Online measurement of water content in candidate reference materials by acousto-optical tuneable filter near-infrared spectrometry (AOTF-NIR) using pork meat calibrants controlled by Karl Fischer titration

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Abstract

Certified reference materials (CRMs) are prepared from a wide variety of matrices. Water removal is an excellent way of achieving increased matrix lifetime and hence CRM stability. High-speed acousto-optical tuneable filter near-infrared spectrometry (AOTF-NIR) has been implemented for measurement of water content in powder matrix reference materials in amber glass vials. Almost 50,000 spectra were collected from 1300 to 2100 nm with a 2 nm increment for powders of meat, rye grass, potato, cotton seed and sugar beet. The AOTF-NIR instrument was placed in a capping machine, with a measurement frequency of 10–15 vials/min and a trigger signal for reproducible collection of spectra. The calibrants comprised 19 pork meat powder samples equilibrated with different hygrostatic solutions or subjected to oven drying to achieve different water concentrations. Mixtures of powders with different water content were also prepared in order to obtain a calibration range from 0.5 to 8.3% water (m/m). All calibration samples were measured by volumetric Karl Fischer titration (V-KFT), accredited under ISO 17025. The calibrants were then measured by AOTF-NIR together with the samples. Multiplicative scatter correction (MSC) was applied to the absorbance spectra in order to correct for the scattering of light in the different powders and scattering effects from the vials. A partial least squares regression model (PLS) based on two principal components was created and applied for prediction of water content in the samples with a standard error of 0.5% water (m/m).

The AOTF-NIR has the potential of rapidly monitoring a large number of samples of different materials with good accuracy as demonstrated by the good agreement with V-KFT. Nevertheless, it is necessary to expand the number of calibration models for different vial sizes as it turns out that MSC cannot correct properly for the influence of scattering of light due to the different vial sizes, in this case 100 mL and 10 mL vials. The influence of the matrix seems not to be critical because the prediction of the water content in a wide variety of matrices was successful using the meat powder as a universal calibrant.
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Keywords: Certified reference materials; Reference material processing; Water content; AOTF-NIR; Karl Fischer titration; MSC; PLS; Pork meat; Cotton seed; Potato; Sugar beet; Rye grass

1. Introduction

Water removal generally renders materials of biological origin much higher long term stability in comparison with wet or fresh materials provided that it is known how the remaining water is bound (Mathlouthi, 2001). Determination of water content alone can therefore not be used as the basis for a long-term stability assessment of a matrix (Mathlouthi, 2001). Before a CRM is released by IRMM, long term stability studies of at least one year are performed to check all analytes to be certified in a matrix over time. A deeper discussion on CRM stability is outside the scope of the present work but nevertheless, a lifetime

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of 4–8 years for a CRM is desirable given the efforts in the production of a material and the laboratory needs to use CRMs for long term comparisons of their performances. The commutability of the CRM may also be in question if the CRM does not behave in the same way as a 'real' sample in the analytical chain, compromises always have to be made between what is technically possible to produce, material life-time and commutability. Therefore, drying is almost always a part of the processing sequence of a matrix reference material. Drying can be achieved by many different means such as freeze drying, vacuum drying, spray drying or oven drying. Common to all drying techniques is that the residual water content has to be controlled by the process control laboratory at regular intervals in the processing sequence. Milling and homogenisation are other activities that are performed on these materials to reduce the particle size in order to achieve reasonable powder properties during mixing and filling and to provide sufficiently low minimum sample intakes. As a process control tool, checking for water content may be relevant at any stage in the process given the hygroscopic nature of some powders. Until recently, however, only 10–15 samples over the filling sequence of the whole production batch of, for example 4000 units, were characterised for their water content. As near-infrared spectrometry provides a non-invasive tool for online measurement of water in every unit leaving the production, it was decided at IRMM to implement such a measurement possibility in order to be able to detect possible trends and to have a better overview of the water content in all units produced. An initial evaluation of different NIR instruments, performed on different powders in amber and transparent glass vials, revealed that a high speed free-space AOTF-NIR instrument, based on measurement of reflected light, could provide a reliable alternative for implementing online measurements of water. As NIR relies on external calibration and partial least squares regression (PLS) models for prediction of water content in the samples, 18 calibrants of pork meat powder, in the range from 0.5% to 8.3% water (m/m) in amber glass vials were used to create a calibration model which was then applied to different powder matrices of meat, rye grass, sugar beet, cotton seed and potato. Various examples exist in the literature describing NIR applications for measuring freeze dried products although the speed of analysis and applicability to more than one material is limited in comparison with this work (Derksen, van de Oetelaar, & Maris, 1998; Roggo, Roeseler, & Ulmschneider, 2004; Stokvold, Dyrstad, & Libnau, 2002; Xiangji, Hines, & Borer, 1998). However, for applications using AOTF-NIR in various fields such as food or pharmaceuticals the readers are directed to the Brimrose homepage http://www.brimrose.com/. The aim of work is the implementation of high speed AOTF-NIR for online monitoring of water content in all vials produced of different candidate reference materials at IRMM using, if possible, one universal calibrant for several different sample matrices.

2. Experimental

2.1. Preparation of meat powder calibrants

At least 15 calibrants are normally required for a robust PLS model. Therefore 1 kg of homogenised freeze dried pork meat powder was obtained directly from the processing facility at IRMM. From this batch, further called "master batch" (MB), 8 samples, as so-called secondary batches (SB) were taken, transferred into 250 mL brown glass bottles and closed with a polythene insert and a screw cap. Two SBs were oven dried at 103 ± 2 °C for 24 h. The other SBs were exposed to different relative humidities, by placing them for 24–72 h in desiccators together with saturated solutions of different salts. The hygrostatic solutions are summarised in Table 1.

After 24–72 h of equilibration, the water content of the different batches was determined using volumetric Karl Fischer titration (V-KFT). To obtain more samples of different water content between 0.5 and 8.3% (m/m), new batches, called mixed batches (MiB), were created by mixing different samples of MB and SBs using a bench-top Turbula mixer (WAB, Basel, CH) for 5 min. Finally, the water content was measured in the 19 resulting batches of MB, SBs and MiBs using a 758 KFD Titrino volumetric Karl Fischer titrator, (Metrohm, Herisau, CH) by a procedure accredited under ISO 17025 (Table 2). Expanded relative measurement uncertainty for V-KFT results of 7.0% was obtained from validation measurements of BCR-384 pork muscle powder. Note that sample J was excluded from the model since the oven drying applied changed the NIR spectrum of this sample and that it was identified as an outlier while included in the model. All calibrants, contained in either 100 mL vials or 10 mL vials were measured at regular intervals during collection of spectra from the different candidate CRMs.

2.2. Implementation of AOTF-NIR in a capping machine and data treatment

The high speed Brimrose Luminar 4030 AOTF-NIR module (Applitek, Nazareth, BE) was attached to a small

Table 1
Overview of the different batches, treatment and hygrostatic salt solutions, MB = master batch and SB = secondary batch

Batch	Treatment[a]
MB	–
SB	Oven dried at 103 ± 2 °C for 24 h
SB	Oven dried at 103 ± 2 °C for 24 h
SB	$LiBr_{(sat)}$
SB	$LiCl_{(sat)}$
SB	$KAc_{(sat)}$
SB	$MgCl_{2(sat)}$
SB	$K_2CO_{3(sat)}$
SB	$NaBr_{(sat)}$

[a] Treatment means that the powders were equilibrated in closed atmospheres in desiccators where the relative humidity was dictated by the saturated salt solutions.

Table 2
Average water content in the calibrants C–U is expressed in % (m/m) as determined by V-KFT based on duplicate measurements. MiB = mixed batch

Batch	Sample code	Mean V-KFT (g/100 g)	Uncertainty (g/100 g)
SB	Sample C	7.58	0.53
SB	Sample D	3.86	0.27
SB	Sample E	4.84	0.34
SB	Sample F	6.00	0.42
SB	Sample G	6.08	0.43
SB	Sample H	8.26	0.58
MB	Sample I	3.69	0.26
SB	*Sample J*	*0.53*	*0.04*
MiB	Sample K	2.58	0.18
MiB	Sample L	1.50	0.11
MiB	Sample M	7.89	0.55
MiB	Sample N	5.46	0.38
MiB	Sample O	1.04	0.07
MiB	Sample P	3.13	0.22
MiB	Sample Q	3.80	0.27
MiB	Sample R	3.36	0.24
MiB	Sample S	3.87	0.27
MiB	Sample T	2.34	0.16
SB	Sample U	5.22	0.37

custom-made aluminium table that could be precisely adjusted in height and distance to the measurement object. The table was then placed next to the star wheel in a capping machine from Bausch und Ströbel (Ilshofen, DE) with an optimum distance of about 60 mm from the AOTF-NIR window to the measurement object. By adjusting the position of the table the raw signal from the material had to reach at least 15,000 counts in order to collect spectra of sufficient signal to noise ratio. On the one hand the measurement spot is only 6 mm in diameter, the initial alignment is therefore rather delicate. On the other hand it is an advantage that measurements can be successfully performed on small amounts of sample if the material homogeneity is ensured in the sample container. The capping machine provided a suitable measurement frequency of 10–15 vials/min for collection of spectra as the star-wheel is moving vials into the capping position. Collection of spectra commenced as soon as a vial had passed an optical sensor which was directly connected to the NIR module with a trigger delay of 200 ms. In this way reproducible collection of 100 spectra from 1300 to 2100 nm with a 2 nm increment could be realised for every sample that was placed in the capping machine. With these settings the collection frequency of spectra amounted to approximately 0.25 Hz. The diffuse reflectance of the transmission spectra from the material was collected in ratio mode (to the internal standard in the AOTF-NIR module). After the samples were measured, the data files containing the average transmission spectra were converted to absorbance spectra and translated into Unscrambler® files using the Brimrose (Baltimore, MD, USA) SNAP!™ 2.03 software. After data conversion all further data treatment was performed using the Unscrambler® software, version 8.0.5 (CAMO, Oslo, NO). The data in the spreadsheets containing absorbance values from 400 wavelength positions and sometimes many thousands of samples per file were transformed using multiplicative scatter correction (MSC) that will correct for differences in offset and slope between spectra making them directly comparable to each other. Once the calibration models were generated using NIR spectra of the calibrants a PLS model using two principal components and one response parameter (water content) was applied to all samples for the prediction of water content. For checking of instrument performance the 18 calibrants were normally
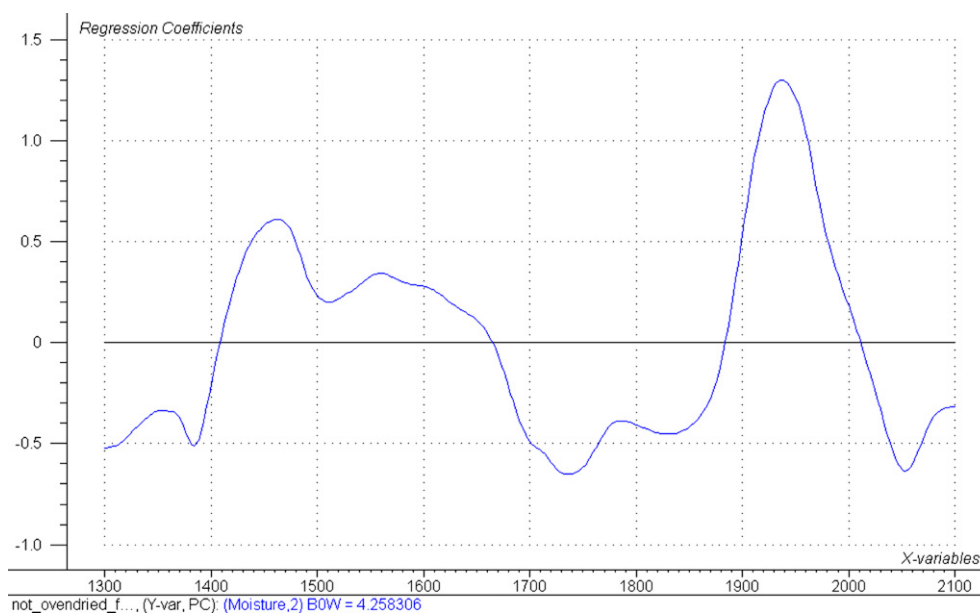

Fig. 1. Regression coefficients for the PLS model as a function of the wavelength for two Principal Components applied to meat powder calibrants in 100 mL vials.
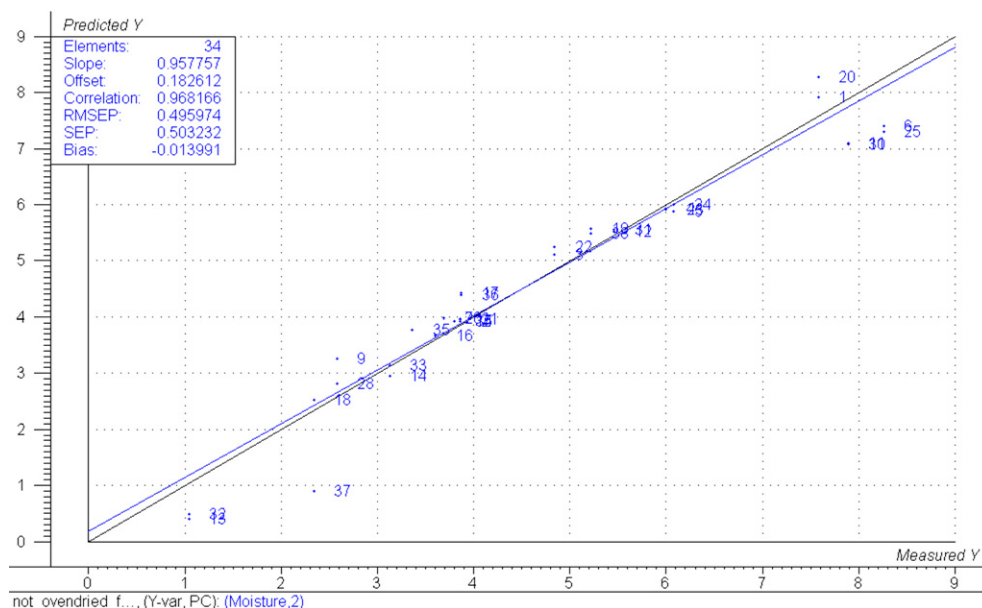
Fig. 2. Predicted (AOTF-NIR) vs. measured (KFT) water content (Y).

3. Results and discussion

3.1. Calibration and PLS model

The regression coefficients as a function of wavelength are depicted in Fig. 1 for two principal components and 18 calibrants in the 100 mL vials. Spectral information relating to water (O–H bands) is present at 1450 and 1950 nm, respectively. By using the PLS model the predicted water content is plotted against the measured (V-KFT) water content in Fig. 2. The correlation coefficient is 0.97 with a reasonably low standard error of prediction of 0.5% water despite the high sample throughput. It should be mentioned that the calibrant J, containing 0.53 % water (Table 2) was excluded from the model as it was identified as an outlier and its NIR spectrum was different from the non-oven dried samples.

3.2. Results for candidate reference materials

In Table 3 all data generated with the AOTF-NIR has been averaged and reported together with results obtained by Karl Fischer titration. The respective standard deviations of the individual measurement results overlap for all investigated samples except one. Hence, there is no statistically significant difference between the results obtained by AOTF-NIR in comparison with KFT. For the non-GMO cotton seed sample there is a small bias between the techniques for which no clear explanation can be given except that the cotton seed samples were notoriously difficult to measure by V-KFT. To give a few examples for

re-run with all sample types at the beginning and in the end of every day of spectral collection.

Table 3
Comparison of results between AOTF-NIR and volumetric KFT in the candidate reference materials where the number of units (n) is mentioned in parentheses

Matrix and candidate reference material and sample mass	Water content in % (m/m) by AOTF-NIR, $\pm$ one SD	Water content in % (m/m) by KFT, $\pm$ one SD
Pork meat 18 g	3.85 $\pm$ 0.33 (3000)	4.00 $\pm$ 0.30 (3)
Rye grass 10 g	2.70 $\pm$ 0.16 (2425)	2.68 $\pm$ 0.01 (10)
Non-GMO potato 1.0 g	1.74 $\pm$ 0.15 (7021)	1.64 $\pm$ 0.08 (2)
GMO potato 0.5 g	1.35 $\pm$ 0.35 (12,772)	1.15 $\pm$ 0.18 (2)
Non-GMO sugar beet 1.0 g	1.40 $\pm$ 0.36 (4402)	1.80 $\pm$ 0.21 (10)
GMO-sugar beet 1.0 g	1.77 $\pm$ 0.34 (5781)	2.11 $\pm$ 0.15 (15)
Non-GMO cotton seed 1.0 g	2.14 $\pm$ 0.25 (4047)	1.42 $\pm$ 0.15 (10)
1% GMO cotton seed 1.0 g	0.60 $\pm$ 0.25 (3442)	0.65 $\pm$ 0.09 (10)
10% GMO cotton seed 1.0 g	0.54 $\pm$ 0.21 (3093)	0.63 $\pm$ 0.07 (10)

The different units measured by KFT were measured at least in duplicate. The first two materials were contained in 100 mL vials and the last seven were contained in 10 mL vials. SD = standard deviation.

whole production batches, the water content has been plotted against the unit number which corresponds to the filling order in Figs. 3a–3d.

3.3. Water content in pork meat and rye grass materials

Results for the meat (3000 samples) and rye grass (2425 samples) are displayed in Figs. 3a and 3b, respectively. For the meat samples the overall result is 3.85 $\pm$ 0.33% (m/m) H_2O for all units which is in good agreement with the 4.00 $\pm$ 0.30% obtained by Karl Fischer titration (Table 3). Similarly, for the rye grass the average AOTF-NIR result is 2.70 $\pm$ 0.16% H_2O (m/m) for all units which is in good agreement with the 2.68 $\pm$ 0.01% obtained by V-KFT (Table 3). No trend for water content in relation to the filling order can be found for neither of the materials
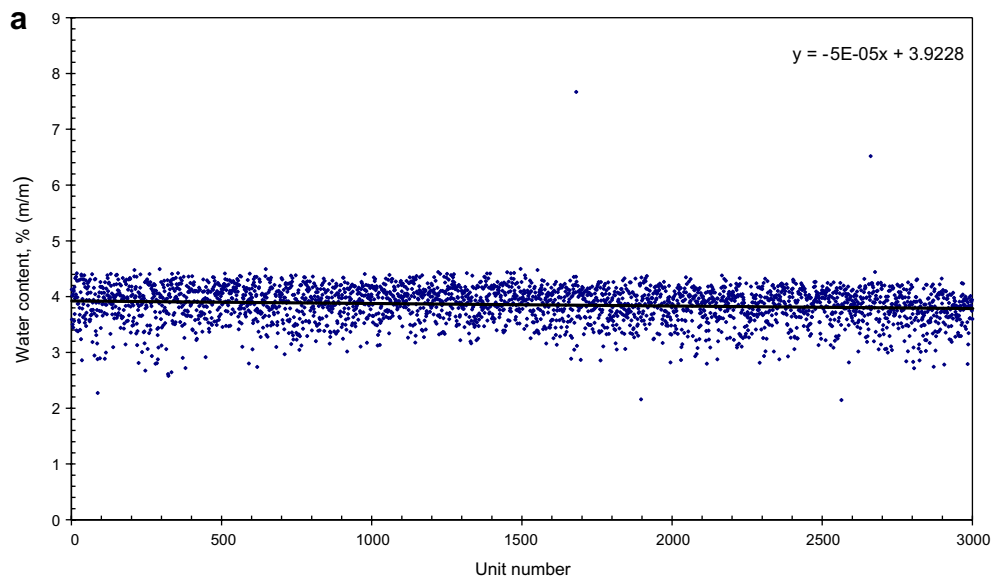
Fig. 3a. Water content in the pork meat material for all 3000 units produced.
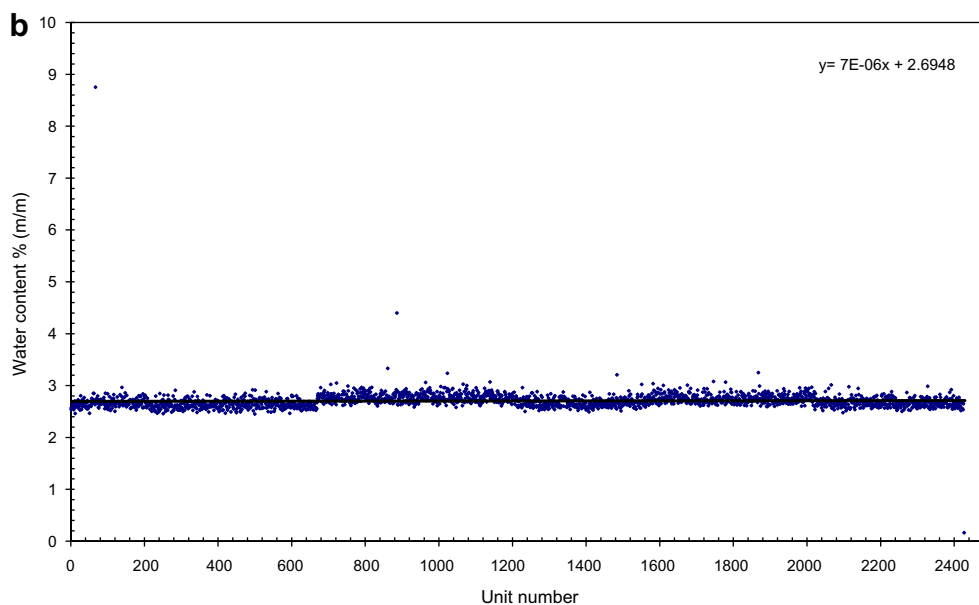

Fig. 3b. Water content in the rye grass material for all 2425 units produced.

and the water content is uniform throughout both production batches as can be seen from the trend lines and their equations. A few very high water content values were measured in both materials using AOTF-NIR. Such spurious results are artefacts associated with corrupt spectra which are induced by dirty surfaces on the vials or a strong reflection due to varying glass thickness too large to be corrected by MSC.

3.4. Water content in non-GMO sugar beet material

The water content as a function of filling order for the non-GMO sugar beets is displayed in Fig. 3c. The three sets of higher values come from the 19 calibrants that were run together with the samples over the two day measurement period. Results covering the last 4400 units of totally 5847 units are displayed. The most interesting feature in Fig. 3c is the sudden shift in water content of about 0.5% (m/m). When looking back into the documentation it can be established that this shift occurs suddenly in the middle of a filling sequence of roughly 1500 units. No explanation can currently be given that relates the difference in water content to anything occurring in the filling station. Fig. 3c provides new information of possible processing problems due to variations in water content using the AOTF-NIR technology which would be very difficult, if not impossible, to see with 10 units distributed over the whole production batch only relying on Karl Fischer
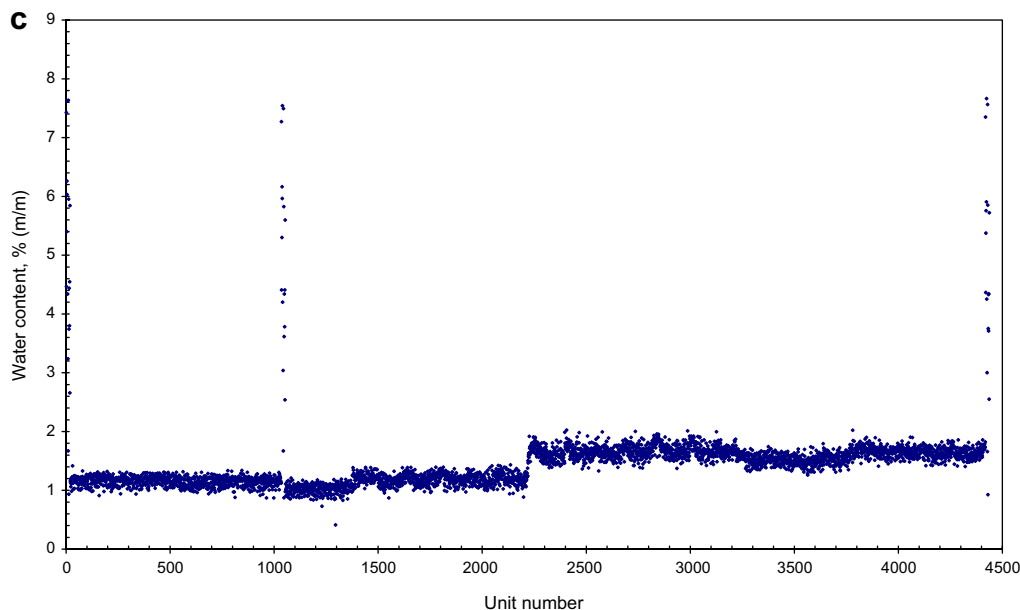
Fig. 3c. Water content in the non GMO sugar beet material showing the last 4400 of 5847 units produced.
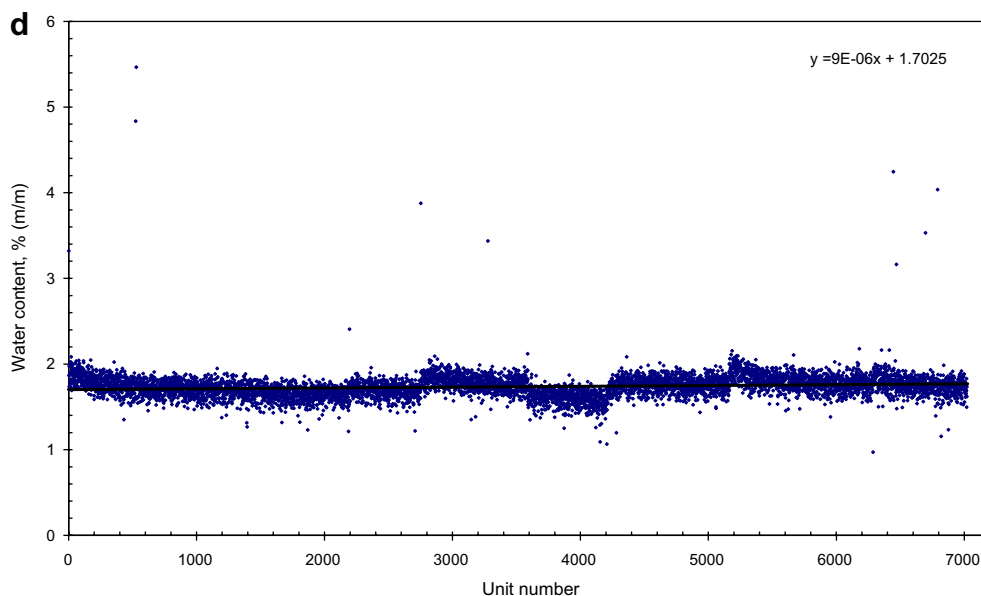

Fig. 3d. Water content in the non GMO potato material for all 7021 units produced.

titration as mentioned above. Moreover the overall result is $1.40 \pm 0.36\%$ H$_2$O (m/m) using AOTF-NIR for the 4400 units measured is in reasonable agreement with the result of $1.80 \pm 0.21\%$ obtained by Karl Fischer titration (Table 3).

3.5. Water content in non GMO potato material

The non GMO potato material is displayed in Fig. 3d. A few very high values for the water content were observed over the 7021 samples which were normally associated with corrupt spectra as described above. The overall result is

$1.74 \pm 0.15\%$ (m/m) H$_2$O for all units which is in good agreement with the $1.64 \pm 0.08\%$ obtained by Karl Fischer titration (Table 3). For this material no trend for water content in relation to the filling order can be found and the water content is uniform throughout the whole production batch. The small but sudden shifts in water content may be associated with the loading of new bags filled with potato powder into the hopper. Unfortunately no records exist to prove this hypothesis but the potato powder was clearly hygroscopic so therefore it was manipulated carefully and it was brought to the filling machine bag by bag which previously had been sealed. It is still possible
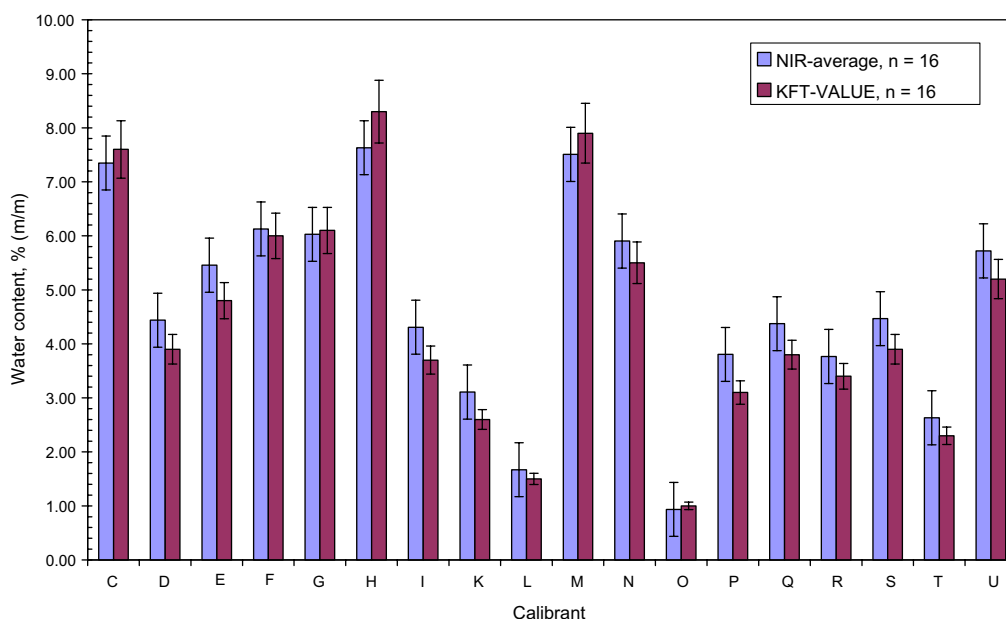
Fig. 4. Water content in the 18 calibrants used for the PLS model measured with AOTF-NIR and Karl Fischer titration. Error bars are standard error of prediction for the AOTF-NIR data and expanded uncertainty for the Karl Fischer data.

that small differences in water content were present between these bags.

3.6. Verification check of re-run calibrants

In Fig. 4 the average water content of the calibrants (measured over a three month period) are displayed in a bar chart and compared with values obtained by V-KFT from Table 2. The results demonstrate good comparability between the water content obtained by V-KFT and the AOTF-NIR for each set of calibrants (excluding calibrant J as mentioned before). Expanded uncertainties for the KFT results and the standard error of prediction for the AOTF-NIR data are of comparable magnitude.

4. Conclusions

The AOTF-NIR clearly has the potential of monitoring a large number of samples with reasonable accuracy at very high speed. Although the standard error of prediction of 0.5% water (m/m) in absolute terms may seem somewhat high, it is certainly fit for purpose. No dramatic effects can be expected for a candidate reference material if it is 0.5% higher or lower in water content than an expected target value. Since certified values normally are reported on a dry mass basis the final analytical result would therefore change 0.5% up or down if the water content can not be determined with a higher precision. This is under normal circumstances negligible in comparison with for example the measurement repeatability which normally accounts for several % RSD. Moreover, the results clearly show good comparability between AOTF-NIR and Karl Fischer titration. It is necessary to expand the number of calibration models for different vial sizes as it turns out that the

MSC cannot correct for the influence of light scattering due to different vial sizes. For instance, a systematic difference of about +1% was found for the non-GMO potato material in comparison with KFT when using the calibration model developed for 100 mL vials for prediction. By refilling the pork meat calibrants in 10 mL vials, a new model could be created that immediately delivered a correct result. The corrupt spectra may be induced by dirty outer surfaces of the vials or a strong reflection due to varying glass thickness too large to be corrected by MSC. This is however a limited problem since it does not occur frequently. The fill-amount of material in the vial is more important for collecting spectra of good quality. For the GMO potato powder only about 0.5 g of material was available in the vial which was really at the limit for collecting of NIR spectra suitable for quantification of water.

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