FINAL PUBLISHABLE JRP REPORT

JRP-Contract number	ENG09		
JRP short name	Biofuels		
JRP full title	Metrology for Biofuels		
Version numbers of latest contracted Annex Ia and Annex Ib against which the assessment will be made	Annex Ia: V1.3 Annex Ib: V1.3		
Period covered (dates)	From 1st June 2010	to	31st May 2013
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Report Status: PU Public

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1 Executive Summary

This project has contributed to the promotion of liquid biofuels in automotive transport, supporting the use of sustainable energy. The proejct has provided validated reference measurement methods that will help ensure the presence of biofuels in EU energy supply.

The Problem

If biofuels are to infiltrate a fuel market dominated by petroleum-based products, they need to be able to mix with traditional fuels and form blends that can be used without affecting vehicle engine performance, reliability or safety.

The issue is that biofuels are expected to have different physical components to fossil fuels, but no internationally-accepted reference methods have been produced, resulting in a lack of harmonisation between geographical regions of current methods.

The Solution

In response to this problem, the project set out to develop more accurate measurements and a greater understanding of biofuel properties by accomplishing the following objectives:

- Provide a reference for the chemical and physical parameters in biofuels
- Develop traceable measurements for the quality indicators of biofuels
- Establish traceability chains for the origin discrimination of biofuels

The Impact

The results obtained represent important steps towards European and International harmonisation of the measurement methods of biofuels and their blends with fossil fuels:

- In the transport sector, clearly defined and internationally comparable quality standards based on traceable measurements and a wider knowledge of properties of biofuels will support further technical developments, facilitating the use of biofuels and leading to greater acceptance from customers and vehicle manufacturers
- The development of references for chemical and physical parameters will provide confidence in international quality assurance and facilitate global trade
- Traceability chains will ensure long-term reliability and global comparability, help prevent economic subsidy fraud, and improve investor confidence
- The availability of reference methods will allow for the provision of traceable reference values, which will eventually be disseminated through the supply of reference materials, proficiency testing schemes and other reference measurements

Such a robust metrology infrastructure, which is above dispute and legal challenge, has the capability to underpin appropriate policies and encourage both the sustainable use of natural resources and the reduction of greenhouse gases, thus fulfilling the requirements of two recent EU directives.

2 Project context, rationale and objectives

2.1 Context

Directive 2009/28/EC1 on the promotion of the use of energy from renewable sources (the "Renewable Energy Directive") established mandatory targets to be achieved by 2020 for a 20 % overall share of renewable energy in the EU and a 10 % share for renewable energy in the transport sector.

At the same time, an amendment to Directive 98/70/EC (the “Fuel Quality Directive") introduced a mandatory target to achieve by 2020 a 6 % reduction in the greenhouse gas intensity of fuels used in road transport and non-road mobile machinery and Directive 2009/30/EC article 7b establishes sustainability criteria for biofuels.

In order to fulfil the requirements of the EU directives, the contribution of biofuels towards these targets is expected to be significant. In this respect, the production processes of biofuels, the characteristics of biofuels feedstocks and end products (blends), the storage, transportation, and distribution conditions must be properly monitored. However, each geographic region has developed its own standards and measurement methods from specifications and test methods originally developed for fossil fuels and industrial or commercial alcohol.

This situation leads to a lack of harmonisation of biofuel specifications at international scale, as highlighted by the “White Paper on Internationally Compatible Biofuels Standards” published on 31 December 2007 by the Tripartite Task Force (BR, EU, USA). Besides, some of the biofuel specifications are method dependent parameters.

To assure long-term reliability and global comparability of analytical data the complete knowledge of the metrological traceability chain to the International System of Units (SI) for biofuel analyses is therefore required. The establishment of the traceability will also contribute to providing the legal and financial regulatory means for “trackability”, determining the bio-origin of the samples/blends and their carbon dioxide contribution.

Along the same lines, the Tripartite Task Force recommends (paragraph 1.4) to “support the development of internationally-accepted reference methods and certified reference materials for improving the accuracy of measurement results that underpin assessment of product quality, and help facilitate trade”.

In 2008, a European FP7 project, REference MAterials for BIOfuel specifications (BIOREMA), was launched. It aimed to produce a batch of homogeneous and stable biofuel test samples (bioethanol and FAME) with well characterised reference values to evaluate the current quality of measurement results (provided by laboratories) by carrying out inter-laboratory comparisons with these materials.

The outcomes of this project highlighted the need for further research in a number of parameters, e.g., electrolytic conductivity and pHe in bio-ethanol and glycerides in biodiesel, with the aim of moving away from operationally defined measurands for these parameters, and therefore providing reliable property values which are traceable to the SI.

The results of the inter-laboratory comparisons organised in the frame of the BIOREMA project also showed that, for a limited number of parameters, the current practice in assessment of biofuels against required specifications by field laboratories was satisfactory. On the other hand, the Inter-laboratory comparison (ILC) results underpin the demand for certified reference materials in support of method validation, quality control, and most importantly for improving the precision and trueness of results of test laboratories tasked with assessing fuel quality against specifications.

2.2 Objectives

The project “Metrology for Biofuels” aimed to provide validated reference measurement methods with ensured traceability of the measurement results for first generation liquid biofuels. This is of prime importance to consolidate a sustainable contribution of biofuels to the European energy supply sources, focusing on the automotive industry.

From a general point of view, improving knowledge on biofuels requires competencies in very different fields, in physical and chemical areas. One of the main challenges of the project was therefore to set-up a metrology infrastructure able to cover the wide range of parameters that needed to be addressed due to their lack of reliability.

The outcomes of the project will benefit to field measurement laboratories for which reliable reference values will be provided, either through proficiency testing schemes or use of (certified) reference materials allowing the dissemination of the metrological traceability.

The project focused on four main objectives:

- Development of references for chemical parameters
- Development of references for physical parameters
- Development of references for quality indicators
- Development of analytical tools for origin discrimination

The activities on chemical parameters focused on measurement methods for those parameters where a need for improved methodologies has been identified amongst others in the "White Paper on Internationally Compatible Biofuel Standards" for the lack of harmonisation between Regions and through evaluation of interlaboratory comparisons in the field.

Some physical parameters are important for legal purposes, others for process control during injection, atomisation, ignition, and combustion of fuel in the engine. A precise knowledge of these parameters is necessary for the optimisation of the combustion process to reach a reduction in consumption, pollutant emission, and noise. Singular measurements of different parameters already existed, but many of them were not traceable to SI and not supported by a chemical analysis of the material. Moreover, the measurement methods were not optimised for biofuels. Reference materials were available for some parameters at standard temperatures and pressures, but not for a sufficiently large range of temperatures. For these reasons the project dedicated part of the work to these topics.

In order to provide a faster, cheaper and more flexible assessment of the quality of biofuels, over-arching quantities (such as pH and electrolytical conductivity) are typically measured for their capability to provide a quick and simple quality assessment for biofuels for some relevant parameters such as corrosion potential and inorganic contamination.However, serious metrological problems with respect to the comparability of measurement results exist, as detailed below, requiring significant research effort to resolve them. The project aimed to develop solutions to address this lack of traceability.

Biofuels are produced all over the world, particularly in Europe, North America, Brazil and Asia. First generation biofuels are vegetable oil, biodiesel and bioethanol. They are produced from a large variety of raw materials such as rapeseed, sunflower, sugar cane, animal fat, and others. Sustainable development and use of biofuels as well as related policies on protection of the European market, climate protection and (world) food affairs require detailed information on the marketed biofuels’ identity such as the country of origin, the way of production and the corresponding raw materials (organic origin). However, this will take effect only if tools for verification are available. A feasibility study on the potential analytical tools based on elemental, isotope and compound-specific isotope fingerprints has been conducted within the project.

3 Scientific and technological results and foreground

3.1 References for chemical parameters

Some of the main achievements of the project are in the development of reference methods for a selected number of important parameters that are required to be tested in biodiesel (as listed in EN 14214, “Automotive fuels – Fatty acid methyl esters (FAME) for diesel engines – Requirements and test methods” and further documentary standards referred to in this document).

The standard methods listed in EN 14214 have been developed in view of their routine use in testing laboratories, usually with the aim of developing cost efficient, fit for purpose methods. The reference methods developed within the project aim to provide a link for the traceability of measurement data generated in biodiesel measurements and have targeted 4 parameters:

- Methanol content
- Glycerol content
- Glycerides content
- Selected FAMEs content.

3.1.1 Methanol content

The upper limit of methanol contained in biodiesels according to EN 14214 is 0.2 % (mass/mass). The methanol concentration has an influence on the flash point of biofuels. The higher the methanol concentration, the lower the flash point will be. The maximum value of methanol content is at the border of the minimum value of the flash point. For the routine determination of the methanol content, the standard EN 14110, *Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of methanol content,* is used. This method lacks a good base of traceability around the specification limit of 0.2 % (m/m).

Within the project, a reference method for methanol determination was developed by VSL, based on a GC (Gas Chromatography) -method with an automated headspace injection system. In parallel, BAM has developed a NIR (Near Infrared) method as a potential on-line or at-line method. Two bilateral comparison campaigns were organised by BAM and VSL in order to test the NIR method developed by BAM for its ability to analyse methanol in biodiesels. During those campaigns BAM and VSL analysed the same biodiesel samples for methanol content, which led to sets of very valuable measurement results and also to i) the proof of principle of the newly developed reference method and ii) the proven versatility and usefulness of the NIR method. The collaborative research within this project enabled these campaigns, and therefore allowed both partners to progress beyond their individual possibilities.

3.1.2 Glycerol content

Free glycerol is another parameter listed in EN 14214. It is closely linked to the determination of the glyceride content. In the project, LGC provided a primary calibrant for glycerol, which enables the traceable calibration of glycerol measurements. The calibrant was distributed to interested partners. The characterization of the glycerol calibrant was carried out jointly by LGC and TUBITAK, each providing data for the purity assessment of the calibrant.

Preliminary analyses and method development were completed to determine the purity of a glycerol to be used as a calibrant. LGC developed a high accuracy IDMS (Isotope Dilution Mass Spectrometry) (method for the quantification of free glycerol in biodiesel. This method was applied to the BIOREMA rapeseed oil biodiesel and a NIST soy based material. The uncertainty, obtained in the preliminary investigation of samples, of the test materials was in the region of 1 %. The values obtained at LGC for the BIOREMA rapeseed oil biodiesel agreed well with the benchmark value previously assigned by project partner JRC. LGC completed the purity assessment of the glycerol calibrant and supplied material to UME for further characterisation. Both institutes used a mass balance approach and qNMR (quantitative Nuclear Magnetic

Resonance). The excellent agreement between the two institutes suggested that this material was fit for purpose for use as a primary standard for the quantification of free glycerol in biodiesel. This material was made available to all partners by LGC.

3.1.3 Glycerides content

By far the most complex issue of the chemical parameters activity was associated with the glyceride measurements. They were challenging, due to the low abundance of glycerides, especially of the triacylglycerides, and due to the large number of individual substances that are summarised in these sum parameters. Alternative solutions to provide improved traceability for glyceride measurements were sought. This resulted in a tiered approach, targeting the different classes of glycerides separately with innovative measurement methods. Initial plans to provide individual calibrants for the larger part of the individual glycerides had to be abandoned. Due to the very different nature of the approaches used, no single mixed solution of mono-, di- and triglycerides would suit all methods under development and partners would each use their own specific calibrants.

The maximum value of EN 14214 for monoacylglycerides contained in biofuels is 0.7 % (m/m). Some monoacylglycerides, especially the saturated isomers, can crystallizs in the storage tank under cold conditions. TThe crystals can therefore block the fuel lines and the engine. The routine method to determine the monoacylglyceride (MAG) content is stated in the standard EN 14105, *Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of free and total glycerol and mono-, di-, triglyceride contents*. In the present EN 14105 no differentiation is being made between the contents contributions from the saturated versus the unsaturated monoacylglyceride isomers, while the saturated isomers seem to be more prone to cause blocking under cold conditions. Furthermore it is suspected that the current MAG content determination following EN14105 is biased because of i) taking into the calculation of the MAG content non-MAG peaks and ii) an overestimation of MAG contents because of overlapping/co-eluting non-MAG peaks.

For the determination of the monoacylglyceride content a multi-dimensional GC system (MDGC) was used to develop a new reference method for the determination of MAG contents, circumventing the problems addressed above.

The newly developed reference method for the determination of monoacylglyceride content proved very useful to not only calculate the MAG contents according to the current EN 14105, but also for the determination of “real” MAG contents (i.e. excluding overlapping/co-eluting isomers in the current EN 14105), which are significantly lower than those determined according to EN 14105, and the determination of the single saturated and unsaturated MAG isomers. As part of the work a purity assessment of commercial MAG isomers was performed and a calibration solution was prepared which can be used for the determination of MAG isomer contents of the most common biodiesels.

A reference method to identify and quantify the level of diglycerides was developed at LGC, using the material already used in the BIOREMA project as a test case. A preliminary investigation into the use of GC-MS (Gas chromatography – Mass Spectrometry) revealed a number of practical issues that made their direct analysis difficult. Following a review of the appropriate methods and approaches available, simultaneous GC-FID (Gas chromatography–Flame Ionization Detector) and GC/MS was selected as the best approach to be used in order to identify and quantify the diglycerides in the material due to its enhanced separation capability over traditional LC or GC approaches. Two methods of analysis were investigated at LGC. One approach was to use conventional wide bore capillary column with a combination of FID and MS detection. A second approach was pre-fractionation of the sample using normal phase chromatography (Silica Column) with subsequent analysis of the fractions by simultaneous GC-FID/MS. Initial results suggested that separation of the diglycerides from the biofuel material using this approach could be achieved. LC and GC conditions, to separate and identify diglycerides were optimised and method development activities completed to accurately quantify diglycerides of different carbon chain lengths and molecular structures.

The standard method in use for the quantification of triglycerides is based on high-temperature GC/FID and the detection of glycerol, mono-, di- and triglycerides. The complexity in the mixtures of different structures is causing overlapping signals, especially difficult in the detection of triacylglycerides. The triacylglycerides also exhibit the lowest mass fraction in biodiesel samples. A reference method that would individually target all those compounds would require an effort that would go far beyond reason. Therefore, an innovative route was chosen at JRC-IRMM that would drastically simplify the analysis of triacylglycerides. A method was developed that consisted of an on-line hydrogenation of the triacylglycerides and the detection of their saturated analogues by GC-FID. This reduces the number of species to be detected drastically and additionally enhances the sensitivity of the method.

This approach was further optimised and eventually expanded to mono- and diacylglycerides.

Another alternative approach consisted of a method for the isolation of triglycerides and quantification after transesterification and analysis by GC/MS/FID against traceable FAME standards prepared by the project. The method was developed at SP and applied on 3 FAME samples provided by the project. The limit of quantification was less than 0.05 % and the detection limit less than 0.01 % triacylglycerides in FAME. The method consists of a separation of triacylglycerides from FAME, separation of mono- and diacylglycerides by RP-LC and online detection by APCI-MS, evaporation of the eluent, transesterification in Na-methanol, analysis of sample by GC/MS/FID before and after transesterification and quantification by an external standard curve.

All the different approaches were finally applied to one and the same sample in the proof-of-concept exercise, allowing to compare results and identify areas for further improvement. This project enabled the partners to try out new and innovative routes for glyceride analysis. Further work will be required in future to select the best approach(es), provide the necessary calibrants and set up a traceability chain to the end-users results.

3.1.4 Selected FAMEs content

FAMEs are the main component of a first generation biodiesel. Their quantification was addressed by developing GC-MS methods based on the use of labelled individual FAMEs for calibration. The project partners LGC, JRC and TUBITAK developed such methods, allowing for a meaningful comparison of the data obtained in the proof-of-concept exercise at the end of the project.

TUBITAK provided the partners with a range of labelled individual FAMEs to be used in isotope dilution GC-MS. Due to some stability issues observed, not all of these could be used by the partners. LGC synthesised a new labelled standard for use in their method development work, while JRC purchased commercially available labelled FAMEs.

3.2 References for physical parameters

Fuels from renewable sources (biofuels) are expected to show differences to fossil fuels when comparing their physical parameters. Thus, the parameters known for fossil fuels and used for volume measurement (legal purpose) and for process control in the engines (industrial purpose) cannot be transferred to biofuels and blends but have to be measured for these new fuels.

The parameter density and its temperature dependence are important for legal purposes (measurement of volume, conversion into a standard volume at defined temperature). The density of blends between fossil fuels and biofuels does not follow a simple linear mixing rule. In order to be able to take into account these differences, accurate measurements of density at various temperatures and with pure materials and blends are required.

The parameter viscosity is a quantity used as a correction factor for some flow meters. Thus, accurate measurements of viscosity at various temperatures are required, too.

The calorific value as a measure for the energy content is the most important quantity describing the amount of substance needed for energy generation and as a result, is of significant importance for economic benefits and influences the impact on environment, especially the carbon dioxide balance.

The parameters density and viscosity are input to the process control during injection, atomisation, ignition, and combustion of fuel in the engine. A precise knowledge of these parameters at higher pressure is necessary for the optimisation of the combustion process to reach a reduction in consumption, pollutant emission, and noise. More information including the heat capacity can be gathered by adding measurements of the speed of sound.

To get reliable results, the physical parameters of biofuels need to be determined by measurements traceable to the SI. At the moment singular measurements of different parameters already exist however many of them are not traceable to SI and this therefore handicaps the comparison of results.

To cover these investigations the project had four tasks:

- Transport properties at normal pressure (density, viscosity)
- Reference materials for density and viscosity at normal pressure and temperatures from -40 °C to +70 °C
- State behaviour at high temperatures and high pressures (density, viscosity)
- Energy content (calorific value)

The materials investigated were fossil Diesel (delivered by Shell Germany), rape seed methyl ester (RME) and soy bean methyl ester (SME) (both delivered by ADM, Germany) and the blends B5, B10, and B15. In addition RME and SME were used for the blending.

3.2.1 Transport properties at normal pressure (density, viscosity)

Density and viscosity have been measured at normal pressure over a broad temperature range between -10 °C and +50 °C. For both quantities two different measurement methods were used. The PTB group measured the density using an oscillation type density meter and the viscosity using a Stabinger viscometer, whereas the LNE group used pyknometers for the density measurement and capillary viscometers for the viscosity measurements. The density measurements show uncertainties in the order of 0.050 kg/m^3, the PTB measurements for viscosity have an uncertainty of 1 %, whereas the capillary measurements of LNE have uncertainties between 0.2 % and 0.6 %.

Additionally, all devices were compared by measurements of the reference material n-nonane which was precisely measured using the PTB primary standard devices for density and viscosity (which is detailed in section below).

These comparisons showed good matching of the measurement devices inside their uncertainties in the temperature range between -10 °C and 50 °C.

The results of the density measurements performed by PTB and LNE on the same fuels showed a good matching with deviations smaller than the claimed uncertainty and give reliable information about the density and its temperature dependence.

But in some cases material changes caused by storage, transport, and handling led to deviations in measuring results about three to four times larger than the claimed uncertainty of the measurements. These deviations were less than 0.2 kg/m^3 in any case; this is a value well below the natural spread of density values when looking for different charges of nominally equal fuels.

The results of the viscosity measurements performed by PTB and LNE on the same fuels showed a very good matching with deviations smaller than the claimed uncertainty and give reliable information about the viscosity and its temperature dependence.

3.2.2 Reference materials for density and viscosity at normal pressure and temperatures from -40°C to +70°C

This task was to deliver a stable reference liquid for the calibration of all measurement devices used for the characterisation of physical properties at the participating laboratories. N-nonane was chosen as a reference liquid as it is a very stable hydrocarbon with parameters near to those of fuels. The density as well as the viscosity was measured in the temperature range between -40 °C and + 70°C with very low uncertainty using primary facilities of PTB.

3.2.3 State behaviour at high temperatures and high pressures (density, viscosity)

In these studies for the first time traceable data on density and viscosity and speed of sound were measured in a broad temperature and pressure region systematically on the same samples and were combined to equations of state including additionally calculated values for the specific heat capacity. All measurements were done at pressures up to 300 MPa and at temperatures up to 80 °C. The density measurements were done using a PVT (Pressure, Volume, Temperature) system, which is a piston in bottle technique; the uncertainty is estimated to be 0.08 %. The viscosity measurements were done using a falling body technique; the uncertainty was estimated to be 2 %. The speed of sound facility uses a double reflector pulse echo technique; the uncertainty is estimated to be 0.3 %.

The measured density and viscosity data show at higher pressures instabilities referred to as “freezing out of parts” of the material. This results in instabilities in the density versus pressure curve. Measuring viscosity is a more sensitive indicator for such freezing out, as the measurements are simply stopped by these precipitations. The gap between the falling body and the surrounding wall is in the order of some micrometers, thus, a further movement of the falling body is stopped by very small precipitations. The effect of having such freezing processes is expected to depend in detail on the chemical composition of the material, and, thus, cannot be generalised to all materials.

The speed of sound data show values at ambient pressure being about 3 % larger for biodiesel than those for fossil Diesel. At a pressure of about 80 MPa there is a turnover, the speed of sound is for fossil Diesel larger above this pressure.

Together with the density data the speed of sound data will allow the calculation of the specific heat capacity of the material.

3.2.4 Energy content (calorific value)

The energy content of the fuels mentioned above was determined as calorific value in comparison to the energy of combustion under standard state conditions. Bomb combustion calorimetry in isoperibol and adiabatic mode was applied by up to three laboratories using two different commercial and one selfmade calorimeter and the data was compared. Deviations between the results are covered by the repeatability and reproducibility limits given in the standards DIN 51900 and ISO 1928.

Furthermore, for the first time a complete uncertainty budget for a calorimetric measurement using bomb calorimetry was estimated. These uncertainty calculations identified the weight and temperature determinations as the main contributors to the uncertainty budget. It turned out that the reproducibility of the water filling of the vessel of a calorimeter, and hence its heat capacity, is also an important contributor. Only by using such complete uncertainty budgets the measurement results become comparable and efforts toward the harmonisation of European combustion standards become possible.

3.3 Traceability for quality indicators

The pH value of bioethanol and the biofuel’s electrolytic conductivity are common measurement quantities, often used for a quick quality assessment. However, serious metrological problems with respect to the comparability of measurement results exist, requiring significant research effort to resolve them.

The pH measurement in ethanol is a method-dependent parameter, so-called pHe, and the usual metrological structure which is well established for pH measurement in aqueous solutions cannot be applied directly to ethanolic solutions. pHe values currently obtained for the routine measurement of bioethanol samples depend on the test method used, and also on the pH electrode design. This situation is exacerbated by the absence of suitable and traceable matrix-matched standards in ethanol for calibrating pHe measurements (usually aqueous solutions are used) and the lack of primary traceability analogous to the current Harned Cell method used for aqueous systems.

Electrolytic conductivity reference standards based on biofuels are inappropriate due mainly to stability issues. Therefore alternative reference standards are typically used. At the application level, cell constants of common electrolytic conductivity measuring cells not only depend on cell design, but also on the kind of solution within the cells. Hence when non-matrix matched reference solutions are used for calibration, conductivity cells of different cell design can provide significantly different measurement results for an equivalent biofuels sample, even if calibrated with the same reference solution.

Therefore, the project had two main tasks related to the use of ‘sum parameters’ as ‘quality indicators’ for bioethanol as a means to assess risk of corrosion and potential damage to engines:

- Improving the comparability of pH measurements of ethanol
- Improving the comparability of electrolytical conductivity measurements

3.3.1 pH measurements of ethanol

A reference method for the measurement of ethanol/water mixtures using the Harned Cell (i.e. the primary system for aqueous pH measurements) has been developed. Because of problems with the solubility of the buffer in the ethanolic solution the preparation of the solutions has been modified for the purposes of the comparison. Firstly, in respect of the aqueous solution, a total buffer molality of 0.03 mol/kg was used instead of typically 0.05 mol/kg in water. Secondly, in respect to the phthalate buffer in water-ethanol mixture, preparation of the solution was undertaken in 2 steps: dissolution in water, followed by addition of EtOH. This dissolution protocol was used because it was noted that when the buffer was dissolved directly (in one step) into the ethanolic solution different results were obtained and there was some evidence of more instability in the solutions produced, possibly owing to different evaporation rates. The differences observed are shown in Figure 1; the same differences were observed at 15 °C, 25 °C and 35 °C.

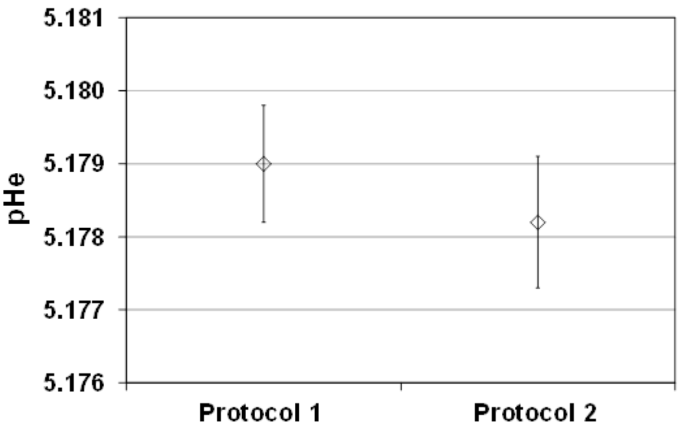

Figure 1. Comparison of the protocol for phthalate buffer preparation (0.05 mol/kg) in 50 % $EtOH-H_2O$ mixture: protocol 1 refers to dissolution of phthalate buffer in 1 step (directly in water-ethanol mixture); protocol 2 refers to the dissolution of the phthalate buffer in 2 steps (dissolution in water then addition of ethanol). Vertical bars correspond to the uncertainty (k=1) of the acidity function (intercept to zero chloride molality)

Furthermore pH measurements of potassium hydrogen phthalate and phosphate buffer in a water-ethanol mixture (50% by weight) at 15 °C, 25 °C and 35 °C using the Harned cell system has been successfully completed. The difference in the values observed are shown in the Figure 2.

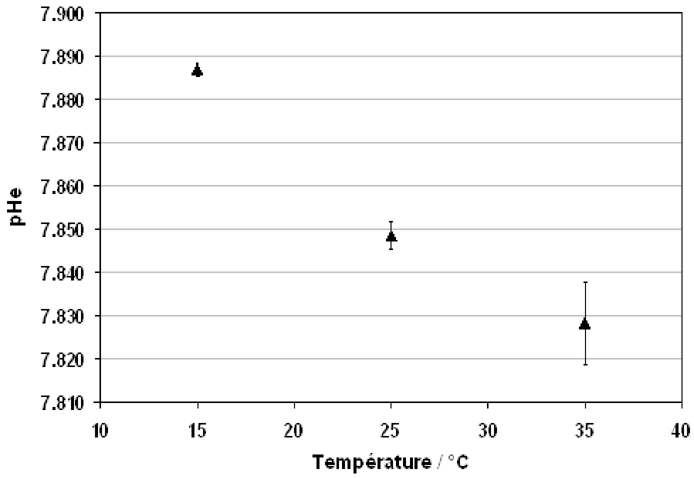

Figure 2. pH measurement using Harned Cell system of 0.03 mol/kg phosphates in 50 % EtOH-H_2O mixture as a function of temperature. Vertical bars correspond to the uncertainty (k=1) of the acidity function (intercept to zero chloride molality).

Overall, satisfactory measurement agreement was found for a given preparation method and for a sample with a given specificity, and furthermore a small influence of bioethanol origin has been found in that slightly different results were observed for real as compared to synthetic ethanol. This work has also determined the influence on standard potentials of the ethanol content of the solutions studied. An increase in the standard potential of 150 µV for every 0.25 % (by weight) increase in ethanol content was observed. This work has resulted in a peer-reviewed publication (Determination of pH values of potassium hydrogen phthalate buffer in water-ethanol mixture (mass fraction 50 %); D. Stoica, P. Fisicaro, P. Spitzer, B. Adel, F.B. Gonzaga, I.C. Fraga, P.P Borges, I. Maksimov, T.Asakai; Proceedings of XX IMEKO World Congress Metrology for Green Growth .

A comparison on ethanol pH measurement of a phosphate buffer in a water-ethanol mixture (50% by weight) using the Harned cell system has been performed between LNE, PTB, INMETRO (collaborator) and NMIJ (collaborator). The results of this comparison are shown in Figure 3.

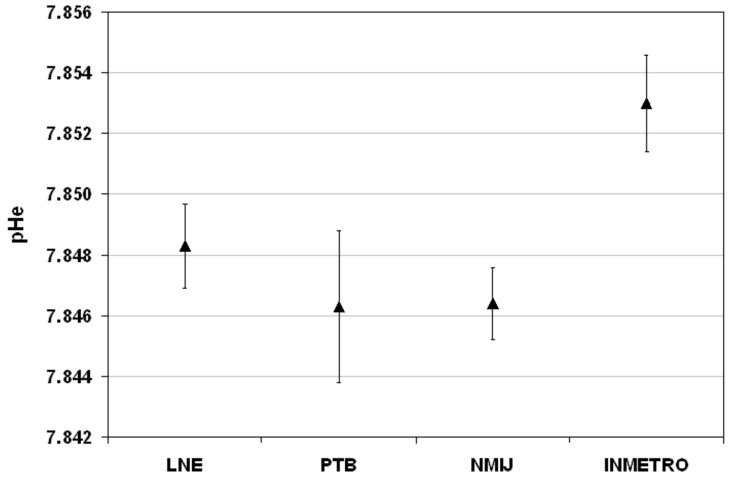

Figure 3. Comparison of pH measurement using the Harned cell system amongst four national measurement institutes of 0.03 mol/kg phosphates in 50 % EtOH-H_2O mixture. vertical bars correspond to the uncertainty (k=1) of the acidity function (intercept to zero chloride molality)

These results have shown promising comparability between the NMIs, at a level similar to the agreement observed with aqueous buffer measurements. However the results have identified several key points which must be satisfied to achieve accurate and traceable measurements of pH of ethanol using the Harned Cell (in general these are the main parameters also affecting aqueous primary measurements of pH). These are, in particular, the accurate determination of E° since this is highly dependent on ethanol content and the need to ensure robust sample handling and sample preparation protocols. Nevertheless, the potassium hydrogen phthalate (0.05 mol/kg-solvent) has been shown to be a suitable standard for traceable pH measurement of hydro-alcoholic solutions.

The work on secondary pH measurements in bioethanol has provided a detailed understanding of the sensitivities and uncertainties of the ASTM D6423 test method for pHe measurement of very high ethanol content (>95 %) solutions. At NPL, assessment of the sensitivities of the critical parameters in the ASTM D6423 documentary standard method for the measurement of pHe in bioethanol has been undertaken in collaboration with other partners. Repeatability of measurements made using the same glass electrode and reproducibility between different glass electrodes have been identified as the main contributors to the uncertainty of the values produced. Strategies to reduce the uncertainty of the measurement have been identified and tested. Both increasing the time after which the pHe measurement is made following immersion in the sample, and rinsing the glass electrode with ethanol prior to immersion in the sample, have been shown to be effective in reducing the uncertainty of the numerical value produced. However, it has also been acknowledged that the values produced using these modified approaches may not be directly compared with those obtained using the documentary ASTM method since pHe is defined operationally by the process used to measure it. This work is shown in Figure 4 and has now been published in the open access peer-reviewed literature (Sensitivities of a Standard Test Method for the Determination of the pHe of Bioethanol and Suggestions for Improvement; R. J. C. Brown, A. C. Keates and P. J. Brewer; *Sensors*, 2010, 10, 9982 - 9993)

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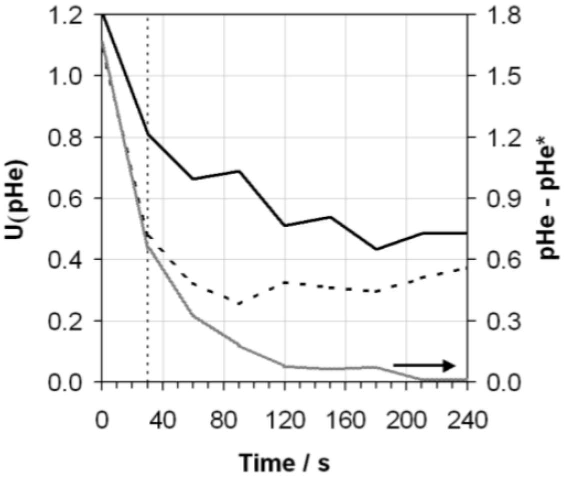

Figure 4. Expanded uncertainty [U(pHe)] calculated as a function of time by combining in quadrature the relevant individual components for the ASTM method (solid line) and the ASTM method but washing the glass electrode with ethanol prior to measurement (dashed line). The difference between the numerical values obtained using the ASTM method (pHe) and the ASTM method but washing the glass electrode with ethanol prior to measurement (pHe*) are also shown (grey line, right hand axis). The ASTM D6423 specified measurement time is indicated (vertical dotted line).

A researcher from LNE spent 3 months at NPL. In this context, the collaboration between LNE and NPL has focused on Ag/AgCl reference electrodes microstructure, especially the porosity characteristics of the electrodes. An exemplar image of the Ag/AgCl electrode fabricated during the placement is shown in Figure 5. This work has now been published in the peer-reviewed literature (Influence of fabrication procedure on the electrochemical performance of Ag/AgCl reference electrodes; D. Stoica, P. J. Brewer, R. J. C. Brown, P. Fisicaro; *Electrochmica Acta*, 2011, 56, 10009 – 10015) as, which also includes the results of the comparison of the response of Ag/AgCl electrodes produced by NPL and by LNE.

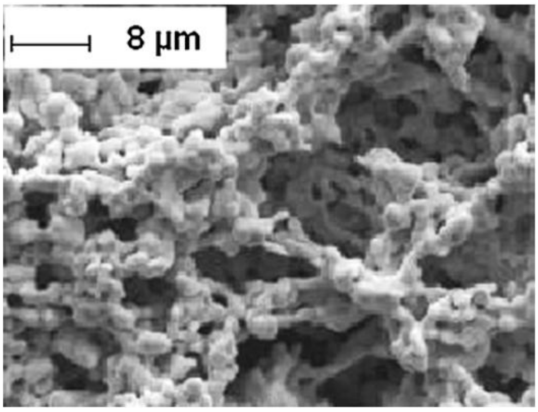

Figure 5. Scanning electron micrograph image of an Ag/AgCl electrode fabricated during the researcher placement.

3.3.2 Conductivity measurements

A reference measurement procedure to measure the conductivity of ethanol traceable to the SI, which in particular includes a reliable method to derive the solution resistance from measured impedance spectra has been developed, based on the use of primary cell systems. Using this procedure the conductivities of a synthetic ethanol sample and bioethanol samples from different origins have been measured, which are for the first time, traceable to SI. Additionally, a detailed uncertainty calculation to assess the significance of the measurement results has been developed. It could be shown, that the conductivities of bioethanol from a Brazilian manufacturer and from a German manufacturer are significantly different (>20 %), meaning that the difference is much larger than the standard uncertainty of the measurement results, which is around 0.3 % for primary measurements and around 1 to 2 % for secondary measurements. Moreover the conductivities of the investigated bioethanol samples are at least about an order of magnitude larger than that from pure ethanol, which indicates a significant amount of ionic contamination. Hence, this indicates that conductivity is an appropriate measurement quantity to assess the quality of bioethanol.

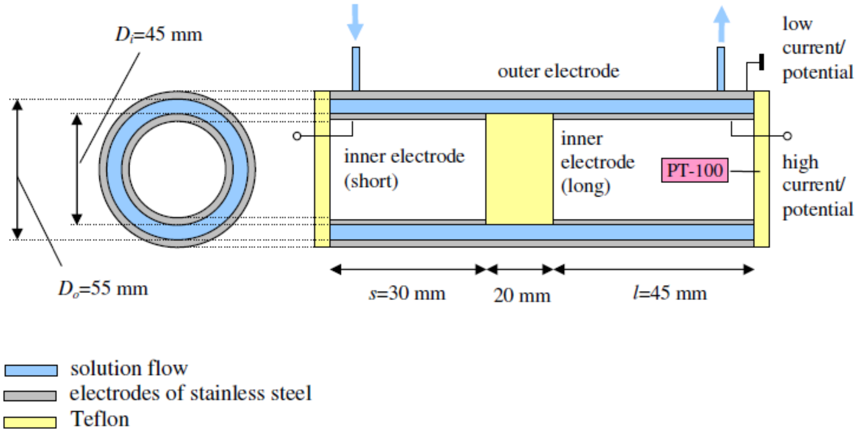

Figure 6. Diagrammatic representation of the primary conductivity measuring cell developed at PTB. The arrows indicate the direction of solution flow.

The measurement results of primary and secondary bioethanol are stable within the stated uncertainty and allow for reliable conductivity results under laboratory conditions. To this end some precautions have to be taken, which are not practical for routine field measurements. Nevertheless, it can be foreseen that an appropriate measurement procedure for field conductivity measurements of bioethanol could be easily established, in particular by keeping the time between sampling and measurement short. In contrast, reference solutions that have been determined based on ethanol or bioethanol are inappropriate for cell calibration purposes due to their instability. The results of the secondary measurements show that a 0.01 mol L^{-1} aqueous KCl solutions and likewise a 130 µS m^{-1} glycerol based KCl can be used for cell calibration. However, the results indicate, that the matrix mismatch causes a change of the cell constant. Therefore, if a two electrode cell of the Jones type is calibrated with such reference solutions, but then used for bioethanol conductivity measurements, an additional uncertainty contribution in the order of roughly 1 % has to be added to the combined standard uncertainty. The 1 % value corresponds to half of the mean, relative deviation observed in the cell constants due to matrix mismatch.

The results of the comparison measurement of bioethanol between the three NMIs taking part is shown in Table 1.

Lab.	t1 (min)	t2 (min)	Drift (% h^{-1})	method	conductivity (µS m^{-1})	stand. uncert. (µS m^{-1})	traceability remarks
PTB	<1	86	non	secondary cell	182.58	0.22	calibration with 130µS m^{-1} KCl(glyc) traceable to SI by primary measurement at PTB
INRiM	<3	0	0.016 (i)	secondary cell	184.5	1.99	calibration with 1mS m^{-1} KCl(aqu) traceable to SI by primary measurement at INRiM
DFM	<1	0	non	secondary cell	162.56	0.09 (only stddev)	calibration with 130µS m^{-1} KCl(glyc) traceable to SI by primary measurement at DFM

Table 1. Bioethanol conductivity intercomparison results (referred to 25 °C). t1 was the time sample was exposed to air before filling, and t2 was time the measurement result is related to with respect to filling of the cell. (i) INRiM observed a change of 0.0289 µS m^{-1} in 10 hours of measurement time, attributed to contamination over time

All institutes have found that the repeatability of measurement results of synthetic ethanol is relatively poor. Hence, it is difficult to assess if the large spread of the reported results is due to poor repeatability, inhomogeneous samples, or differences in the measurement and data evaluation procedures. However, the purpose of measuring synthetic ethanol is to get a reference conductivity value to assess the contamination of bioethanol by conductivity measurements. Even though the mean of the reported values (2.46 μS m^{-1}) has a large relative uncertainty of around 10 %, this can still serve as an estimate for this purpose, since the absolute uncertainty of about 0.2 μS m^{-1} to 0.3 μS m^{-1} is relatively small compared to the conductivity values of bioethanol.

The deviation of the bioethanol conductivity value reported by DFM is significant. It can neither be explained by the measurement uncertainties, nor by the homogeneity limits. Furthermore, DFM and PTB used the same calibration solutions, which were calibrated using their primary calibration procedures. Therefore, the large difference in the bioethanol conductivity values cannot be easily understood currently. This could only be explained if the differences in the current pathway, due to differences in the electric field distribution, for bioethanol and the calibration solution deviate significantly in the cells used. However, both cell geometries are such, that the pathways are mostly restricted to the volume between the electrodes, so it is not clear whether the discrepancy lies here or not. However, from the experience gained in this comparison and from further follow-up measurements at the PTB it is clear that better compatibility is achievable in future by implementing some simple improvements such as: use of improved preparation procedure; filling of the cell under an argon atmosphere that is saturated with ethanol; performance of characterisation measurements with at least five samples; determination of the temperature coefficients using a wider temperature range; each institute should at least measure three samples from the three bottles; each institute should report at least 5 measurement results, measured within a period of at least 4 hours to demonstrate the drift; and all participants should use the same calibration solution, preferably the 130 μS m^{-1} KCl(glyc) solution, since its value is closer to that of bioethanol.

3.4 Analytical tools for origin discrimination

Sustainable development and use of biofuels as well as related policies on protection of the European market, climate protection and (world) food affairs require detailed information on the marketed biofuels' identity such as the country of origin, the way of production and the corresponding raw materials (organic origin).

This information can be obtained for the majority of biofuels by enacting a labelling regulation such as EC 1760/2000 for beef or EC 392/2004 for organic products. However, this will take effect only if a tool for verification is available. One of the objectives of the project was therefore the development of such tools.

Due to the large variety of feedstock and end-products, the origin determination could only be carried out as a feasibility study in the frame of this project. Therefore, the project had five main tasks related to this topic:

- Selection and collection of representative samples
- Bulk $\delta^{2}H$, $\delta^{13}C$, $\delta^{18}O$ analysis
- Isotope and fingerprint analysis of inorganic elements
- Fingerprint analysis of organic traces
- Compound-specific isotope analysis of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{18}O$

3.4.1 Selection and collection of representative samples

TUBITAK and LGC organised collection of raw materials (with corresponding oil and biodiesel) from different geographical and biological origin namely Brazilian, Mexican and Turkish soy bean and European (pool), Turkish and USA rapeseed. LGC's raw samples were provided by ADM, Germany. It was very difficult to access a wider range of materials of known geographical origin and production chain. The materials were prepared, bottled and distributed by LGC and TUBITAK to all partners. Alternatively, sugar beet from Germany and sugar cane from Kenya with the corresponding bioethanol samples have been obtained, prepared, bottled and distributed by PTB to the partners. PTB also collected sugar beets obtained from specific and well known geographical sites in Europe (as part of a campaign) for which Sr isotopic data for the site specific mineral water is available.

3.4.2 Bulk δ^{2}H, δ^{13}C, δ^{18}O analysis

LGC completed bulk δ^{13}C measurements in biodiesels, oil and raw materials using Elemental Analyzer (EA)-IRMS (Isotope Ratio Mass Spectrometry) . Three bottles of each material were measured in different days (4 sample replicates of each bottle/day). A fourth bottle was measured in five different days (4 sample replicates/day). The measured δ^{13}C values of the sample materials were normalised to the VPDB scale using the certified and measured values of 4 CRMs (IAEA NBS-22 mineral oil, IAEA CH6 sucrose, USGS 40 l-glutamic acid and USGS 41 l-glutamic acid). The combined uncertainties associated with the δ^{13}C values for the investigated samples were calculated by combining the measured δ^{13}C values of the samples and CRMs used for normalisation, as well as the uncertainties of the certified δ^{13}C values of the CRMs using the Kragten (1994) approach. The mean measured δ^{13}C values across all sequences were used for these calculations. The results obtained are summarised and represented in Figure 7.

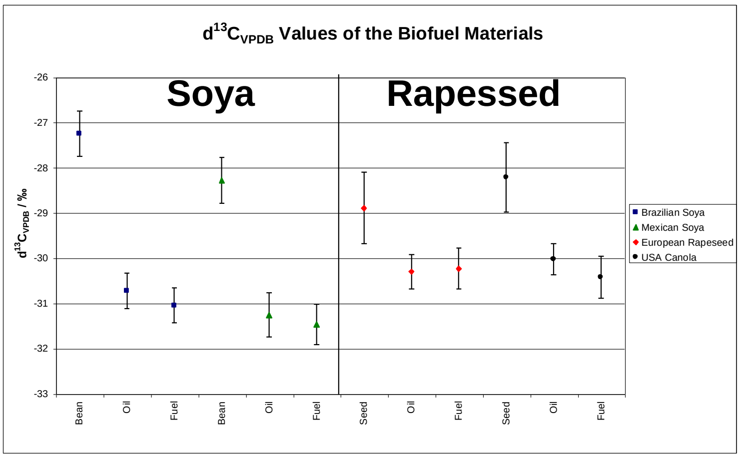

Figure 7. Bulk δ^{13}C values of the four biofuels with their corresponding seeds/beans and extracted oils. Vertical bars represent the combined measurement uncertainty. Canola is a type of rapeseed.

The data show that bulk C IR measurements does not seem to be a good tool to discriminate between rapeseed fuels of different geographical origin (e.g. Europe and USA). This was supported by the bulk δ^{13}C values obtained by TUBITAK on the same samples using EA-IRMS and five IAEA CRMs (NBS-22 mineral oil, CH$_6$ sucrose, Biotite NBS 30, Sodium Nitrate USGS35 and Barium Sulfate NBS 127) to normalise the measured δ^{13}C values of the sample materials to the VPDB scale. For example, the mean normalised δ^{13}C values and associated standard deviations (n = 4) obtained by TUBITAK for European rapeseed biodiesel and USA canola biodiesel were -28.57 **‰** (SD = 0.10 **‰**) and –28.45 (SD = 0.03), respectively. For all the materials investigated, it was interesting to note that the δ^{13}C values for the oil and corresponding biodiesel agreed quite well within the associated uncertainty but they were significantly different from those obtained for the corresponding raw materials.

TUBITAK also performed bulk δ^{2}H and δ^{18}O IR analyses of oils and corresponding biodiesels for the samples provided by LGC and those purchased from local supermarkets using EA-IRMS. The same CRMs as used for bulk C IR measurements were used to normalise the measured δ ^{2}H and δ ^{18}O values. The data suggest that unlike δ^{13}C measurements, both δ^{2}H and δ$^{□8}$O measurements have great potential to differentiate between oils and biodiesel of different types of raw material and geographical origin. For example, the δ $^{□8}$O and δ ^{2}H values showed differences in ≥ 10 ‰ and ≥4 ‰, respectively, between canola oil and soybean oil, as purchased in a local supermarket. Tables 2 and 3 summarise bulk δ^{2}H and δ^{18}O values and their associated SD (n = 4 or 5) for European rapeseed and USA canola biodiesels, as provided by LGC. Differences in ≥30 ‰ and ≥4 ‰, respectively, were observed. It was also interesting to note that the δ^{2}H values for the oil and corresponding biodiesel agreed well but significant differences were observed between the δ^{18}O values of the same pair of samples.

Sample Name	**Measured Values (‰)**	**Corrected Values (‰)**	**Mean of Corrected Values (‰)**	**Standard Deviation of Corrected Values (‰)**
EUROPEAN RAPESEED BIODIESEL	-160.505	-154.85	-154.86	0.10
	-160.438	-154.79		
	-160.749	-155.04		
	-160.510	-154.85		
	-160.445	-154.80		
USA CANOLA BIODIESEL	-202.851	-188.82	-189.93	0.72
	-204.233	-189.92		
	-204.601	-190.22		
	-205.315	-190.79		
	-204.208	-189.90		

Table 2 δ^{2}H Isotope Ratio Values of Biodiesel Samples (obtained from LGC)

Sample Name	**Measured Values (‰)**	**Corrected Values (‰)**	**Mean of Corrected Values (‰)**	**Standard Deviation of Corrected Values (‰)**
EUROPEAN RAPESEED BIODIESEL	16.128	17.58	17.39	0.14
	15.915	17.35		
	15.824	17.25		
	15.941	17.38		
USA CANOLA BIODIESEL	11.513	12.56	12.58	0.04
	11.543	12.59		
	11.580	12.63		
	11.499	12.54		

Table 3 δ^{18}O Isotope Ratio Values of Biodiesel Samples (obtained from LGC)

3.4.4 Isotope and fingerprint analysis of inorganic elements

PTB developed and validated methodology based on the use of Thermal Ionisation Mass Spectrometry (TIMS) for Sr isotope ratio measurements in sugar raw materials. Evaluation of their feasibility as tools for origin discrimination has shown differences in the δ (R(^{87,86}Sr)) value (referred to the NIST SRM 987) of >1 ‰ for sugar cane from Kenya and sugar beet from Germany (see Figure 8). Relative expanded uncertainties for δ (R(^{87,86}Sr)) ranged from u_{rel} = 0.052 % to 0.250 %.

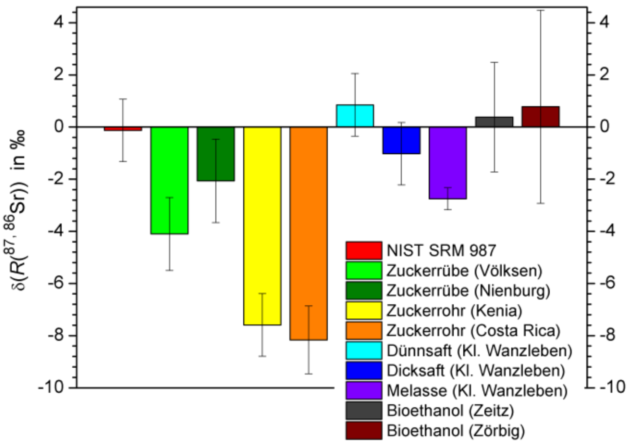

Figure 8. δ (R(^{87}Sr/^{86}Sr) results referred to NIST SRM 987 (obtained using TIMS)

Application of the Sr isotope ratio methodology to sugar beets obtained from specific and well known geographical sites in Europe (for which, Sr isotopic data for the site specific mineral water is available) did not show correlation of the geology of the locations where the beets were grown and the measured Sr isotope ratios. It was found difficult to define the area extension for which elemental abundances and isotopic signatures are representative.

JRC worked on the identification of possible biomarkers of origin discrimination by using elemental mass fraction ratios obtained for the comparison between four different raw materials (provided by LGC) and for one of the raw materials (USA Canola) compared with its corresponding oil and final product (biodiesel). To achieve this, sector field double focusing ICP-MS after sample digestion was applied. Main challenges were the low elemental concentrations in oils and biodiesel, the difficulty of digesting biodiesels and oils using large subsamples and microwave closed digestion due to generation of huge amounts of fumes and the control of procedural blanks. Sources of contamination during sample treatment were investigated and after taking precautions to minimise contamination, procedural blank signals and reproducibilities improved by factors of 5.9 and 12, respectively. Oils and biodiesels were digested using a high pressure asher. Calibration was performed by matrix matching and internal standardisation. The criteria for selection of the candidate elements to be monitored and quantified, as considered present in the samples included i) verification of the absence of severe spectral interferences by multi-isotope/isotope ratio monitoring, ii) detection of blank corrected isotope signals higher than 10 times the standard deviation of the blank signal and iii) achievement of signal RSD (n = 2) lower than 10 %.

Figure 9 compares the 4 raw materials analysed. Ratios have been normalised (Norm. ratio = $\mathrm{Ratio}_{\mathrm{raw\ material}\ i}/\mathrm{Ratio}_{\mathrm{Average}}$) to compare all results on a scale centred around the unity. A direct correlation was observed between ratios of mass fractions found for samples from the same kind of raw material whereas an anti-correlation was observed between different raw materials (e.g. SB from Brazil and CS from USA).

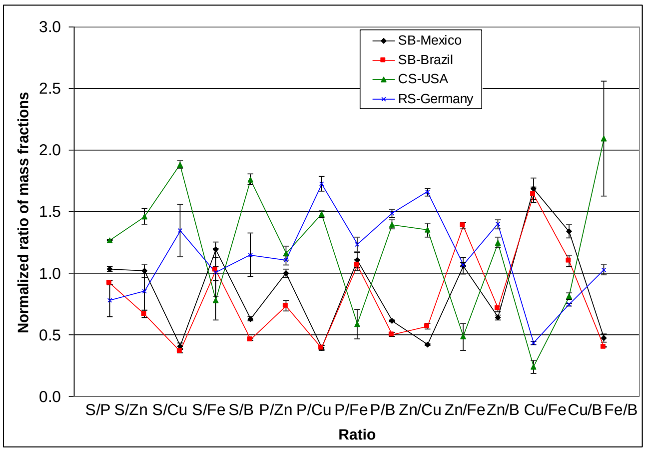

Figure 9. Normalized ratio of elemental mass fractions obtained for raw materials by ICP-MS. vertical bars correspond to the standard deviation of three replicates

JRC completed characterisation of a method (e.g. for selectivity, measurement uncertainty, etc) for Cu and Zn isotope ratios in biofuels. Cu isotopic measurements, when combined with Zn isotopic measurements, were found promising to discriminate between materials (raw materials) of different biological and geographical origin. No delta-scale isotope ratio measurements of Zn in biodiesel could be obtained due to the high and irreproducible procedural blanks. No agreement between the results obtained for the raw plant materials and the biofuels was observed for δ (‰) values obtained for n(65Cu)/n(63Cu).

3.4.5 Fingerprint analysis of organic traces

UT (Metrosert – University of Tartu) developed sample preparation/preconcentration and LC-ESIMS./MS methodology for screening of fatty acids in biodiesel. Evaluation of the feasibility of the developed methodology to demonstrate the potential of main free fatty acids (FFA ratios) as tools for the biological origin discrimination of biodiesel suggested that the ratio of some fatty acids, e.g. linoleic acid/oleic acid, may have potential to distinguish between biodiesels of different source or biological origin (e.g. soy biodiesel from canola/rape biodiesel).

3.4.6. Compound-specific isotope analysis of δ^2H, δ^{13}C and δ^{18}O

Compound-specific δ^{13}CVPDB values of the individual FAMEs within the biofuels have been determined by LGC using GC-C-IRMS. Results of the application of compound specific δ^{13}C values (see Figure 10) did not allow discrimination upon the basis of geographical origin of fuels derived from the same plant species. The FAME distribution, as determined by GC-C-IRMS, within the biofuels does allow discrimination between plant species; however this can be determined through more simple analytical techniques such as GC or GC-MS.

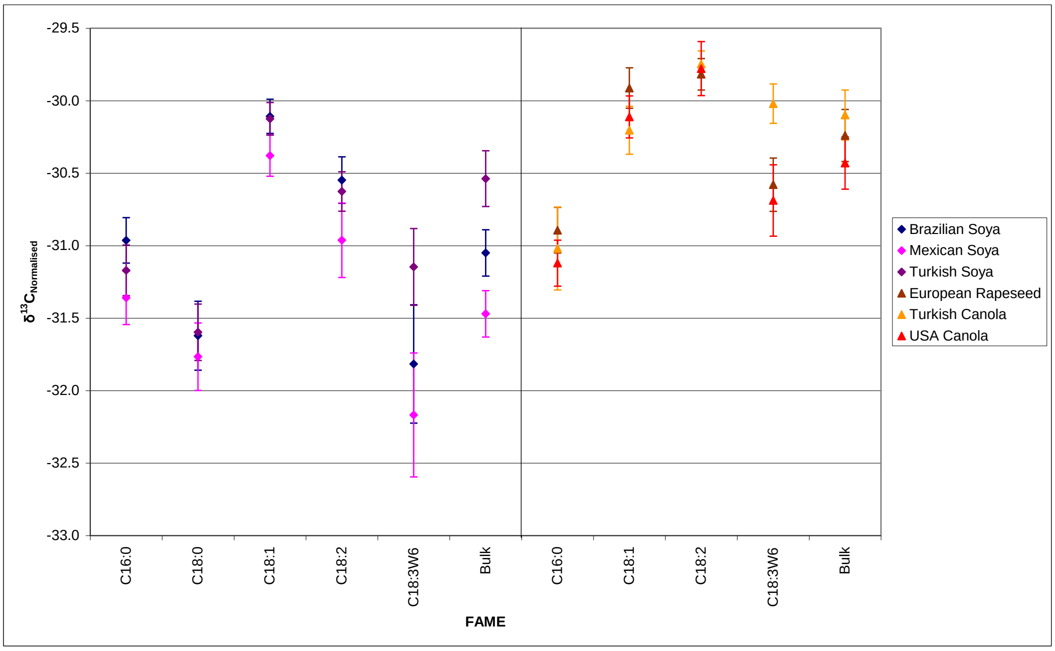

Figure 10. Normalised $\delta^{13}C$ values of the biofuel FAMEs with the bulk $\delta^{13}C$ value for comparison. Vertical bars represent ± the expanded uncertainty (k=2).

Overall achievements related to the origin discrimination can be summarised as follows:

- For the first time, isotope ratio data able to discriminate between biofuels of different origin is provided using validated and well characterised multi-methods.

In particular, the scientific results achieved are:

- Either, bulk and compound-specific C isotope ratios using EA/GC-IRMS were not proven to be good tools to discriminate between fuels of different geographical origin (e.g. Europe and USA).
- Good agreement of LGC with TUBITAK $\delta^{13}C$ values for biodiesel samples from different origins (e.g. European rapeseed biodiesel and USA canola biodiesel) within the measurement uncertainty were achieved.
- Unlike $\delta^{13}C$ measurements, both $\delta^{2}H$ and $\delta^{18}O$ measurements showed great potential to differentiate between oils and biodiesel of different types of raw material and geographical origin.
- δ (*R*(87Sr/86Sr) measurements were found to be a suitable tool for discriminating between sugar beets and sugar cane, both used to produced bioethanol. However, application of the Sr isotope ratio methodology (*U*rel < 0.052 %) to sugar beets obtained from specific and well known geographical sites in Europe (for which, Sr isotopic data for the site specific mineral water is available) did not show correlation of the geology of the locations where the beets were grown and the measured Sr isotope ratios. It was difficult to define the area extension for which elemental abundances and isotopic signatures are representative.
- Elemental mass fraction ratios for certain element pairs, measured in the raw materials, were found to be potential tools for discrimination between biofuels of different biological (source) origin.
- The combined use of *δ (‰)* values obtained for n(66Zn)/n(64Zn) and n(65Cu)/n(63Cu) was also found promising to differenciate between the investigated different raw plant materials.
- The ratio of specific fatty acids (e.g. linoleic acid/oleic) may have the potential to discriminate between final products (biodiesels) coming from raw materials of different biological origin.

- The withdrawn observations and conclusions, although relevant to the identification of biomarkers of biofuel origin, have to be interpreted with caution since the number of investigated samples is somewhat limited.

It is important to note that without the joint effort from TUBITAK and LGC to provide samples for all the partners, project delivery would not have been possible. PTB provided bioethanol and corresponding raw materials to JRC for elemental /isotopic method development and validation. The knowledge transfer from JRC to other partners, providing consistent and effective ways of presenting and interpreting the data achieved with these tools has been invaluable to the project success.

4 Actual and potential impact

4.1 Metrology achievements

Concerning the chemical characterisation of biodiesel, the reference methods for the following parameters have been validated within this project:

- Methanol content
- Glycerol content
- Glycerides content
- Selected FAMEs content.

In the field of physical characterisation of biodiesel, the reference methods for the following parameters have been validated:

- Transport properties at normal pressure (density, viscosity)
- State behaviour at high temperatures and high pressures (density, viscosity)
- Energy content (calorific value)

The methods for both chemical and physical parameters have been applied to characterise a reference material which is expected to be released in late summer/autumn 2014.

Moreover, the methods are described in scientific papers (see list in section 4.2) which allow the user community to have access to them.

In the field of references for quality indicators of bioethanol, pH and conductivity measurements have been validated. Also these methods are published. Moreover, two pilot studies are planned in the frame of the BIPM CCQM EAWG on these two measurements. This will allow the knowledge developed during the project to be shared with other NMIs.

Finally, some of the outputs of the feasibility study conducted to develop analytical tools for origin discrimination of biofuels have also been published and other are on the way to be published.

4.2 Dissemination activities

4.2.1 Scientific publications

The project has generated twelve high impact publications in key journals and four are in preparation. These incorporate the significant scientific outputs of the project. A list is provided in section 6.

4.2.2 Conferences and relevant fora

The work carried out in the project has already reached both the wider scientific audience in general conferences such as *Euroanalysis*, *BMSS* or *IMEKO World Congress* as well as targeted audiences in specialised conferences such as the *Biofuels International Conference* (Antwerp, BE, November 2012) and the *International Conference on Biofuels Standards* (Gaithersburg, USA, November 2012).

In total, two invited lectures have been given by the coordinator, and fourteen oral presentations as well as six poster presentations have been given by the partners during the life time of the project. Positive reactions were received to all these contributions, attracting discussions and comments.

Presentations of the outcomes of the project have also been given at a number of key fora, in particular at EURAMET and BIPM-CCQM working groups' meetings, where it has resulted in the influence of future strategy ensuring on-going impact of the project into the future.

In particular, at CCQM level, a pilot study is foreseen on pH and electrolytic conductivity on ethanol, in order to demonstrate the feasibility of ensuring SI traceability for these parameters.

Furthermore, the activities on references for quality indicators also has significant synergy with EMRP project ENV05 "OCEAN" where methods of the measurement of pH and electrolytic conductivity in seawater are being investigated. The generic methods developed under this project will feed through to the work of ENV05 in this area, providing significant added value for the EMRP endeavour.

4.2.3 Stakeholder Engagement and Standards

The main objective of the project was the development of reference measurement procedures of high order. These analytical methods are not directly applicable as standard "routine" methods and therefore not directly transferable to standardisation bodies. However, they are a fundamental tool to ensure the metrological traceability of the results of routine measurements and will allow method validation and quality assurance.

Furthermore, the results obtained on physical parameters are a strong support for the legal metrology, for which a robust data base for thermal conversion factors and for energy content to ensure fair trade and fair calculation of tax is needed.

Care was therefore taken during the life time of the project to keep regularly informed the standardisation bodies of the project activities and outcomes.

LNE is member of the *Bureau National du Pétrole* P08 and P06 committees, which are the mirror committees of CEN TC19 (gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin). The project has been presented to the working groups in March and September 2012. In this context, some results obtained on density measurements have partially been used to draft a French standard (FD M 08-037). This document has been published in December 2012 by AFNOR (the French Standardisation Body) and could be now used to draft a CEN or ISO standard.

In February 2013 VSL presented the newly developed "Reference GC method for methanol in FAME" to CEN/TC19/JWG1, including the measurement results and conclusion of the bilateral comparisons of BAM and VSL with respect to comparing the newly developed GC method with BAM's newly developed NIR-method during the analysis of the methanol contents in various biofuels. Furthermore the (then) almost finished newly developed "Reference GC method for MAG in FAME" was presented. The audience consisted of 20 experts, who welcomed the work performed within the project, as this work not only set a much improved calibration structure for checking the methanol content against the existing specification, but also showed a very promising new device to do this under field conditions at-site. Furthermore the experts were very happy with the progress then already made in quantifying the MAG contents in a thorough and much improved way.

The activities on references for quality indicators have been presented and have stimulated debate in CEN and ISO (in particular ISO TC28/SC7/WG4 'Ethanol Test Methods') about the creation of an improved test method for pHe which might at a future date be incorporated into an updated European specification for bioethanol quality. Participation continues on in ISO TC28/SC7/WG4 as NPL has been actively involved, through injecting knowledge on the metrological concepts, defining the measurand and evaluating uncertainty, in drafting a new standard method under a new work item entitled ISO/FDIS 17315 "Ethanol-Determination of total acidity by potentiometric titration". This standard is in its final draft registered for formal approval and should be published in few months. This will allow the worldwide harmonisation of the measurements for the total acidity of bioethanol.

In the United Kingdom, there has been interaction with relevant BSI standardisation committees who are interested in the outcomes of the project and some preliminary discussion about whether some new British Standards could be developed using the work on the improvement of the method for pHe.

The measurement methods developed in the frame of the activities related to origin discriminations and the need for biofuel RMs for quality control were discussed at the UK Energy Institute's Testing Measurement and Standards Committee (TMS meeting held in London in February 2013). This committee addresses measurement methods and reference materials for all areas of the petroleum and biofuels industries. Through its sub committees, specialist technical panels undertake collaborative development and testing of a wide range of industry standard measurement methods. This work inputs directly to BSI standards making committees and TMS members whom are active participants in many CEN, ISO and ASTM technical committees. No standard exists so far for the origin discrimination but the outputs of the feasibility study are encouraging to support future developments in this respect.

The entire project has been presented by the coordinator at the 4th International Conference on Biofuels Standards, organised by NIST in Gaithersburg, USA, in November 2012 (invited lecture). At this event, representatives of USA, Brazilian and European Standardisation Bodies were present. Positive reactions and encouragements were received, in particular from the Secretary of the CEN TC19 (gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin).

The outcomes of the project have also been discussed with the chair of the ISO/PC 248 “Sustainability criteria for bioenergy”, which scope is the standardization in the field of sustainability criteria for production, supply chain and application of bioenergy, including terminology and aspects related to the sustainability (e.g. environmental, social and economic) of bioenergy. The outputs of the project will contribute to the reflexion of the definition of the sustainability criteria.

CECOD (Committee of European Manufacturers of Petroleum Measuring and Distributing Equipment) is developing a software tool for legal metrology to take into account the presence of a fraction of biofuels in the fuels delivered at the pump. For this tool, the results on physical parameters were used. The software is currently still not finalised, but the interaction between the partners involved in this topic and CECOD is still active.

4.2.4 Workshops

Two stakeholders workshop have been organised during the life time of the project. During the first one, held in Paris in March 2012 in conjunction with the periodic meeting of the project, about thirty participants attended the meeting, including representatives of analysis laboratories, Proficiency Testing schemes providers, academic groups and research laboratories in the field of fuels. One day was dedicated to the presentation of the project results to the participants. Two invited speakers (from IFPEN and University of Alicante) presented their activities in the field of biofuels and in particular the advancements into the new generations.

The second workshop, entitled “The role of metrology in technical and economic development of biofuels” was held in Paris in May 2013, to mark the end of the project. About forty participants including biofuels producers, measurement instruments manufacturers and researchers met to exchange on the needs in the field of biofuels characterisation. A representative of the European Commission (DG Energy) gave an overview of the forthcoming regulatory perspectives for biofuels market. During this intervention, it was underlined the importance of the work conducted on the origin discrimination, as one of the main concerns of the EC in support of the sustainability of the biofuels.

A member of CECOD presented the software under development (see previous section). CECOD was particularly interested in the developments on the physical parameters, which will be useful for the improvements of their equipments.

Representatives of non-European NMIs were also present. In particular a representative of INMETRO and Chair of the ISO/PC 248 committee on Sustainability criteria for bioenergy gave an overview on the challenges and achievements for the sustainability standards.

For both the workshops, the delegates showed very positive interest about the outputs of the project, in particular emphasising the role of the NMIs in the development of metrological standards, needed to ensure the comparability on the field measurement results.

4.3 Effective cooperation between JRP-Partners

The European Metrology Research Programme (EMRP) is a metrology-focused European programme of coordinated R&D aimed at facilitating closer integration of national research programmes and ensuring collaboration between National Measurement Institutes, reducing duplication and increasing impact.

This project has been a good example of the implementation of this programme, gathering twelve NMIs/DIs and four academic partners (three Research Excellent Grant researchers and one linked third party) from ten European Countries and from the EC. Some NMIs from countries which are smaller contributors to the EMRP are also involved in the project. Furthermore, INMETRO (Brazilian NMI) and NMIJ (Japanese NMI) have also collaborated on specific tasks of the project.

Moreover, this project is one of the first collaborative projects in the field of metrology involving partners which have a range of expertise from both chemical and physical metrology.

The participation of a large number of research groups from several countries shows the general interest of the topic and represents the first valuable impact created by the project.

Many exchanges between the partners have taken place. This is shown in the frame of two “Research Mobility Grants” one researcher from LNE has spent three months at NPL (on the activities for the development of references for the quality indicators of bioethanol) and one researcher from PTB has spent 4 months at LNE (on the activities for the development of energy content measurements).

Several tasks have taken benefit from the collaboration between the partners, as demonstrated by the several joint publications and presentations (see below) and different analytical methods have been validated thanks to the joint collaboration of partners.

At the end of the project, a proof-of-concept exercise has been organised and JRC provided a rapeseed oil based biodiesel material which is a candidate certified reference material (CRM), to be certified for a number of parameters of EN 14214. While the actual CRM was produced outside the scope of this project, the same material was used to organise the proof-of-concept exercise, as the material had known stability and homogeneity for a large number of parameters of interest to the project. The material was offered to all the partners and measurement data has been provided for a number of chemical and physical parameters. The resulting data has been summarised and will be adequately referenced and acknowledged in the CRMs certification report, once the material is released by JRC.

4.4 Examples of early impact

Standards and regulation:

The French standard FD M 08-037 has been drafted using data results obtained on density measurements. This document has been published in December 2012 by AFNOR (the French Standardisation Body) and could be now used to draft a CEN or ISO standard.

User uptake:

CECOD (Committee of European Manufacturers of Petroleum Measuring and Distributing Equipment) is developing a software tool for legal metrology to take into account the presence of a fraction of biofuels in the fuels delivered at the pump. For this tool, the results on physical parameters were used. The software is still not finalised, but the interaction between the partners involved in this topic and CECOD is still active.

A certified reference material is currently under characterisation by the JRC-IRMM. This material is expected to be released in late summer/autumn 2014.

Scientific uptake and impact

The work undertaken in the frame of the feasibility study for the origin discrimination has attracted large interest by both the EC and the scientific community.

Two pilot studies are planned at BIPM CCQM level based on the work undertaken in the frame of the quality indicators (pH and conductivity measurements of bioethanol). These inter-laboratory comparisons will involve about 10 NMIs, i.e. much more than only those involved in the project. These comparisons will allow the scientific knowledge developed in the frame of the project to be shared outside Europe on these two parameters.

The development of reference methods for physical parameters and quality indicators is also part of a tripartite collaboration between LNE, PTB and INMETRO outside the scope of the project, which will support the development of certified reference materials for biofuels in Brazil.

4.5 Potential impact

Dissemination of the traceability concept to field laboratories through the metrology infrastructure put in place is a major outcome of the project. This will be achieved through the provision of reliable reference values either for proficiency testing schemes or for the certification of reference materials. The development of references for chemical and physical parameters will contribute to the harmonization of measurement and documentary standards across borders, which is obviously essential for confidence in international quality assurance, needed to facilitate global trade.

In the transport sector, clearly defined and internationally comparable quality standards based on traceable measurements and a wider knowledge of properties of biofuels are needed to support further technical developments which will facilitate the use of biofuels (e.g. improvement of engines and materials, development of techniques for a more efficient use of biological energy sources) and lead to more acceptance by customers and vehicle manufacturers.

This project, providing reliable analytical methodology suitable for biofuels, intended to contribute to the development of sustainable energy with the reduction of greenhouse gases, reinforcing the establishment of the new European directive on the use of energy from renewable sources.The results obtained on a number of parameters are important steps towards European and International harmonisation of the measurement methods of biofuels and their blends with fossil fuel. In particular the data obtained for physical properties will give a strong support to the legal metrology, for which a robust database for thermal conversion factors and for energy content to ensure fair trade and fair calculation of tax is needed.

It is hoped that all of these outcomes will in future have a direct influence on the documentary standard specifications for defining the quality of biofuels and in addition how these measurement can be made in future.

5 Website address and contact details

A public website has been open, where the main public deliverables have been made available for the end-users and keep them informed about project meetings and events: www.french-metrology.com/jrp/metrology-biofuels.

A partners' restricted area has also been created, in order to give the possibility to all the partners to share work documents and deliverables: https://pilotage-metrologie.lne.fr (the password is available on request).

The contact person for general questions about the project, is Dr Paola Fisicaro, LNE (paola.fisicaro@lne.fr).

The contact person for the development of references for chemical parameters is Dr Andrea Held, JRC-IRMM (Andrea.HELD@ec.europa.eu).

The contact person for the development of references for physical parameters is Dr Henning Wolf, PTB (Henning.Wolf@ptb.de).

The contact person for the development of references for quality indicators is Dr Richard Brown, NPL (richard.brown@npl.co.uk).

The contact person for the development of analytical tools for origin discrimination is Dr Heidi Goenaga-Infante, LGC (Heidi.Goenaga-Infante@lgcgroup.com).

6 List of publications

[1] A novel application of Recursive Equation Method for determining thermodynamic properties of single phase fluids from density and speed-of-sound measurements
S. Lago and P.A. Giuliano Albo
J. Chem. Thermodynamics, In press (DOI: http://dx.doi.org/10.1016/j.jct.2012.10.016)

[2] Sensitivities of a Standard Test Method for the Determination of the pHe of Bioethanol and Suggestions for Improvement
R. J. C. Brown, A. C. Keates and P. J. Brewer
Sensors, 2010, 10, 9982 - 9993. (http://www.mdpi.com/1424-8220/10/11/9982/pdf)

[3] Influence of fabrication procedure on the electrochemical performance of Ag/AgCl reference electrodes
D. Stoica, P. J. Brewer, R. J. C. Brown, P. Fisicaro
Electrochmica Acta, 2011, 56, 10009 - 10015.
(http://www.sciencedirect.com/science/article/pii/S0013468611013363)

[4] Evaluation of standard potential of Ag/AgCl electrode in a 50 wt% water-ethanol mixture
D. Stoica, C. Yardin, S. Vaslin-Reimann, P. Fisicaro
Journal of Solution Chemistry, 2011, 40, 1819 - 1834. (http://link.springer.com/article/10.1007%2Fs10953-011-9758-3)

[5] Electrolytic Conductivity as a Quality Indicator for Bioethanol
S. Seitz, P. Spitzer, H.D. Jensen, F. Durbiano
Proceedings of XX IMEKO World Congress Metrology for Green Growth
(http://www.imeko.org/publications/wc-2012/IMEKO-WC-2012-TC20-O12.pdf)

[6] Determination of pH values of potassium hydrogen phthalate buffer in water-ethanol mixture (mass fraction 50 %)
D. Stoica, P. Fisicaro, P. Spitzer, B. Adel, F.B. Gonzaga, I.C. Fraga, P.P Borges, I. Maksimov, T.Asakai
Proceedings of XX IMEKO World Congress Metrology for Green Growth
(http://www.imeko.org/publications/wc-2012/IMEKO-WC-2012-TC24-O4.pdf)

[7] Fatty acid composition and chemotaxonomic evaluation of species of Stachys
Ahmet C. Gören, Ekrem Akçicek, Tuncay Dirmenci, Turgut Kilic, Erkan Mozioğlu, Hasibe Yilmaz
Natural Product Research, 2012, 26, 84–90

[8] Density, viscosity and specific heat capacity of diesel blends with rapeseed and soybean oil methyl ester
N. Glen, M. Clerck, H. Wolf, R. Pagel, S. Lago, P. A. Giuliano Albo, P. Ballereau
Fuel, submitted

[9] Experimental speed-of-sound measurements of pure fatty acids methyl ester, mineral diesel and blends in a wide range of temperature and for pressures up to 300 Mpa
P. A. Giuliano Albo, S. Lago
Fuel, 115, 2014, 740-748

[10] On-line hydrogenation of biodiesel—Toward a reference method for the determination of glycerides and total glycerol.
L. Ruano Miguel, M. Ulberth-Buchgraber, A. Held
Journal of Chromatography A, 2014, 1338, 127–135

[11] Examining the possibility of using ratios of element mass fractions as markers for origin determination of soya and canola based biodiesels. Measurement procedure validation to underpin this study.E. Paredes, S.Z. Can, C.R. Quétel

Fuel 123, 2014, 248–255

[12] Combustion energy and calorific value of n-dodecane: A case study applying different calorimeter types. R. Pagel, Dz. Zaitsau
Journal of Thermal Analysis and Calorimetry, Submitted

Furthermore, four publications are under preparation, covering

- the joint research results of VSL and BAM on the development and validation of both a reference and a NIRS method for methanol in biodiesel,
- the development of a qNMR method for methanol (BAM),
- the development and validation of a reference method for the FAMEs in biodiesel (joint paper of TUBITAK, LGC and JRC-IRMM),
- the joint research results on origin discrimination (joint paper of LGC and JRC-IRMM, TUBITAK, Uni Tartu)