19ENG09 BIOFMET (D7)

New metrological methods for biofuel materials analysis

Good Practice Guidelines on uncertainty assessment of biofuel measurements

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Contents

1. SCOPE

The purpose of this guideline is to provide guidance in how to estimate the uncertainty related to biofuels measurement.

The guide considers measurement of water content and impurities in liquid and solid biofuels. Guidelines for estimating the uncertainty due to sampling is provided and the potential in using machine learning techniques for optimising calibration curves is discussed.

2. INTRODUCTION

This document provides guidance for interpreting the requirements of ISO 17025:2017 clause 7.6 "Evaluation of measurement uncertainty" [1] and how they apply to the testing and calibration that is carried out in accredited laboratories. It also outlines the fundamental principles and potential methods for evaluating measurement uncertainty for quantitative testing.

The guide considers measurement of water content and impurities in liquid and solid biofuels. The guide include advice in estimating uncertainty due to sampling and discuss the potential in using machine learning techniques for optimising calibration curves.

The development of this document is made as part of the 19ENG09 BIOFMET project and the document provides information on the developed analytical methods for determination of impurities in selected liquid and solid biofuels with accent on on-line and laboratory methods for industrial application. Analytical methods developed, optimized, and validated within the course of the project have been defined in terms of the measuring principle and mathematical models used to generate data. The associated measurement uncertainties follow the developed models and consider all recognized uncertainty sources.

3. TERMS AND DEFINITIONS

- **Analytical acceptance criteria:** Performance criteria applied to results obtained from the analysis performed. These criteria are pre-defined and are dependent on the nature of the product, the analytical procedure and the specification limits given in the monograph or in the marketing authorisation.

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- **Bias (measurement bias):** estimate of a systematic measurement error [2].
- **Combined standard uncertainty:** standard uncertainty of the result of a measurement when the result is obtained from the values of several other quantities, equal to the positive square root of a sum of terms, the terms being the variances or covariances of these other quantities weighted according to how the measurement result varies with these quantities [3].
- **Coverage factor, *k*:** numerical factor used as a multiplier of the combined standard uncertainty to obtain an expanded uncertainty, which is typically in the range 2 to 3. The choice of the factor *k* is based on the level of confidence required and on the set of data available. At the approximate level of confidence of 95 %, the *k*-value is usually set to 2, for normally distributed data. However, a correction factor (i.e. *t*-Student value) should be applied in the calculation of the standard uncertainty depending on the number of measurements [3, 4].
- **Expanded uncertainty, *U*:** quantity defining an interval around the result of a measurement that may be expected to encompass a large fraction of the values that could reasonably be attributed to the measurand. It is calculated from a combined standard uncertainty and a coverage factor *k* [3].
- **Level of confidence:** a number expressing the degree of confidence in a quoted result, e.g. 95 %. It represents the probability that the value of the measurand lies within the quoted range of uncertainty [3].
- **Machine learning:** the use and development of computer systems that are able to learn and adapt without following explicit instructions, by using algorithms and statistical models to analyse and draw inferences from patterns in data.
- **Measurand:** quantity intended to be measured [2, 6].
- **Standard uncertainty:** uncertainty of the result of a measurement, expressed as a standard deviation [3].
- **Systematic error (Systematic measurement error):** component of measurement error that in replicate measurements remains constant or varies in a predictable manner [2].
- **Type A evaluation (of uncertainty):** method of evaluation of uncertainty by the statistical analysis of series of observations [3].
- **Type B evaluation (of uncertainty):** method of evaluation of uncertainty by means other than the statistical analysis of series of observations [3].
- **Measurement uncertainty (*MU*):** a parameter associated with the result of a measurement that characterises the dispersion of the values that could be reasonably attributed to the measurand [6].
- **Uncertainty evaluation procedure:** the procedure used for estimating the overall uncertainty [6].
- **DUT**: the Device under test, i.e. the device being calibrated.

4. WATER CONTENT

4.1 Systems for water-content measurements

There exist several methods for measuring the water content in solid materials.

- In-line measurement: Measurements taken within a process or system while it's operational. Provides continuous real-time data for monitoring and control purposes.
- On-line measurement: Measurements made with an instrument that is connected to the process either continuously or in intervals, real-time or quasi-real-time monitoring and data collection.
- Off-line measurement: Measurements conducted in a controlled environment, such as a laboratory, on samples taken from the process or system.

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- At-line measurements: Measurements are taken close to the process using portable instruments, allowing real-time feedback and adjustments without interrupting the process.

This report will mainly focus on inline and online measurements. Methods applicable to inline/online measurements of water includes infrared (IR), radiofrequency (RF), microwave (MW), and acoustic techniques.

4.2 Uncertainty contributions related to the calibration of the measurement instrument

Calibration of equipment for online measurements of the water content of solid biofuels requires that the equipment is compared with a reference method on appropriate test sample material.

The calibration uncertainty includes the following contributions:

- Uncertainty associated with the reference measurement (using a reference method or transfer standard)
- Uncertainty associated with the in-homogeneity of the sample material (if only part of the test material is used for the reference measurements)
- Uncertainty associated with the calibration curve, usually determined using regression techniques
- Uncertainty due to short term stability of the DUT

Reference measurements

The reference values for the calibration are provided by either a transfer standard (e.g. an electromagnetic resonant cavity) or a reference method (e.g. based on the evolved water vapour technique). In either case, the method should have an uncertainty budget on its own. The combined uncertainty from this budget must be transferred here as $u_{reference}$.

Inhomogeneity / Sampling

The uncertainty of the water content, originating from the inhomogeneity in the material is found by extracting a number of samples, *n*, from the batch of biofuel. The water content of each sample is measured. Using the results, the average water content of the entire batch can be found, as well as the standard deviation of the water content.

For this exercise, at least three samples must be taken, although more samples are recommended. The samples must be taken randomly throughout the batch, making the probability of extracting any particular volume of biofuel identical.

The contribution from the inhomogeneity of the batch is found as the standard deviation of the measured values over the square root of the number of samples:

$$u_{inhomogeneity} = \sqrt{\frac{\sum_i^n (x_i - \bar{x})}{(n-1)\cdot n}} = \frac{s_{inhomogeneity}}{\sqrt{n}}. \quad (1)$$

Calibration curve determined using regression techniques

The relation between the reference values and the indication of the sensor being calibrated (DUT) is usually made using linear regression. The regression have an uncertainty contribution. The uncertainty of a linear regression is found as:

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$$u_{regression} = \sqrt{\frac{\sum_{i=1}^{n}\left(f_{x_i} - y_i\right)^2}{n-2}}, \quad (2)$$

where f_{x_i} is the value obtained from the DUT after correction, y_i is the reference value, and n is the number of samples used during the calibration.

Alternatively, if the average value of the readings using the working device is the measurand, rather than the individual value measured on a specific sample the uncertainty contribution is:

$$u_{regression} = \frac{1}{\sqrt{n}}\sqrt{\frac{\sum_{i=1}^{n}\left(f_{x_i} - y_i\right)^2}{n-2}}, \quad (3)$$

where n is the number of calibration samples.

Short Term Stability of DUT

If the instrument does not show the same result, when measuring repeatedly on the same sample, an uncertainty component must be associated, which takes this repeatability into account.

This uncertainty component can be minimized by measuring on the sample multiple times and taking the average value, where the uncertainty of this average value becomes:

$$u_{repeatability} = \sqrt{\frac{\sum_{i}^{n}(x_i - \bar{x})^2}{(n-1)\cdot n}} = \frac{s_{repeatability}}{\sqrt{n}}, \quad (4)$$

where x_i is the measurement result from a single measurement, $\bar{x}$ is the average value of all the measurements, and n is the number of measurements.

4.3 Additional uncertainty contributions related to inline/online measurements

Besides the calibration uncertainty, there will be additional uncertainty contributions in normal operation of the working device, which add to the total uncertainty of inline/online measurements.

- Long-term stability of DUT
- Type of biofuel
- Purity of sample

Long-term stability of DUT

Often, a significant drift over time of a measurement device occurs, where it will slowly start giving different readings when measuring the same quantity. This effect, known as drifting, should be taken into account by regular calibrations of the device.

If regular calibrations of the device are performed, the drift of the instrument can be determined as:

$$u_{drift} = \frac{e_i - e_{i-1}}{\sqrt{3}}, \quad (5)$$

where e_i and e_{i-1} are instrument errors (difference between instrument reading and reference value) on two succeeding calibrations. This uncertainty component needs to be updated after each calibration.

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Type of biofuel and Purity of sample

On top of the uncertainty components defined by the measurement equipment, the material analysed also adds to the final combined uncertainty. For example, additional uncertainty contributions may arise from different wood species or different concentration of impurities. Since most facilities will not have the possibility of changing settings for the measurement equipment whenever a new load of material is measured on, the variation on sensor readings, based on sample material must be included.

The effect of sample type and purity is quantified by measuring several different test samples, which are analysed offline. The uncertainty component from the variability of the biofuel is found as

$$u_{sample} = \sqrt{\frac{\sum_i^n (x_i - \bar{x}_{DUT})^2}{n-1}} = s_{sample},$$

where x_i are the offline measurement results and $\bar{x}_{DUT}$ are the corresponding results of the working instrument.

Ideally, the measurements result of the working instrument may be corrected for the average error, i.e. $\frac{1}{n}\sum_i^n (x_i - x_{DUT})$, which will result in a smaller uncertainty, however it requires frequent test measurements whenever a new sample type occurs.

4.4 Combined uncertainty

Once all the uncertainty components have been found, the combined uncertainty is found according to JCGM 100:2008 [3] as

$$u_{comb} = \sqrt{\sum u_{individual}^2} \tag{6}$$

$$= \sqrt{u_{reference}^2 + u_{inhomogeneity}^2 + u_{regression}^2 + u_{repeatability}^2 + u_{drift}^2 + u_{sample}^2}.$$

The expanded uncertainty (k = 2 or approximately 95 % confidence) is consequently

$$U = 2 \cdot u_{comb}. \tag{7}$$

5. SAMPLING

5.1 Introduction

To obtain a representative value during testing, it is necessary to test on a representative sample. Since biofuels are often highly inhomogeneous, multiple steps must be taken to gain a representative sample. A further complication is, that it usually only is possible to measure on a small fraction of the combined biomass bulk. This has the consequence, that material sampling must be performed, followed by a reduction of the sample to a volume, which can be handled by the test equipment.

To obtain a representative sample, the following fundamental principles should be considered:

1. Principle of statistical regularity
2. Principle of inertia of large numbers

The first principle states, that all samples should be equally probable. I.e. there can be no bias in how the samples are collected, be it from top or bottom of the batch, large or small particles,

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etc. In other words, the sampling must be random.

The second principle states, that the more samples are used, the more accurate the result is. Practically this means, that if one sample is obtained from a batch, then the result is less valid, than if a result is obtained as an average of 100 samples (given that the 100 samples follow the first principle).

On top of that, sampling of biofuels should in general follow the standard "ISO 18135:2017: Solid Biofuels – Sampling". The following considerations should mainly be seen as one possible implementation of this guideline, along with some best practices investigated in this project.

5.2 Manual vs. Automatic sampling

Sampling can be performed both manually and automatically. Both have advantages and disadvantages regarding establishing costs, safety, and representativeness.

Representativeness: As stated in the first fundamental principle of sampling, all samples should be random, meaning that all parts of the batch should have equal probability in being sampled. While this can be achieved using manual sampling methods, humans do have a higher than zero probability towards taking "interesting" samples rather than random samples.

Automatic sampling does not find specific parts of the batch to be more interesting than others. As such, it is easier to avoid a bias towards certain parts of the batch than others, as the locations at which the samples are extracted can be programmed to be random, after which the sample extractor/robot will use these locations, without asking questions. Although this might leave out volumes, which seem suspicious to a human, it will allow for an unbiased measurand.

Safety: Batches of biofuels are often delivered by truck, where the sample is obtained from a falling stream of the back of the truck. Obtaining samples this way requires that the operator is secured by a harness and rope, as well as other safety measures. These safety measures are there for a reason, and things can still go bad in the sampling situation, resulting in serious injuries or death.

Establishing costs: One area, where automatic sampling falls short of manual sampling is in the establishing costs. Where manual sampling principally only requires a shovel and a bucket (depending on how you extract the sample), automatic sampling requires a much bigger and more expensive setup. This might restrict some locations from ever being able to install automatic sampling, as the potential savings in determining the correct water content cannot justify the costs of installing an automatic sampling system.

5.3 Sampling uncertainty

The uncertainty of the batch can be found after the samples have been collected. The uncertainty in the estimation of the water content in a batch of biofuels is almost identical to the uncertainty estimation of the water content found in the previous section. The only difference between the expressions found here is the addition of a term taking the inhomogeneity of the batch, s_{batch} into account.

As such, the uncertainty for water content in a batch of biofuels is:

$$u_{biofuel} = \sqrt{u_{comb}^2 + \frac{s_{batch}^2}{n}} \tag{8}$$

where u_{comb} is the uncertainty found in section 4, s_{batch} is the standard deviation between samples extracted from the batch of biofuel, and n is the number of extracted samples.

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From the above equation it is seen that the uncertainty in the estimation of the water content of the batch decreases with the number of samples extracted.

6. MACHINE LEARNING for water-content determination

The technology of machine learning allows to extract the information from so-called big data. As an example of big data, the training data for water content measurements could be a set of data, giving the water content along with additional measurement data from different sensors. Using machine learning it is possible find and quantify the effect of correlations between different measurements (e.g. data from a near infrared sensor (NIR) or/and a microwave sensor (MW), local temperature, humidity etc.), which are not established in advance and would be difficult or impossible to find otherwise. Thus, machine learning makes it feasible to combine all the readings to determine the water content of biofuel with improved precision compared to using traditional analytic techniques. In addition, the sensor data from multiple sensors can be used to create an outlier detection model. In that case, the correlation between all the relevant measurements is found, and a probability distribution of the outcomes is created. If for some reason, measurements start falling outside of the probability distribution, it is a good indicator, that the reliability of the measurement is low (large uncertainty) and something needs to be checked, be it a sensor which is drifting or broken, or an extreme change in the material measured on.

Machine-learning models do not necessarily learn to produce real physical functions, but just empirical approximations of the real relationship between the model input and its outcome. As an approximation, the accuracy of the model's output is limited by the model's parameters, hyperparameters, input variables, and available data points. There are many sources of uncertainty in a machine learning project, including variance in the specific data values, the sample of data collected from the domain, and the imperfect nature of any models developed from such data.

6.1 Total uncertainty when using machine learning

Typically, the machine-learning procedure will replace part of the traditional data-analysis procedure. Therefore, machine learning will add new uncertainty contributions that replaces some of the contributions resulting from a traditional analysis. The machine learning standard uncertainty contribution, u_{ML}, can be expected to replace the contribution from *repeatability*, *regression*, and *sample inhomogeneity*. In that case, the resulting combined uncertainty can be determined by

$$u_{comb} = \sqrt{\sum u^2_{individual}}$$

$$= \sqrt{u^2_{reference} + u^2_{inhomogeneity} + u^2_{repeatability} + u^2_{drift} + u_{ML}}. \quad (9)$$

As above, the expanded uncertainty (95 % confidence) is determined by equation (7).

6.2 Uncertainty contribution from machine learning

The fact that machine-learning algorithms are not explicitly based on physical principles has significant impact on the performance of the algorithm and the uncertainty analysis. Usually, machine learning has an advantage over traditional data analysis when it comes to the ability of reproducing the learning data, i.e., reference dataset using for training the machine-learning algorithm. On the other hand, the ability to predict new results under different circumstances

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is likely poor, due to the lack of link to physical principles. For the same reason, it is not possible to determine the uncertainty contribution from machine learning using the uncertainties of the model input parameters and sensitivity coefficients derived from the machine-learning model, and instead the method described below must be employed.

To determine the uncertainty contribution related to a machine-learning as defined in the previous section the available dataset is divided into subsets.

1. Training: Only data in this group are used for training (i.e., optimising) the machine-learning model.
2. Optimisation: The goal of this group is to optimise the machine-learning model, e.g., by adding additional (virtual?) parameters or correlations.
3. Validation: This is the control group of data, that are used for the actual determination of the uncertainty contribution.

If the optimisation subset is not relevant, the data are divided into only two subsets. It is important that the validation subset is sufficiently large to yield a reliable value for the uncertainty contribution. This can be tested by repeating the training of the model several times, with different random divisions of the dataset. As a guide, the training and validation subsets should have equal size in order to secure equal weight in the analysis. See Figure 1.

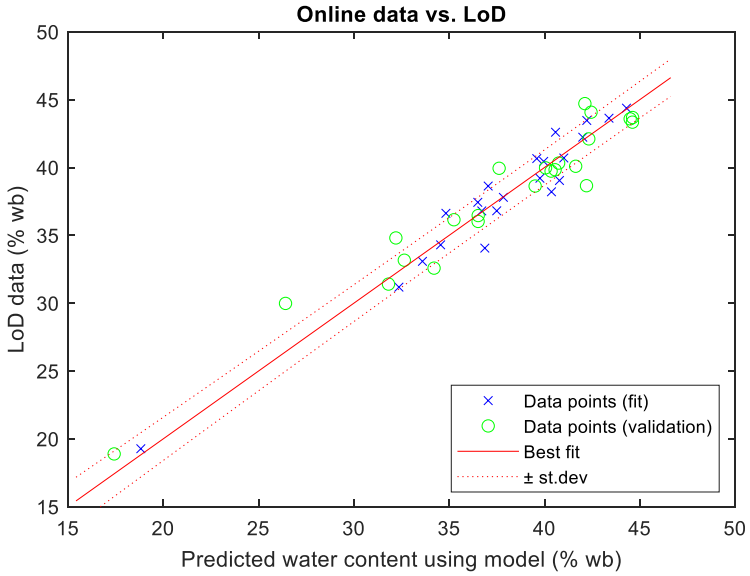

Figure 1. Splitting the machine-learning data into several subsets to determine the uncertainty related to machine learning. Standard uncertainty of learning subset (fit): 1.2 %; Standard uncertainty of validation subset (validation): 1.6 %.

The calculation of the standard uncertainty of the machine-learning model, u_{ML}, is performed using the residuals, i.e. the difference between the results of the machine-learning model, $x_{ML,i}$, and the corresponding reference values, $x_{Ref,i}$, using

$$u_{ML} = \sqrt{\frac{\sum_i^n \left(x_{ML,i} - x_{Ref,i}\right)^2}{n-1}}, \tag{10}$$

where n is the number of reference measurements.

The division into the subsets should be random (Monte Carlo). Furthermore, the reference data points should be distributed approximately equally over the tested parameter space (i.e., should cover the relevant water-content range approximately homogenously). If this is not the case data points should be omitted to obtain an equal distribution.

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7. INORGANIC IMPURITIES AND MAJOR COMPONENTS OF SOLID AND LIQUID BIOFUELS

7.1 Atomic emission spectrometry

The analytical method of atomic emission spectroscopy (AES) measures the intensity of light produced by the atoms in excited states and is used to quantify metal atoms. An excited atom produces radiation with a certain wavelength as it descends to the ground state. Excitation (radiation absorption) and de-excitation (radiation emission) of electrons are both involved in atomic emission spectroscopy. To excite electrons to greater energy levels, a certain wavelength must be absorbed when heated to a high temperature. When the excited species depart from the high-temperature zone, they emit radiation in the form of distinct wavelength packets as a means of cooling back down to the ground states. Before reaching the detectors, these emissions pass a monochromator or filter.

For obtaining the best results, all instruments must be calibrated before analysis. Usually, a minimum of five standard solutions with the increasing concentration similar to those in the sample solutions and a with known concentration of the metals to be examined are required for calibration. These solutions are named “primary standards”.

All data read from the calibration curves are further processed for their standard deviation under repeatability and reproducibility conditions and all dilutions and other manipulative activities samples undergone are taken into account.

When the samples have a complex matrix and matrix reference materials are available, an empirical approach in estimating the measurement uncertainty is usually applied due to its comprehensiveness and uniformity in application.

An empirical method for the determination of the measurement uncertainty is based on the principle that reliability of results = precision + accuracy, and the overall combined measurement uncertainty can be expressed using the following equation:

$$u_c = \sqrt{u_{prec}^2 + u_{bias}^2} \tag{11}$$

A critical concept for analytical chemists, precision, is described in VIM [2] as the degree of agreement between indications or measured quantity values acquired by repeat measurements on the same or comparable objects under prescribed conditions. Different conditions are utilized by analysts for various quality-related tasks, such as method validation, internal quality control, collaborative trials, time of measurement, measurement performer (analyst), etc. The variability of results can be assessed under repeatability and reproducibility conditions. Reproducibility in this context means that different variables/conditions under which the measurements were taken were applied on the same analytical sample(s).

Bias on the other hand as a measure of systematic error can be estimated when optimized conditions of certain analytical methods are applied for the measurement of known amount of analyte my means of reference materials or interlaboratory studies, or by application of different but comparable analytical methods (one validated with prove of capability to generate accurate results).

When assessing measurement uncertainty of selected elements in solid/liquid biofuels or resulting ash content the following sources should be taken into account: overall precision of a method as a reproducibility of data generated by different analysts, on different days and different subsamples and analytical calibration lines; linearity of calibration curves applied; bias – systematic error estimated by use of certified reference materials for both preparation of calibration curves and matrix CRM for the estimation of analytical results accuracy, as well as the sources from other measuring instruments such as balance and/or volumetric labware

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and instruments (automated pipettes) if samples and calibration standards are prepared gravimetrically or based on accurate volume, respectively.

$$u_c = \sqrt{SD_{pooled}^2 + u_{CRM\ cal}^2 + u_{matrix\ CRM}^2 + u_{cal}^2 + u_{mass\ xi}^2 + u_{volume\ xi}^2} \quad (12)$$

The expanded measurement uncertainty is then calculated by multiplying the combined measurement uncertainty with the appropriate coverage factor that can be calculated from the effective degrees of freedom. A coverage factor close to 2 for 95 % confidence can be expected for large number of measurements (normal distribution).

7.2 Fluorescence X-ray spectrometry (wavelength or energy dispersive)

Atoms in a specimen are activated by primary photons from outside sources, such as an X-ray tube, radioactive source, or synchrotron beam, to create primary fluorescence. This technique is known as direct excitation. An alternative method is indirect excitation, in which photons or particles (electrons) are generated by direct excitation or other secondary processes within the material, resulting in the observable fluorescence as a secondary process. The electromagnetic radiation known as X-rays is produced when atoms are struck by high-energy particles. Wave-particle duality characterizes this radiation. For material composition analysis and chemical state study, X-ray fluorescence (XRF) spectroscopy employs primary X-ray photons or other tiny particles to excite the atoms in the test sample and create secondary XRF.

Two main techniques WD XRF and ED XRF can be applied to measure the elemental composition of solid and liquid samples. WD-XRF systems are based on Bragg's law, which states that crystals will reflect X-rays of specific wavelengths and incident angles when the wavelengths of the scattered X-rays interfere constructively. While the sample position is fixed, the angles of the crystal and detector can be changed in compliance with Bragg's law so that a particular wavelength can be measured. Only X-rays that satisfy Bragg's law are reflected. Collimators further improve resolution by providing different angular divergences to restrict unwanted secondary X-rays from reaching the detector. Larger collimators can be used when high intensity is favoured over resolution. WD system delivers rapid quantitative determination of major and minor atomic elements, from beryllium (Be) through uranium (U), in a wide variety of sample types. WDXRF uses crystals to disperse the fluorescence spectrum into individual wavelengths of each element, providing high resolution and low background spectra for accurate determination of elemental concentrations. The types of crystals used in WDXRF include minerals, metallic, organic and synthetic multi-layers. Synthetic thin film multilayer crystals are increasing in popularity because they offer higher sensitivity and resolution for enhanced light element analysis.

According to Moseley's law, the EDXRF spectrometer was created. A power supply, a light path subsystem, a control circuit, and a personal computer (PC) make up the spectrometer. The X-ray tube receives high-voltage electricity to emit a primary X-ray, which irradiates the sample. The XRF is then detected by an XRF detector once the sample has been induced to emit it. The detector sorts the incoming photons into groups based on their energy and counts how many of each kind there are. The PC then completes the qualitative and quantitative analysis after receiving the findings from the detector.

Empirical approach for estimating measurement uncertainty is proved to be very helpful for XRF methods as it provides possibility to assess bias and precision individually. Since no ILC or CRM for solid and liquid biofuels or ash material designed for XRF methods for determination of elements in such matrices, within the project the ref material were prepared and the values for selected elements together with measurement uncertainties were assigned. These materials were used to calibrate the XRF instruments and to assess the bias

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component of the method uncertainty. Precision was estimated as reproducibility pooled standard deviation of measurements on the same sample (different subsamples) by different analysts and on different days.

The following sources have been taken into account: overall precision of a method as a reproducibility of data generated by different analysts, on different days and different subsamples and analytical calibration lines; bias – systematic error estimated by use of assigned values and uncertainties for ash reference materials by means of primary method for the estimation of analytical results accuracy, as well as the sources from other measuring instruments such as balance since samples are prepared gravimetrically by mixing certain mass of sample and binding material.

$$u_c = \sqrt{SD_{pooled}^2 + u_{matrix\ CRM}^2 + u_{balance}^2} \tag{13}$$

The expanded measurement uncertainty is then calculated by multiplying the combined measurement uncertainty with the appropriate coverage factor that can be calculated from the effective degrees of freedom. A coverage factor close to 2 for 95 % confidence can be expected for large number of measurements (normal distribution).

8. ORGANIC IMPURITIES AND MAJOR COMPONENTS OF LIQUID BIOFUELS

The main organic impurities in liquid biofuels are total glycerol, free glycerol and residual mono-, di- and triglycerides contained by fatty acid methyl esters (FAME) resulting from the transesterification of mineral oils.

Qualification and quantification of organic impurities can be made using the chromatographic method. The principle of the method consists in the transformation of free glycerol and mono-, di- and triglycerides into much more volatile and stable derivatives in the presence of pyridine and N-methyl-N-trimethylsilylfluoroacetamide (MSTFA). After silanization, the samples are analysed by gas chromatography on a short capillary column with a low stationary phase deposition, with the introduction of the sample directly into the capillary column (on-column) and the detection of the compound with a flame ionization detector (FID).

After a calibration procedure of the gas chromatograph equipped with an injector as allow the introduction of the sample directly into the chromatographic column, the quantification of free glycerol is performed in the presence of the internal standard 1,2,4-butanetriol and mono-, di- and triglycerides are directly quantified in the presence of internal standards for each category of glycerides.In the equations below the following standards are employed:

- mononononadecanoin (Mono C19) for monoglycerides
- dinonadecanoin (Di C38) for diglycerides
- trinonadecanoin (Tri C57) for triglycerides

8.1 Measurement uncertainty for organic impurities: Glycerides

The mass concentration of mono-, di- and triglycerides in % (m/m) is calculated using the following equations:

$$M = (A_{Mono} \backslash A_{MonoC19}) \cdot (M_{MonoC19}/m) \tag{14}$$

$$D = (A_{Di}/A_{DiC38}) \cdot (M_{DiC38}/m) \tag{15}$$

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$$T = (A_{Tri}/A_{TriC57}) \cdot (M_{DiC38}/m) \quad (16)$$

Explanation of symbols:

- M, D, T – the concentration of mono-, di- and triglycerides in the sample, in % (m/m)
- A_{Mono}, A_{Di}, A_{tri} – the sum of the areas corresponding to the peaks of mono-, di- and triglycerides in the sample
- $A_{MonoC19}$ – the peak area corresponding to the internal standard Mono C19
- $M_{MonoC19}$ – the mass corresponding to the internal standard Mono C19, in mg
- A_{DiC38} – the peak area corresponding to the internal standard Di C38
- M_{DiC38} – the mass of the internal standard Di C38, in mg
- A_{TriC57} – the peak area corresponding to the internal standard Tri C57
- M_{TriC57} – the mass of the internal standard Tri C57, in mg
- m – the mass of the biodiesel sample

8.1.1 Monoglycerides uncertainty

According to equation (14), the standard uncertainty associated with the concentration of monoglycerides in the sample is:

$$u(M) = C_{Mono}\sqrt{\left(\frac{u(A_{Mono}/A_{MonoC19})}{A_{Mono}/A_{MonoC19}}\right)^2 + \left(\frac{u(m_{MonoC19})}{m_{MonoC19}}\right)^2 + \left(\frac{u(m)}{m}\right)^2}, \quad (17)$$

with

- $u(A_{Mono}/A_{MonoC19})$ – the uncertainty of the ratio $A_{Mono}/A_{MonoC19}$
- $u(m_{MonoC19})$ – the uncertainty of the mass of the internal standard Mono C19 (mg)
- $u(m)$ – the uncertainty of the mass of the biodiesel sample (mg)
- C_{Mono} – mass concentration of monoglycerides in biodiesel (%)

8.1.2 Diglycerides uncertainty

According to equation (15), the standard uncertainty associated with the concentration of diglycerides in the sample is:

$$u(D) = C_{Di}\sqrt{\left(\frac{u(A_{Di}/A_{DiC38})}{A_{Di}/A_{DiC38}}\right)^2 + \left(\frac{u(m_{DiC38})}{m_{DiC38}}\right)^2 + \left(\frac{u(m)}{m}\right)^2}, \quad (18)$$

with

- $u(A_{Di}/A_{DiC38})$ – the uncertainty of the ratio A_{Di}/A_{DiC38}
- $u(m_{DiC38})$ – the uncertainty of the mass of the internal standard Di C38 (mg)
- $u(m)$ – the uncertainty of the mass of the biodiesel sample (mg)
- C_{Di} – the mass concetration of diglycerides in biodiesel (%)

8.1.3 Triglycerides uncertainty

According to equation (16), the standard uncertainty associated with the concentration of triglycerides in the sample is:

$$u(T) = C_{Tri}\sqrt{\left(\frac{u(A_{Tri}/A_{TriC57})}{A_{Tri}/A_{TriC57}}\right)^2 + \left(\frac{u(m_{TriC57})}{m_{TriC57}}\right)^2 + \left(\frac{u(m)}{m}\right)^2}, \quad (19)$$

with

- $u(A_{Tri}/A_{TriC57})$ – the uncertainty of the ratio A_{Tri}/A_{TriC57}
- $u(m_{TriC57})$ – the uncertainty of the mass of the internal standard Tri C57 (mg)

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- $u(m)$ – the uncertainty of the mass of the biodiesel sample (mg)
- C_{Tri} – the mass concetration of triglycerides in biodiesel (%)

8.2 Free glycerol uncertainty

The standard uncertainty, u, for determining the concentration of free glycerol is composed of a accuracy component, u_R, given by the internal repeatability of the method, a systematic error, u_{bias}, highlighted by the bias (the difference between the reference value and the experimentally determined value) of the method, and the laboratory and a component resulting from chromatographic determination u_{chr},

$$u = \sqrt{u_R^2 + u_{bias}^2 + u_{chr}^2}. \quad (20)$$

8.2.1 The uncertainty associated with the accuracy of the method (u_R)

The accuracy component, S_r, was estimated by means of the standard deviation of the repeatability of the differences between the experimentally determined values for the same sample,

$$S_r = \sqrt{\frac{\sum d^2}{2n}}, \quad (21)$$

where

- S_r – standard deviation of repeatability
- d – standard deviation of repeatability
- n – the number of determinations

8.2.2 The uncertainty associated with the trueness of the method (bias)

Systematic error, u_{bias}, was determined from the experiments used to validate the trueness of the method as a combination of the difference between the concentrations of the analyzed standard solutions and the concentrations determined experimentally and the uncertainty of the preparation of the standard solutions,

$$u_{bias} = \sqrt{RMS_{bias}^2 + u_{C_{ss}}^2}, \quad (22)$$

where

- RMS_{bias} – the difference between the glycerin concentration of the standard solutions and the glycerin concentration determined experimentally
- $u_{C_{ss}}$ – the standard uncertainty of preparation of standard glycerin solutions

8.2.3 The uncertainty associated with chromatographic analysis

The uncertainty resulting from the chromatographic analysis, u_{chr}, is due to the differences (residual values) between the concentration values used to express the linearity of the calibration curve and the concentration values obtained by calculation using the obtained linear regression equation,

$$u_{chr} = \sqrt{u_{C_0}^2 + u_{C_{G.sol.std.cal}}^2}, \quad (23)$$

where

- u_{C_0} – the standard uncertainty of determining the concentration of the analyte by direct reading on the calibration line

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- $u_{C_{G.sol.std.cal}}$ – the standard uncertainty associated with the concentration of glycerin in the standard calibration solutions

8.2.3.1 The standard uncertainty of determining the concentration of the analyte by direct reading on the calibration line

The standard uncertainty, u_{C_0}, of determining the concentration of glycerine by direct reading on the calibration line represents the standard deviation of the concentration of glycerine calculated with the calibration data,

$$u_{C_0} = S_{x_0}. \tag{24}$$

The standard deviation of determining the concentration of glycerin by direct reading on the calibration line is calculated with the calibration data according to the equation:

$$S_{x_0} = \frac{S_{y/x}}{b}\left\{\frac{1}{m} + \frac{1}{nk} + \frac{(y_0 - \bar{y})^2}{b^2 \sum (x_i - \bar{x})^2}\right\}^{1/2}, \tag{25}$$

where

- $S_{y/x}$ – residual standard deviation
- a – the distance from the origin of the regression line
- b – the slope of the regression line
- m – the number of replicates for the sample
- n – the number of calibration points ($n = 4$)
- k – the number of replicates for standard calibration solutions ($k = 3$)
- y_0 – the signal corresponding to the analyte in the sample
- $\bar{y}$ – the average of the signals obtained for all calibration points
- x_i – the concentration of the analyte in the standard calibration solutions
- $\bar{x}$ – the average of the analyte concentrations from all standard calibration solutions
- $y_j = A_G/A_{SI}$, where
- A_G – the area corresponding to the glycerin peak
- A_{SI} – the area corresponding to the internal standard peak;

Furthermore,

$$y_0 = \frac{\sum_{j=1}^{m} y_{sj}}{m}, \tag{26}$$

i.e. sum of all y_{sj} {(from $j = 1$ to m (the number of replicates of the samples)} presented (calculated) above divided by the number replicates of the sample (m).

8.3 Total glycerol uncertainty

The percentage of total glycerol in the sample, GT, (in %, $/m$) is calculated with the equation:

$$GT = G + 0.255M + 0.164D + 0.130T. \tag{27}$$

Thus, the standard uncertainty associated with the concentration of total glycerol in the sample is in accordance with:

$$u(GT) = \sqrt{u(G)^2 + \left(0.255u(M)\right)^2 + \left(0.164u(D)\right)^2 + \left(0.130u(T)\right)^2}. \tag{28}$$

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9. BIBLIOGRAPHY

[1] ISO/IEC 17025:2017 “General requirements for the competence of testing and calibration laboratories”

[2] International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM 3rd edition - 2008 edition with minor corrections) [JCGM 200:2012]

[3] Evaluation of measurement data - Guide to the expression of uncertainty in measurement, JCGM 100:2008

[4] Keith Birch, British Measurement and Testing Association: Measurement Good Practice Guide No. 36, Estimating Uncertainties in Testing,

[5] An Intermediate Guide to Estimating and Reporting, Uncertainty of Measurement in Testing, 2003 8. S L R Ellison and A Williams (Eds).

[6] Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012) ISBN 978-0-948926-30-3.

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