of the endpoint. The detecting electrode should be cleaned by rinsing with ultrapure high resistivity water and gentle wiping with a kimwipe or equivalent. To remove the electrode, the instrument must be turned off. Care must be taken not to cause

an extreme change in the distance between platinum electrodes. Scratching the electrode must also be avoided.

ii. *Solvent becomes brown before endpoint or dark brown at endpoint* – This is usually a sign of the buildup of side reaction products. Place the titrator in standby mode by pressing the **titration** key on the keypad and press down on the fluid line toggle switch on the left-hand side of the titration unit behind the reaction vessel. Once the unit is empty, refill it by pressing up on the fluid line toggle switch until the tip of the electrode is completely immersed in titration solvent. Press the **titration** key again to allow the titrator to dehydrate the solvent.

iii. *Cleaning the double hollow needle* – When not in use, the double hollow needle should be stored in a water-methanol mixture. The needle should be cleaned after each use by forcing methanol through it. This is accomplished with a common laboratory wash bottle filled with methanol. The tip of the bottle should be wide enough to slide it down over the inner needle. The wash bottle tip should be made flush with the flange on the double needle. The bottle is squeezed to start flow through both the inner and outer needles. If solvent flow is impeded, sonication in methanol/water for one hour might be necessary to remove foreign material from the needle assembly.

iv. *Changing the sieve material in the VA-230 dessicant tube* – the molecular sieve material should be replaced every 8 to 12 weeks. Degradation in system performance due to exhaustion of the sieve material is evidenced by increasing problems achieving endpoints and maintaining dehydrated conditions in the titration vessel. The molecular sieve is packed in the dessicant tube by lining the bottom with approximately ½ inch of glass wool. The tube is then filled about ¾ volume with the sieve material. This is covered with another half inch layer of glass wool. The top is replaced and the dessicant tube placed back onto the VA-230.

v. *Cleaning the titration vessel* – if the vessel becomes visibly dirty, it can be removed for cleaning. Care should be taken to turn the power off to the titrator prior to disconnecting the titration vessel. Soap and water should be used for cleaning followed by a methanol rinse. The unit should be dry before placing it back onto the titrator.

Sources of Uncertainty

1. Sample handling (exposure to laboratory atmosphere).
2. Weighing errors during sample preparation.
3. Titer degradation over time (see the measurement preparation section of the procedure to check the titer of the titrant).
4. Spent dessicant in nitrogen drying tube allows trace levels of water from the nitrogen talk to influence the experiment. The dessicant should be changed every 8 to 12 weeks.

Calculations

The amount of water present in the sample is based on the concentration of iodine in the titrant and the volume of titrant consumed in the oxidation reaction:

$$\text{Moisture Content \%} = \frac{\text{titrant consumed (ml)} \times \text{titer} \left(\frac{\text{mg}}{\text{ml}}\right)}{\text{sample quantity (g)} \times 1000} \times 100$$

The "titer" is used to determine the quantity of water from the amount of reagent titrated. It shows the equivalent concentration of water (mg/ml) and is based on the 1:1 reaction of iodine and water. The titer is determined experimentally for each bottle of titrant prior to analysis of samples.

Reporting Results

Report the results as follows:

Component	Sample Type	Units	Precision	Format
water	tobacco	weight percent	1 decimal	x.X

Safety Notes

Consult the MSDS for any chemicals that are unfamiliar. All chemicals should be considered hazardous – avoid direct physical contact.

References

Instruction Manual for (Mitsubishi) Moisture Meter (VolumetricTitration) Model KF-200, Control No. ZKFEMAE-06

"Karl Fischer Reagents Technical Manual" API Corporation (Mitsubishi Chemical Group). http://www.mcckf.com/english/manual_E.pdf.

Required Training, Certification, and Recertification

1. Training in the operation of the Mitsubishi Chemical Analytech KF-200 Moisture Meter fitted with the VA 230 Water Vaporizer.
2. Initial certification by running 7 LCS samples (Kentucky reference cigarette 3R4F) with results of precision and reproducibility in agreement with the results of the validation package titled "Moisture Content in Tobacco Products by Volumetric Karl Fischer Titration".

3. Periodically, chemists are retested for competency (e.g. every 5 years) and/or given proficiency testing.

Revision History

Rev 1 – New test method for implementation (4/11/2013)