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Vaporisation coulometric Karl Fischer titration: A perfect tool for water content determination of difficult matrix reference materials

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Abstract

Volumetric Karl Fischer titration (V-KFT) is the reference method to determine the water content in a whole range of organic and inorganic matrices. The method has several important drawbacks e.g. a high solvent consumption and a rather large measurement uncertainty when determining the water content in reference and candidate reference materials containing starch (e.g. maize, toasted bread, etc.). It is also labour intensive for routine measurements. In order to overcome these disadvantages, a relatively new method of coulometric Karl Fischer titration, equipped with a fully automated oven system, has been established and optimised.

The objective of this study was to establish the robustness of the vaporisation coulometric Karl Fischer titration method (vap-C-KFT) and to compare measurement uncertainties with those obtained by V-KFT. The overall results obtained with the proposed vap-C-KFT methods correlate well with the results obtained by V-KFT ($R^2 = 0.998$). Due to the good repeatability and intermediate precision of the vap-C-KFT methods compared to V-KFT, significant lower measurement uncertainties were obtained from vap-C-KFT measurements.

Keywords: Karl Fischer titration; Vaporisation; Oven sample processor; Certified reference materials; Uncertainty

1. Introduction

The long-term stability of certified reference materials (CRMs) is a crucial issue within their certification process. This is particularly the case for biological and clinical matrix CRMs, where enzymatic and microbiological activity are known to be the most important causes of product deterioration and degradation (Rückold, Grobecker, & Isengard, 2001a, 2001b; Rückold, Isengard, Hanss, & Grobecker, 2003). Since water is the driving force behind both degradation activities, dehydration techniques such as freeze or spray drying have to be applied primarily to lower the water content and with it the water activity below the level of microbial growth ($a_W < 0.6$) and to guarantee almost infinite shelf life and long-term stability of the CRMs (Fellows, 2002; Isengard, 2001; Mathlouthi, 2001). The Institute for Reference Materials and Measurements (IRMM) of the Directorate General-Joint Research Centre (DG-JRC) of the European Commission (EC) is one of the main CRM producers that distribute chemical, environmental, clinical, biological, food, nuclear and industrial CRMs to control and research laboratories for instrument calibration, method validation and quality control. The CRM production activities at IRMM are accredited according to ISO Guide 34 (2000). For the majority of the biological and environmental CRMs, the assigned value is directly related to the dry mass and therefore, efficient and precise water content determinations are of utmost importance (De Caro, Aichert, & Walter, 2001; Vogl & Ostermann, 2006; Yazgan, Bernreuther, Ulberth, & Isengard, 2006).

As homogeneity and stability are essential prerequisites for the end product, routine water content determinations are performed through all phases of the preparation/production process (from control of base materials to

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long-term stability studies). The techniques used and accredited according to ISO 17025 (2005) at IRMM, are volumetric Karl Fischer titration (V-KFT) and coulometric Karl Fischer titration (C-KFT). The chemical characteristics of Karl Fischer reaction mechanisms and reagents have already been frequently documented (Supartono, Rückold, & Isengard, 1998; Grünke, 2001; Schöffski, 2001). For most of the biological CRMs, V-KFT is applied as it is an internationally accepted reference method (ASTM E203-01, 2001; ISO 760, 1978) and yields reliable and reproducible results. However for difficult matrix CRMs (e.g. potato, maize, toasted bread, rice, etc.), V-KFT gives rather high measurement uncertainties, due to the starch matrix. To improve measurement repeatability and thus decrease uncertainty for these difficult matrix CRMs, vaporisation coulometric Karl Fischer titration (vap-C-KFT) was improved and optimised.

Vap-C-KFT uses an automated oven sample processor connected with a diaphragm-free coulometric titration cell. The working principle of vap-C-KFT is based on heating a closed glass vial containing a pre-weighed sample and transferring the evaporated water by means of a stream of dried air or inert gas into the anolyte present in the titration cell. The amount of water is then stoichiometrically determined. The method of vap-C-KFT has already been used successfully for water content determination in coal, motor oils, etc. (Margolis, Vaishnav, & Sieber, 2004a, 2004b) as well as in gliadin (Bernreuther & Klein, 2005) and pharmaceutical lyophilisates (Mihatsch & Isengard, 2001).

This study assesses robustness and measurement uncertainty of vap-C-KFT for determining the water content in eight different candidate and certified biological matrix reference materials. The primary robustness variables tested were type of carrier gas, gas flow rate, sample size and oven temperature. The oven temperature has proved to be the most critical variable. Low oven temperatures lead to underestimated water contents and overheating may accelerate non-enzymatic browning reactions and/or decomposition that lead to additional water formation (Anese, Nicoli, Massini, & Lerici, 1999).

In a full validation study, measurement uncertainties and their correlation for both Karl Fischer methods were estimated. Each sample was analysed in triplicate during five different days. Uncertainties for repeatability and intermediate precision were obtained by ANOVA (analysis of variance). Both individual uncertainty components were then combined and multiplied by a coverage factor ($k = 2$; level of confidence of 95%), to arrive at the expanded uncertainty.

Furthermore, samples were analysed by vap-C-KFT and checked for residual moisture and water by means of thermogravimetry and near infrared spectrometry (NIR) (ASTM E 1868-04, Derksen, Van de Oetelaar, & Maris, 2004; Derksen et al., 1998; May, Wheeler, & Grim, 2001; Reh, Gerber, Prodolliet, & Vuataz, 1986; Zhou, Hines, & Borer, 2006; Zhou et al., 1998).

2. Materials and methods

2.1. Samples

The following freeze-dried candidate reference materials and CRMs were produced and obtained from IRMM: rye flour (ERM®-BD381, candidate RM), wheat flour (ERM®-BD382, candidate RM), skim milk powder (BCR-685), toasted bread (ERM®-BD273), cabbage powder (BCR-679), GMO maize powder MIR604 (ERM®-BF423c), pork muscle (ERM®-BB384, candidate RM) and cotton seed powder (ERM®-BF422a).

2.2. Volumetric Karl Fischer titrations (V-KFT)

V-KFT measurements were carried out at a constant temperature of 40 °C using a 758 KFD Titrino titrator (Metrohm, Herisau, Switzerland), equipped with a titration stand 703 and a thermostatic titration vessel. The solid matrix samples were directly added to the vessel containing approximately 40 mL of dry methanol (Merck, Darmstadt, Germany) and titrated with a one-component reagent, Composite 5 (Merck, Darmstadt, Germany). The solvent was changed after each triplicate measurement. The performance of the titration method was checked by determining the water content of sodium tartrate dihydrate with a certified water content of 15.66 g/100 g (Sigma–Aldrich, Seelze, Germany). Titration conditions were a minimum extraction time of 180 s, a start and stop drift of 10 μL/min, a polarisation current of 50 μA and an endpoint detection voltage of 250 mV. Sample masses ranged between 0.2 and 0.8 g.

2.3. Vaporisation coulometric Karl Fischer titration (vap-C-KFT)

A 774 Oven Sample Processor connected with a 756 KF Coulometer (Metrohm, Herisau, Switzerland) was used for vap-C-KFT determinations. A C-KFT water standard with a certified water content of 0.0998 g/100 g (Sigma–Aldrich, Seelze, Germany) and an oven standard with a certified water content of 1.00 g/100 g (Merck, Darmstadt, Germany) were used to verify the method and instrument performances of the coulometer and the oven, respectively. Iodine was generated in a diaphragm-free cell at a constant current of 10 μA and the endpoint of the titration was set at a voltage of 50 mV. Other titration variables were a minimum extraction time of 600 s, a start drift of 10 μg/min and a stop drift of 20 μg/min.

Sample masses of 0.3 ± 0.1 g were accurately weighed into 7 mL sample vials, the sample vials were hermetically closed using crimp caps (containing a septum). Double dried air was used as carrier gas (constant flow of 50 mL/min) to transfer the water released by the heated sample into the titration vessel. Different oven temperatures (110 to 140 °C) were applied in the isothermal mode.

2.4. Thermogravimetric analyses (TGA)

Thermal stability of the samples was investigated using a SETSYS TG-DSC 800/1600 analyser (Setaram, Caluire, France). The instrument was kept at 15 °C and purged with a constant flow of argon; measurements were performed under helium atmosphere. Approximately 30 mg portions of the samples were accurately weighed in open standard aluminium crucibles (40 μL); an empty crucible was used for reference. Thermograms were determined by heating the samples from 25 °C up to 140 °C with a heating rate of 30 °C/min, followed by an isothermal step for 2500 s.

2.5. Acousto optical tunable filter near infrared spectroscopy (AOTF-NIR)

Before and after vap-C-KFT measurements, samples were transferred into 100 mL amber glass vials and scanned by a Luminar Model 4030 AOTF-NIR spectrometer (Brimrose, Baltimore, MD, USA) for detection of residual water. 100 spectra between 1300 and 2100 nm were collected over 5 s.

3. Results and discussion

3.1. Robustness of vap-C-KFT

Instrument stability was found to be one of the most important and critical issues for reliable and accurate vap-C-KFT results. In general, the instrument performance is consequently checked by determining the water content of a certified oven standard. Fig. 1 shows the influence of measuring three blanks before the first sample, in this case an oven standard with an absolute water content of 1.00 g/100 g. The first 18 measurements were each time obtained as the first sample of a sequence, thus with measuring only one blank in advance. The 18 other measurements were each time preceded by three blanks. The approach of measuring three blanks in advance clearly shows that these three blanks have a significant influence on the performance, stability and reproducibility of the instrument, as the relative standard deviation could be reduced from 4.3% to 1.2%.

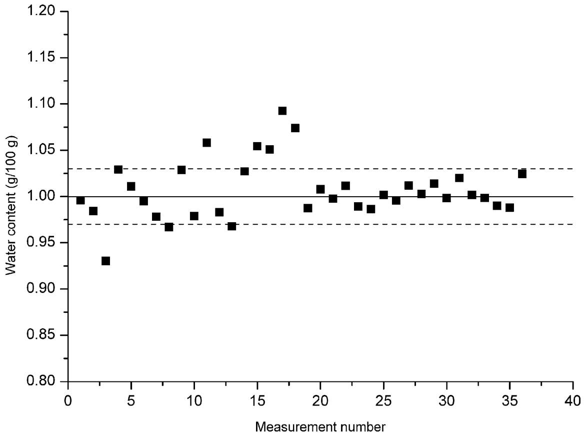

Fig. 1. 36 instrument performance qualifications of vap-C-KFT by measuring an oven standard with certified water content (1.00 g/100 g; uncertainty is 0.03%) without blanks (measurements 1–18) and preceded by three blanks (19–36).

The minimum sample mass determined for the oven standard correlates with an absolute water content of approximately 2 mg.

To examine potential sources of variability, the water contents of skim milk powder and maize were measured under varying individual operating variables (gas type, gas flow rate and sample size). Three different gas flow rates (20, 50 and 100 mL/min) and three different sample sizes (100, 300 and 500 mg) were tested in triplicate for maize. Significantly lower water contents ($P < 0.05$) were found for the highest gas flow rate and the lowest sample size. The latter agrees with the minimum sample intake as the absolute water content of the samples was below 2 mg.

The influence of the gas type (air and nitrogen) was investigated for skim milk powder. Non-enzymatic browning (Maillard reaction) (Anese et al., 1999), which is known to be the most important mode of deterioration in milk powders, is initiated by a thermal condensation reaction between an amino compound and the carbonyl group of a sugar. One could suppose that a combination of high oven temperatures and air atmosphere could accelerate these condensation reactions. Therefore, the influence of an inert gas (nitrogen) was tested at an oven temperature of 110 °C. No significant deviations were observed.

3.2. Oven temperature optimisation

The most critical variable for vap-C-KFT is the oven temperature. On one hand, the oven temperature should be high enough to allow evaporation but on the other hand overheating may result in additional water production caused by condensation reactions. Unfortunately, a universal oven temperature for all materials cannot exist, since all matrix materials behave in a different way when exposing them to heat.

Rye and wheat flour were both tested at different oven temperatures (110, 120, 130, 140 and 150 °C). A temperature of 130 °C resulted in the best accuracy and acceptable repeatability compared to V-KFT. The optimum oven temperature found for skim milk powder and maize was 110 °C. Cotton seed and pork muscle were both measured at 130 °C.

Toasted bread is one of the materials, which releases water very slowly during V-KFT. Therefore, vap-C-KFT has been optimised for toasted bread by evaporating the water at different oven temperatures (Fig. 2). Temperatures below 130 °C were found to be insufficient because of very long extraction times (>120 min). Above 150 °C, increased water contents were observed due to thermal

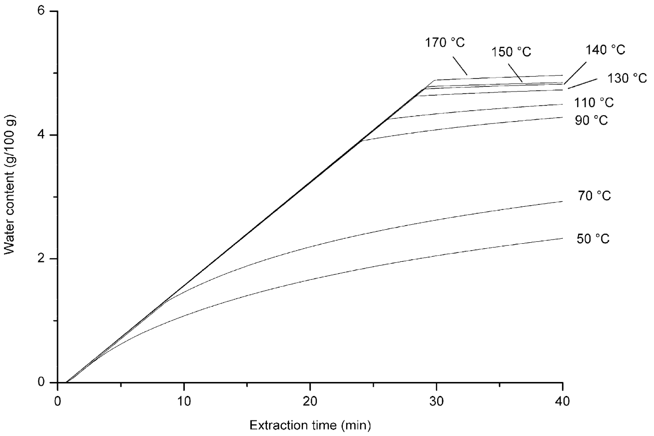

Fig. 2. Vap-C-KFT isothermal programs for toasted bread.

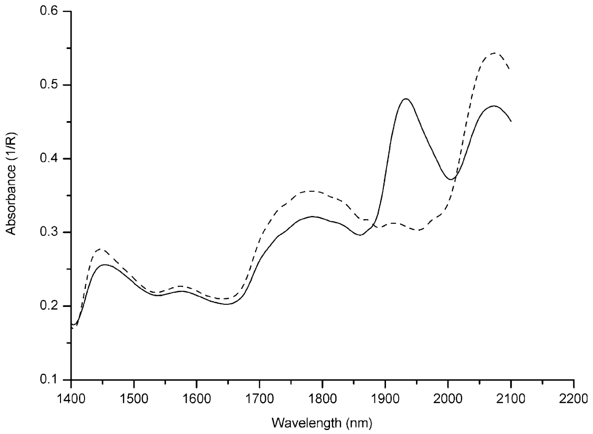

Fig. 4. NIR spectra of native toasted bread (solid line) and toasted bread after vap-C-KFT (dashed line).

decomposition. The optimum oven temperature for toasted bread samples proved to be 140 °C.

The selection of the optimum vap-C-KFT oven temperature was supported by checking the sample for residual moisture and water using thermogravimetric analysis (TGA) and acousto optical tunable filter near infrared spectroscopy (AOTF-NIR).

Fig. 3 presents the TGA mass loss profiles for both native toasted bread and toasted bread which was measured in advance by vap-C-KFT (isothermal at 140 °C). Both samples were heated from 25 °C to 140 °C at 30 °C/min and exposed isothermally (140 °C) for 40 min. The data clearly indicate that toasted bread samples measured by vap-C-KFT at an isothermal temperature of 140 °C are thermally stable. Moreover, all the water has been evaporated since changes in mass loss could not be observed.

Results of TGA were confirmed by AOTF-NIR. Overlaid spectra of native toasted bread and toasted bread after vap-C-KFT for the wavelength region 1400–2200 nm are shown in Fig. 4. It is evident from Fig. 4 that there is a significant decrease of absorbance values at about 1950 nm for the sample after vap-C-KFT; this may serve as a marker band for water. The absence of a peak at this particular wavelength can be related to very low water contents or only traces of water (Stapelfeldt, Meisen, & Skibsted, 1997). This peak was not found in the sample which had been measured by vap-C-KFT.

For production control, AOTF-NIR can be a powerful tool for the on-line water content monitoring of samples in sealed vials (e.g. reference materials, pharmaceuticals, etc.). The most important advantages of this approach are the non-destructive character of the method and the high measuring speed. Nevertheless, AOTF-NIR cannot replace Karl Fischer completely since a reliable calibration model is necessary to predict the final water content in the vials. The use of AOTF-NIR to estimate the water content in certified reference materials during production will be discussed elsewhere (Kestens, Charoud-Got, Bau', Bernreuther, & Emteborg, in press).

3.3. Method validation and uncertainty estimation

The purpose of this study was to evaluate statistically the performance of the vap-C-KFT method. In the first part, accuracies of water content determinations obtained by V-KFT and vap-C-KFT were compared. As can be seen in Fig. 5, for almost all tested materials an excellent correlation was achieved ($y = 0.172 + 0.977x$; $R^2 = 0.998$) except for toasted bread. V-KFT results were significantly lower possibly due to matrix effects. Therefore, the results from toasted bread were not taken into account for the linear regression.

Once the oven temperature for the vap-C-KFT was optimised for each matrix material, the water content was determined under intermediate precision conditions (5 days with 3 replicates per day). By means of one-way ANOVA (analysis of variance) the matrix-dependent measurement uncertainties were calculated, which are graphically presented in Fig. 6 (relative expanded uncertainties, based

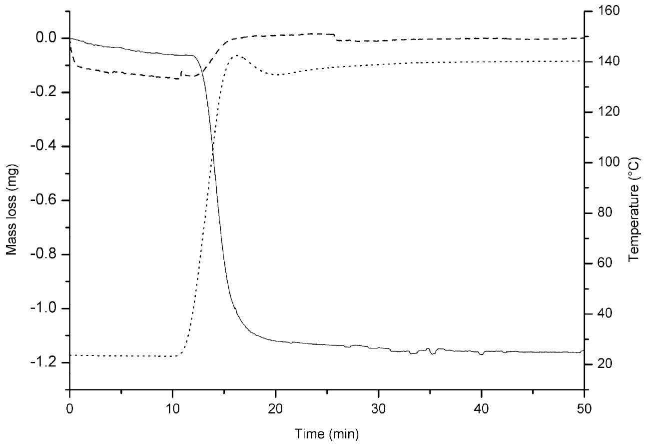

Fig. 3. Thermogram of native toasted bread (solid line) and toasted bread after vap-C-KFT (dashed line), temperature program (dotted line).

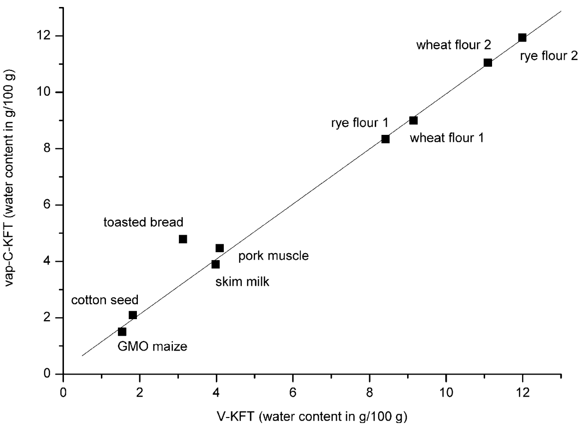

Fig. 5. Correlation between V-KFT and vap-C-KFT water content determinations (results for toasted bread were not taken into account).

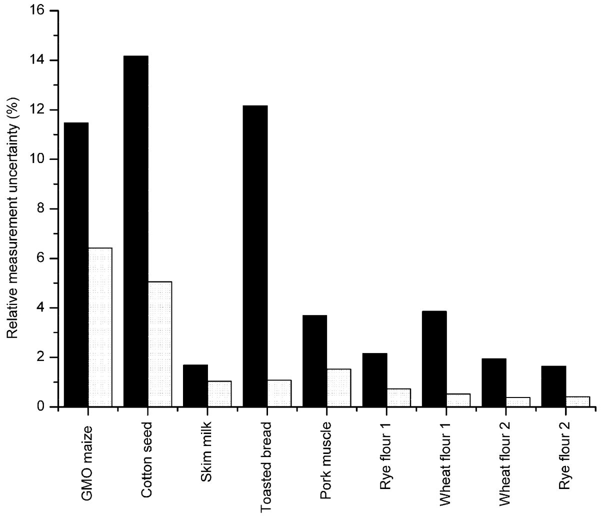

Fig. 6. Relative measurement uncertainties ($k = 2$; $n = 3$) for V-KFT (black) and vap-C-KFT (grey).

on triplicate measurements with a coverage factor $k = 2$ for a confidence level of about 95%). For most of the tested materials, the vap-C-KFT results are accompanied with significantly smaller uncertainties than the V-KFT results.

4. Conclusion

Vap-C-KFT can be fully automated with less reagents, working time, sample size, waste and with improved repeatability and intermediate precision. Therefore, vap-C-KFT becomes increasingly attractive and feasible for water content determination of materials, such as reference materials containing starch. Further investigation is ongoing for other difficult matrix reference materials (e.g. cabbage, potato, rapeseed and sugar beet). In addition, environmental matrices such as sewage sludges, soils and sediments as well as clinical materials (e.g. human serum) will be included in the further development of vap-C-KFT methods.

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