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Automated Karl Fischer titration for liquid samples – Water determination in edible oils

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Abstract

In the field of food analysis, rapid measurements and results are generally of high importance. The Karl Fischer titration (KFT), a chemical method for determining water content, and its automated performance enhance rapidity by giving the opportunity to deal with more samples in less time; automated sequences can include different determination methods and sample treatments. Moreover, automation can improve reproducibility and precision.

In this work water determination methods were established with an automated KFT system using both the volumetric and coulometric KF technique. Aspiration and transfer of the sample are freely defined in the methods, which consist of different combinations of so-called transfer volumes, air bubbles, special liquids and sample volumes. Commercially, available liquid water standards were used as samples for designing basic methods. Furthermore, applications for different edible oils were developed.

Keywords: Automated Karl Fischer titration; Water content; Edible oils

1. Introduction

The Karl Fischer titration (KFT) is a chemically selective method for determining water content in different matrices. Thus, the complete water but solely the water of a sample is being detected. This results from a chemical reaction between the sample water and an alcohol, imidazole as a base, sulphur dioxide and iodine, according to the following reaction equations (Scholz, 1984):

$$\mathrm{ROH} + \mathrm{SO_2} + \mathrm{R'N} \rightarrow \mathrm{ROSO_2^-} + \mathrm{R'NH^+} \qquad \text{(step 1)}$$

$$\mathrm{ROSO_2^-} + \mathrm{I_2} + \mathrm{H_2O} + 2\mathrm{R'N} \rightarrow 2\mathrm{R'NH^+} + \mathrm{ROSO_3^-} + 2\mathrm{I^-} \qquad \text{(step 2)}$$

Complete equation:

$$\begin{aligned}&\mathrm{ROH} + \mathrm{SO_2} + 3\mathrm{R'N} + \mathrm{I_2} + \mathrm{H_2O}\\ &\quad\rightarrow 3\mathrm{R'NH^+} + \mathrm{ROSO_3^-} + 2\mathrm{I^-} \quad \text{with } \mathrm{R'N} = \text{base},\\ &\quad \mathrm{R} = \text{–}\mathrm{CH_3} \text{ or } \text{–}\mathrm{CH_2}\text{–}\mathrm{CH_3}\end{aligned}$$

The expenditure of time for water content determinations can be reduced by using the KF method, as these reactions proceed much faster in comparison to traditional drying techniques (Isengard, Felgner, Kling, & Reh, 2006).

Furthermore, with the possibility to automate KFT, manpower can be used more efficiently and results can be obtained in shorter time periods. Automated KF sequences may include different determination methods for water equivalent of the titrating agent, blank values and different samples. Even various sample treatments can be performed, like adding of solvents. Moreover, automation can improve reproducibility and accuracy (Bruttel & Schlink, 2003).

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For the investigations in this work volumetric and coulometric KF techniques were used. The volumetric technique is preferably applied for samples containing higher water contents from approximately 1% up to 100%. For performing the volumetric experiments a titrating agent containing iodine is used for titration with a burette. The water content of the sample is calculated from consumption of iodine and water equivalent of the titrating agent. The coulometric KFT, applied for smaller water contents in the range of few µg/g, uses electrochemically generated iodine, produced from iodide by anodic oxidation at a generator electrode directly in the titration vessel (Scholz, 2006).

For both techniques, methods were developed using commercially available liquid water standards as samples. In addition, applications using the coulometric KFT for different edible oils were developed.

2. Methods

A Karl Fischer system of Metrohm AG, Herisau, Switzerland, was used, based on an 815 Robotic USB Sample Processor XL including an 801 magnetic stirrer and as titrating devices an 841 Titrando (for volumetric measurements) and a 756 KF coulometer (for coulometric measurements). For liquid handling, several dosing devices (800 Dosino with 807 dosing unit) were used, one for transferring and aspirating and one for adding and levelling the working medium; for the volumetric technique one more dosing device for adding titrating agent is necessary. The coulometric setup of an automated KF system is shown in Fig. 1.

All chemicals noted in the following were kindly provided by Sigma–Aldrich Laborchemikalien, Seelze, Germany.

Volumetric determinations were performed via the one-component technique using Hydranal-Composite 2 (containing all reactive compounds) as titrating agent and Hydranal-Methanol dry as working medium. The water equivalent (= titre) of the titrating agent was determined weekly. For end-point detection the bivoltametric method was applied using a polarizing current of 50 µA and stop voltage of 250 mV. A drift rate of 10 µL/min served as stop criterion; an extraction time of 120 s was applied.

Fig. 1. Setup of automated coulometric Karl Fischer titration.

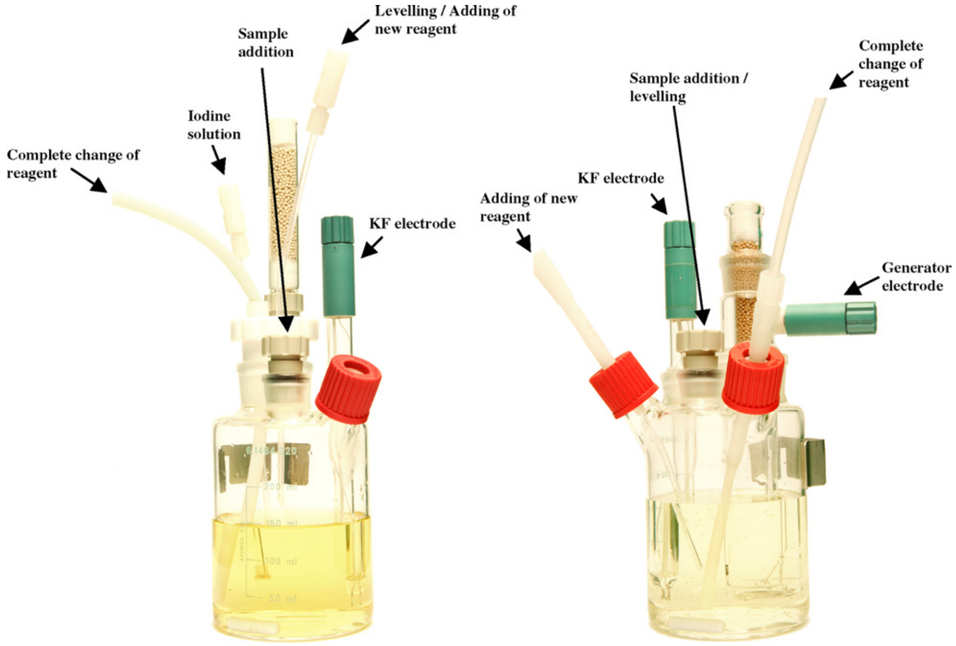
Coulometric determinations were conducted using a generator electrode without diaphragm (generator current 400 mA), with Hydranal-Coulomat AD (reagent for coulometric KFT for cells without diaphragm) as working medium for determination of water standards and Hydranal-Coulomat AG-H (reagent for coulometric KFT in long-chained hydrocarbons; free of halogenated hydrocarbons) as working medium for determination of edible oils. The accuracy of the system was checked weekly. End-point detection was performed by the bivoltametric method using a polarizing current of 10 µA and a stop voltage of 50 mV. As stop criterion served a drift rate of 10 µg/min; extraction times of 120 s for water standards and 660 s for edible oil samples were applied. The higher extraction time for edible oils is caused by a longer sample transfer time, thus the titration cannot stop before all the sample is transferred.

All results of the automated determinations were calculated referring to the obtained water contents by manually conducted KFT and are mostly displayed as recovery rate in %. Also included in those calculations was the parameter of density of each fluid. Determination of density of all samples was performed on a DMA 5000 density meter from Anton Paar Germany GmbH, Ostfildern, Germany. Measurements were taken at respective room temperatures.

3. Procedures and parameters

To obtain a mean value for the water content of a sample, a complete sequence of measurements is necessary, consisting of seven (10 including blank values) separate measurements: first a start dummy, which is run at the beginning of the sequence to rinse the system; then – if necessary – three blank values (their average being deducted from the sample results) and last follow the measurements of the actual sample by $n = 6$. The system was checked for memory effects by performing long series of measurements consisting of six to nine different samples (with $n = 6$ for each sample), which were determined not in a row but mixed among each other. No memory effects were observed.

The robustness of the system was also examined by taking measurements in varying laboratory conditions in temperature and humidity through different seasons and weather situations. The resulting effects on coulometric KF measurements have to be eliminated by including blank values in the experiments. However, for volumetric measurements, the laboratory conditions influence the measurements in a dimension of 0.0001%, which falls within the standard deviation and therefore is negligible. Thus no blank values are needed, as long as the sample is



Volumetric vessel

Coulometric vessel

Fig. 2. Setup of volumetric and coulometric titration vessels.

not dissolved externally in a solvent before titration. For the coulometric measurements however, an additional special liquid is used in the methods. It is aspirated in the course of sample taking in between other liquids to ensure separation of these liquids and to guarantee complete transfer of the sample into the titration vessel by better rinsing of the tube. This is especially important for the coulometric determinations since the examined water contents are very small, therefore no sample must be left in the tube. For water standards as samples methanol is used as special liquid and *n*-hexane is used for the determination of edible oils. Therefore, blank values are required to exclude the water content of the special liquid from the results.

The samples are filled in 6 mL glass vials and sealed with aluminum silicone septum caps. For aspirating and transferring fluids, a double hollow transfer needle is used, with small vent holes at top and bottom of its outer shell. The transfer needle is attached to a tube of 10 mL volume (transfer tube) which itself is attached to a dosing device.

Before running the start dummy the transfer tube is “prepared”, i.e. completely filled with water free reagent out of the titration vessel. This preparation of the transfer tube has to be done before every sequence start and again during long sequences to prevent remaining water in the tube from entering the titrations.

For performing a measurement, first an air bubble, then a defined so-called transfer volume (consisting again of water free reagent out of the vessel) followed by another air bubble is aspirated through the needle into the transfer tube. Then the defined sample volume is taken up followed by another air bubble taken out of the headspace inside the vial. For coulometric measurements, the special liquid is aspirated before the sample volume, separated from the other liquids by additional air bubbles. Now the sample and parts of the transfer volume are transferred into the vessel while the titration is started.

Another important role plays the fluid level in the titration vessel. There is a risk of carrying over liquids of any kind through the vent holes of the transfer needle, thus the fluid level in the vessel must not exceed a defined level. For this levelling as well as for the complete changing of the working medium, the sequences described in the following were developed, slightly differing between volumetric and coulometric assembling. For levelling the fluids in the vessel, a variable in the software adds up the volumes. After addition of more than 40 mL of fluid levelling takes place and the fluid level returns to its initial position. Depending on the water content of the sample, levelling can be performed up to four times before the working medium has to be changed completely (by using the membrane pump unit of the sample processor) and the levelling process restarts. As shown in Fig. 2 different titration vessel setups are employed for volumetric and coulometric examinations.

For optimum sizes and volumes of air bubbles and fluids, different alternatives were examined in preliminary tests. The results of the optimized methods are given in Section 5.

4. Samples

Samples used for developing the automated KF methods:

- Hydranal Methanol Water Standard 5.00 (containing methanol; 5.00 ± 0.02 mg H_2O/mL).

- Hydranal Water Standard 1.00 (containing anisole; 1 mg H_2O/g).
- Hydranal Water Standard 0.10 (containing xylene; 0.10 mg H_2O/g).

The exact water content of these standards is individually indicated on a certificate.

Edible oil samples:

- Soy bean oil, P. Brändle GmbH, Empfingen, Germany.
- Vegetable oil from rapeseed, EUCO GmbH, Hamburg, Germany.
- Sun flower oil, olive oil (refined), sesame oil and pumpkin. seed oil, Gustav Heess Oleochemische Erzeugnisse GmbH, Stuttgart, Germany.

5. Results and discussion

The aspiration of air bubbles in between the different fluids has proven crucial, as they form buffer areas and thereby prevent diffusing and transferring of fluids between the different zones. Furthermore, the air bubble size is relevant: too small bubbles may result in transfer of parts of the fluids between zones; oversized bubbles may cause compression of volumes and therefore falsify the results.

The special liquid aspirated between sample and transfer volume seems to be only relevant for coulometric KF measurements, as it helps to rinse the complete sample amount into the titration vessel, which is obviously very important when investigating very small water contents.

One of the most important parameters is the speed of aspiration and transfer of fluids and air bubbles, which depends primarily on the viscosity but also on the water content of the sample as for higher water contents the error obviously diminishes. Preliminary tests showed that for samples containing more than approximately 1% water in volumetric determinations a speed of 5 mL/min is adequate, whereas for coulometric determinations of samples with less than approximately 1% water a slower speed of 2 mL/min should be applied. For the coulometric determinations with the highly viscous edible oils a speed of even 1 mL/min is necessary to get reasonable results and prevent mixture of fluids in the transfer tube.

Another factor for optimization of the methods is the amount of volume transferred into the titration vessel. There may be influences on the resulting water contents by water from the environment remaining in the transfer tube. Therefore various tests were performed, leaving different parts like air bubbles or part of the transfer volume out of the titration.

In Table 1 the optimized parameters resulting from all preliminary tests for both KF techniques are given. For the coulometric measurements, the sample volumes were 1 mL for Hydranal Water Standard 1.00 and 2 mL for Hydranal Water Standard 0.10, respectively. The edible oils were examined using the coulometric KF technique, as they show small water contents approximately between 100 and 700 μg/g. The coulometric method was slightly modified for the determination of oils: increased transfer volume, special liquid (0.1 mL *n*-hexane) for better solution of the oils and therefore better rinsing of the transfer tube, aspiration and transfer speed decreased to 1 mL/min. The sample volumes were adjusted according to the water contents of the oils.

In Table 2 the recovery rates for the established KF methods using different samples are displayed; they refer to the manually determined water content. The density of the samples is also taken into account. As mentioned above, every data bar consists of the mean of six measurements. The results shown are the results of the two most suitable methods of the preliminary tests, one for the coulometric and one for the volumetric KF technique. According to Scholz (2006), recovery rates for Hydranal Water Standard 1.00 should be found between 97% and 103% and for Hydranal Water Standard 0.10 recovery rates between 90% and 100% can be expected.

The results of the determinations of edible oils are given in Table 3. For each oil two sequences of measurements ($n = 6$) were carried out using different amounts of sample

Table 1
Parameters of volumetric and coulometric KF methods

Technique Sample	Volumetric KFT Liquid water standard	Coulometric KFT Liquid water standard	Coulometric KFT Edible oil
Aspiration of (AB = air bubble)	AB 0.05 mL/transfer volume/AB 0.02 mL/sample volume	AB 0.05 mL/transfer volume/AB 0.05 mL/special liquid/AB 0.05 mL/sample volume/AB 0.05 mL	AB 0.05 mL/transfer volume/AB 0.05 mL/special liquid/AB 0.05 mL/sample volume/AB 0.05 mL
Transfer into titration vessel of	Half transfer volume/AB 0.02 mL/sample volume	All	All
Aspiration and transfer speed	5 mL/min	2 mL/min	1 mL/min
Transfer volume	3 * sample volume	3 * sample volume	10 mL (complete tube volume) less air bubbles, special liquid and sample volume
Sample volume	0.5 mL	1/2 mL	0.5–4 mL
Special liquid	None	Methanol 0.05 mL	Hexane 0.1 mL

Table 2
Recovery rates and standard deviations of volumetric and coulometric KFT methods using different liquid water standards; $n = 6$ for every value

Recovery rates (%)	Measurement 1	Measurement 2	Measurement 3
Volumetric KFT Water Std 5.00	100.2 ± 0.5	99.4 ± 0.4	99.7 ± 0.6
Coulometric KFT Water Std 0.10	98.6 ± 1.0	98.7 ± 1.2	99.0 ± 1.6
Coulometric KFT Water Std 1.00	99.0 ± 0.2	98.7 ± 0.3	99.7 ± 0.2

Table 3
Results of determinations of edible oils given as recovery rate (%) (referred to manual KFT result) and water content (μg/g); $n = 6$ for every value; CV = coefficient of variation

Sample/sample volume	Results by automated KF method			Results by manual KF method in μg/g
	Recovery rate in %	Water content in μg/g	CV	
Soy bean oil/3 mL	129.5 ± 1.8	102.5 ± 1.5	1.4	79.2 ± 1.5
Soy bean oil/4 mL	123.2 ± 3.7	97.5 ± 2.9	3.0	79.2 ± 1.5
Sun flower oil/3 mL	104.2 ± 1.8	202.1 ± 3.5	1.7	194.0 ± 8.2
Sun flower oil/4 mL	103.5 ± 3.1	200.7 ± 6.1	3.0	194.0 ± 8.2
Olive oil/2 mL	101.4 ± 0.6	210.2 ± 1.1	0.6	207.3 ± 3.2
Olive oil/3 mL	102.1 ± 2.4	211.7 ± 4.9	2.3	207.3 ± 3.2
Rapeseed oil/1 mL	97.8 ± 1.5	425.6 ± 6.7	1.6	435.0 ± 1.7
Rapeseed oil/2 mL	99.0 ± 1.4	430.6 ± 6.3	1.5	435.0 ± 1.7
Sesame oil/1 mL	100.6 ± 1.1	538.2 ± 6.1	1.1	534.7 ± 3.8
Sesame oil/2 mL	100.5 ± 0.3	537.2 ± 1.5	0.3	534.7 ± 3.8
Pumpkin seed oil/0.5 mL	100.7 ± 4.7	689.0 ± 32.1	4.7	684.3 ± 2.1
Pumpkin seed oil/1 mL	99.6 ± 1.6	681.8 ± 11.0	1.6	684.3 ± 2.1

volumes adjusted to the water content of the oil. The results are given as recovery rates in % as well as water contents in μg/g, including standard deviation and coefficient of variation. For olive, rapeseed, sesame and pumpkin seed oil the recovery rates are very good, between 97% and 103%. For sun flower oil, the results lie slightly outside of these limits. For soy bean oil the recovery rates are less good, but a look at the results of the water contents indicates very small amounts of water in these samples, below 80 μg/g according to manual determination. The explanation for this lies in the assembly of the instrument used: For investigation of such small absolute amounts of water and especially oils as samples, a coulometric generator electrode with diaphragm is required, otherwise the found results will be too high, according to the supplier. For easier handling of automation and change of reagents, a generator electrode without diaphragm was used in these experiments.

6. Summary

For liquid samples and samples easily soluble in adequate solvents, the water determination according to Karl Fischer can easily be automated using the methods established in this work obtaining very good results.

Volumetric and coulometric automated KF methods were established. By aspiration and transfer of different combinations of air bubbles and fluids, the methods have been optimized. For the coulometric method, in addition to the basic settings methanol as a special liquid is used in the transfer tube to ensure complete rinsing of the sample into the titration vessel.

For water content determination in edible oils the automated coulometric KFT is applied. Because of the high viscosity of these samples slight modifications are necessary: reduction of aspiration and transfer speed and use of *n*-hexane as special liquid.

Likewise, the levelling and changing of reagent in the titration vessel proceed automatically, especially required when performing sequences with high numbers of samples. By using dosing devices, excess reagent is removed to maintain the fluid level in the vessel. After several times of removing (depending on the water content of the introduced sample), the complete reagent is removed and the vessel is filled with new reagent.

For potentially solid samples that have to be dissolved in a solvent before determination (complete dilution is required), the blank value of the solvent has to be determined before the measurement of the samples and deducted from the water content of the sample. Addition of solvents and determination of blank values can be implemented in sequences and therefore performed as automated procedures.

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