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Validation of direct methods for biogenic fraction assessment in fuels on a liquid scintillation counter

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Abstract
Recently developed direct LSC method for the biogenic fraction determination in biodiesel samples was evaluated. Intercomparison samples had the unknown composition of biomaterials/fossil fuels and a broad range of quench levels. Reliable results were obtained with the direct two-step LSC method for the samples with a quench level of roughly 50 channels above the SQP(E) limit of the method's applicability. The Internal Standard method for the detection efficiency determination provided better accuracy, additionally lowering the SQP(E) limit of the method's applicability. The adapted two-step method's calibration via Internal Standard technique was tested on samples with sunflower seed biocomponent.

Keywords Biodiesel · Biogenic fraction · Liquid scintillation counting (LSC) · ^{14}C content

Introduction

The Directive (EU) 2018/2001 concerning the European Union's gross final consumption of energy established a 32% target for the overall share of energy from renewable sources to be achieved by the year 2030, following a cut of at least 40% of carbon emissions [1]. Liquid biofuels from very diverse sources (agricultural materials, originating from food and non-food energy crops, residues from forestry sources, etc.) play a major role in the global transition to renewable and sustainable energy [2]. The increasing production and promotion of different fossil/biogenic fuel mixtures has been followed by the establishment of methods for the accurate validation of the biomass fraction in liquid fuels.

Accelerator Mass Spectroscopy (AMS) and Liquid Scintillation Counting (LSC) are the most commonly used techniques based on radiocarbon (^{14}C) method which are able to make a distinction between fossil and bio-based fuel matrices [3, 4]. The instrument cost for AMS is ~2,500,000 USD, with rather expensive analysis of (300–500) USD per sample, with over a 1 week turnaround time before the results are obtained, whereas LSC instrument cost is ~150,000 USD [5], the cost of analysis per sample is roughly 4 times less expensive than by AMS, and results of sample analysis can be obtained in 1–2 days or less.

Radiocarbon ^{14}C is a cosmogenic isotope, continuously generated in the upper atmosphere, in collisions of atmospheric ^{14}N and free neutrons that were created in cosmic-ray transformations [6, 7]. Together with stable carbon isotopes, ^{13}C and ^{13}C, ^{14}C is oxidized to carbon dioxide CO_2, which is than incorporated into plants through photosynthesis, and animals by eating the plants [8]. Biogenic material is mostly equilibrated isotopically with the ^{14}C content of the atmosphere. Carbon exchange with the environment ceases when the organism dies, but radioactive decay of ^{14}C is still undergoing with a half-life of 5730 years [9]. The detection of remaining ^{14}C (i.e. radiocarbon dating) is possible in materials about ~50,000 years old [6]. ^{14}C is an ideal tracer for the biogenic component in fuel blends since all recent natural products (plant or animal materials) are effectively pre-labelled with ^{14}C [6]. The physical basis of a biomass percentage determination in fuel blends lies in the fact that fossil fuels (oil, coal) are millions of years old, do not contain ^{14}C, while the ^{14}C content of bio-based modern-day

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materials, including liquid fuels, is approximately proportional to the biogenic fraction [8, 10].

Biomass quantification by AMS requires expensive equipment and assumes relatively complex measurement procedures [10] which is the reason many authors have investigated LSC methods as an alternative. In the last two decades, the development and successful implementation of several slightly different direct LSC techniques for the biomass fraction determination in liquid fuels have been reported [3, 5, 8, 11–14]. Besides the direct LSC method, there are two other, more complex LSC methods that involve benzene synthesis and carbon dioxide absorption in amine [13]. A few studies have conducted comparisons of the performance of all three LSC methods during the analysis of fuel samples [15, 16]. The LSC-benzene synthesis method has high precision and sensitivity, but the sample preparation is expensive, time consuming [13], and involves work with hazardous materials. The LSC-CO_2 method is less expensive and less complicated, although its precision and accuracy are poor, and its sensitivity is not adequate for the measurement of the lowest ^{14}C activities in fuel samples [13, 15].

The direct LSC method involves mixing of the fuel sample with a scintillation cocktail prior to counting. Its advantages include quick, simple and low-cost sample preparation, as well as satisfying accuracy and sensitivity of ^{14}C content determination required for the quantification of bio-component in fuel samples [4, 5, 13–15, 17]. However, during LSC experiments, quench phenomena (chemical and color quench dominantly) are the most important factor that can seriously diminish the counting efficiency for a given sample/cocktail mixture [18]. Chemical substances present in the sample can absorb nuclear decay energy in the scintillation process, obstructing the transfer of nuclear decay energy to the scintillation cocktail solvent. Color quench occurs if the sample has visible color, absorbing photons of light in the vial before their detection by the photomultipliers [19]. Quench effects could be compensated by correct determination of detection efficiency via quench calibration curves, i.e. the counting efficiency versus quench-indicating parameter, which could be applied for each measured sample [19].

One drawback of direct LSC method during biodiesel analysis lies in the large diversity and different colors of commercial biofuels on the market [8], which implies that they pose quenching levels in a wide range. Consequently, the calibration of the instrument is often applicable only for the assessment of biofuels of certain biogenic or fossil fuel composition. The most accurate results are obtained when biofuel carbon mass fraction and biofuel feedstock of the analyzed sample are known [14].

The purpose of this paper was to validate recently developed direct LSC methods and to investigate the possibilities and limitations of the "one-step" and the "two-step" LSC methods for the characterization of biofuels. In the one-step method, the mass percentage of biofuel is determined from the measured count rate in ^{14}C beta spectrum. The two-step method involves determination of detection efficiency based on the quench level of the analyzed sample, which is followed by determination of the bio-mass percentage based on the ^{14}C activity concentration in the fuel mixture [12, 20]. The calibration of a Quantulus 1220 LS counter was carried out with biodiesel produced from two feedstock materials: sunflower seed and lard fat. The entire optimization procedure has been published recently [20], where calibration of the instrument was demonstrated for several combinations of biobased/fossil fuel blends. This paper presents one generalized calibration of the instrument that encompasses all the previously obtained data. Its application was tested on real fuel samples with unknown composition. The main research goal was to investigate whether our generalized two-step method would derive acceptable results of bio-component quantification in intercomparison samples (seven samples that were part of an inter-laboratory method comparison) containing unknown biomaterials. The paper offers detailed discussion on the present accuracy and the uncertainty of the two-step method and the possibilities of its improvement in the future work. Lastly, we demonstrate the adaptation of the two-step method's calibration to the analyzed biofuels via an Internal Standard technique, which has been further tested on several samples with sunflower seed biocomponent.

Experimental

Equipment

An Ultra Low Level Liquid Scintillation Spectrometer Wallac Quantulus 1220 (PerkinElmer, Finland) was used for the counting of all samples. This instrument ensures an ultra-low background level since it contains both passive and active shield [21]. Lead, copper, and cadmium layers are distributed all over the vial chamber, generating the detector's passive shield. Detection of radiodecay events (in a mixture of the sample and scintillation cocktail) is enabled via two photomultiplier tubes positioned around the sample vial that operate in coincidence. Furthermore, the active shield is composed of a mineral oil scintillator that encircles the vial chamber with an additional pair of photomultiplier tubes. This pair works in anticoincidence with two photomultiplier tubes that record events coming from the vial chamber, thus efficiently discriminating the majority of background events.

The Quantulus 1220 can approximate counting efficiency in the analyzed samples using the quench indicating parameter Spectral Quench Parameter of the External Standard (SQP(E)). Namely, Quantulus is equipped with external standard ^{152}Eu, and SQP(E) is a channel number that corresponds to 99th percentile of the ^{152}Eu spectrum

[21]. The presence of quench in samples is reflected in a spectral shift towards lower channels, consequently lowering SQP(E) value. It was confirmed that SQP(E) measured for each sample for 10 min ensures precise quench level determination [22].

Pulse Amplitude Comparator (PAC) circuit, if adequately adjusted, additionally decreases the background level that is generated by the spectral interference, such as fluorescence or Cherenkov events [21]. The basis for PAC operation is the comparison of the amplitudes of recorded events in two photomultiplier tubes around the vial chamber that work in coincidence. Pulses that originate from ^{14}C decay in a sample vial will not have significant differences in amplitudes of recorded coincident pulses from the two photomultiplier tubes. Background pulses, however, would be recorded with a relatively large amplitude difference in coincidence pulses. The selected PAC level therefore discriminates pulses with a certain amplitude ratio of coincident pulses, which the user can adjust manually. This circuit is the most efficient when ^{14}C detection is conducted in low-quenched samples that are prepared in glass vials. It was determined that $50 < PAC < 150$ setting provides low background level and precise ^{14}C spectrum generation, which are adequate for ^{14}C activity determination in fuel samples [20].

The analysis of biogenic fraction was conducted with the following protocol: after sample preparation, all samples have been counted by selecting the detector's default ^{14}C counting protocol. A high coincidence bias was chosen, and PAC parameter was adjusted to 100. Spectral acquisition and analysis were performed using EasyView and WinQ software. After the counting, the count rate and SQP(E) parameter for the each sample were obtained for determination of biogenic fraction in the analyzed fuel sample.

Materials and intercomparison samples

All samples have been prepared in polyethylene vials (20 mL of the total volume) by direct mixing of 10 mL of the analyzed fuel sample with 10 mL of Ultima Gold F scintillation cocktail and have been counted in three cycles, each for 1000 min. Calibration samples (as well as samples for the modified two-step method test listed in Table 2) were prepared by combining biofuels FAME (Fatty Acid Methyl Esters) produced from two feedstock materials - hybrid sunflower oil and animal fat - and two commercial fossil fuels (diesel with winter and summer season additives). The reasons for their selection, their preparation, as well as the properties of all used biodiesel and fossil diesel, are displayed and discussed in detail in the previous publication [20].

The background count rate has been obtained as 1.034(31) cpm in 230–540 the channel range, which is the average value of different matrices of fossil fuels (diesel-premium quality, diesel without additives, diesel with winter additives, diesel with summer additives, petroleum ether, oil, and gasoline).

For the efficiency determination via Internal Standard method, a standard radioactive source (aqueous ^{14}C solution) produced by PerkinElmer was used (catalog/part no. 6,002,135), with a certified activity $A(^{14}C) = (9.19 \cdot 10^5 \pm 0.82\%)$ dpm mL^{-1} on reference date 09/07/2008.

Seven intercomparison samples originate from the international intercomparison study ILC/2018 Content of biocomponent in liquid fuel samples, organized in 2018 by the Institute of Ceramics and Building Materials (Opole, Poland). Seven fuel samples with an unknown composition have arrived at the Laboratory for Low-level Radioactivity, Ruđer Bošković Institute (RBI) in Zagreb, which officially participated in intercomparison. The results of these measurements have been published recently [23]. From there, the samples have been distributed at the Department of Physics, University of Novi Sad (DP-UNS), for the purpose of unofficial internal intercomparison and validation of methods that had been developed at the Laboratory for Testing Radioactivity of Samples and Doses of Ionizing and Non-Ionizing Radiation at DP-UNS, Serbia. Methods that have been evaluated at RBI and DP-UNS institutions during this intercomparison are both direct LSC methods, however, the methodologies and optimization procedures are different and have been published in previous works [8, 20].

Methodology overview

Direct methods involve mixing of fuel samples with scintillation cocktails. Fuel samples do not require any pretreatment procedures before preparation. When mixed, they are left for one day in the dark in order to allow chemiluminescence and photoluminescence to dissipate prior to being counted with the LS counter. Duration of the counting is an important variable that influences the minimal detectable biogenic content in a fuel sample. Longer counting times reduce the ^{14}C activity concentration that can be detected in a sample as demonstrated in previous research [20], thus reducing the detection limit of the percentage of biogenic mass. The reports in the literature assure the adequate accuracy for 360 min of counting time [10], i.e. 330 min of counting time for the precision < 3% [13]. The counting time of 1000 min has been recommended in some reports as well [4]. The obtained limits of detection strongly depend on the quench level of analysed sample, which will be demonstrated later in this research.

The mass percentage of biofuel represents a ratio of the mass of bio-component and the total mass of the sample (biogenic mass + mass of fossil fuel) [24]. Laboratory at the University of Novi Sad, Department of Physics, has developed two variations of direct LSC method: the so-called

one-step and two-step LSC methods for the biomass fraction determination in biodiesel samples [20].

The one-step method requires the creation of a correlation function between the mass percentage of biofuel and the count rate from the ^{14}C beta spectrum. The main drawback of the one-step method is the assumption that the counting efficiency (i.e. the quench level) is similar in all fuel samples. Although this approach is simple, it neglects the varying quench levels of fuel blends, which can significantly alter the obtained count rates and detection efficiency. Therefore, its application guarantees accurate results only for the analysis of samples with the same chemical composition of biobased and fossil matrix as the calibration samples [4]. This paper offers an investigation into the limitations and the range of application of the one-step method with a generalized calibration curve obtained from several different fuel blends.

The two-step method, on the other hand, comprises the quench correction during biomass fraction determination. It involves the usage of two calibration curves: the established correlation between the quench level indicated by the SQP(E) value and detection efficiency, followed by the mass percentage of biofuel in a sample versus ^{14}C activity concentration correlation [12]. Therefore, after detection efficiency determination based on the quench level in the sample, the obtained count rate is converted to ^{14}C activity concentration, which is correlated with the biofuel fraction. The two-step procedure offers general application and reliable characterization of biogenic component for fuel blends with a broad range of quenching properties. The quality and applicability of the quench correction curve is better when used calibration samples are diverse, i.e. contain different components (additives, different types of biofuel materials etc.). Additionally, two-step method in a combination with Internal Standard method can improve the accuracy of the obtained results, which will be demonstrated and discussed in the following text.

According to the Internal Standard method, a known amount of non-quenched ^{14}C standard should be pipetted after the initial counting of the sample, and the sample should be recounted [25]. The efficiency is calculated from:

$$\varepsilon = \left(R' - R\right)/A \tag{1}$$

where R' [cpm] is the count rate of the sample spiked with ^{14}C standard, R [cpm] is the count rate of the sample before ^{14}C addition, and A [dpm] is the added ^{14}C activity (which is recommended to be much higher than that of the sample for good counting statistics).

Results and discussion

One-step and two-step methods' unified calibration

For blends with two different feedstock materials as biogenic component (sunflower seed and lard fat) and for commercial fossil fuels with different additives (winter and summer package), calibration equations of the one-step method were obtained separately. Therefore, four linear functions were obtained as calibration equations that would fit properly if samples of the same composition were analyzed [20]. Those data were used to generate one unified equation in order to investigate the potential and constraints of the one-step method during the analysis of unknown samples.

Furthermore, for the one-step method, the essential parameter, the optimal spectral window, Region of Interest (ROI), was previously chosen in the channel range 230–540. The optimal spectral window was decided based on the highest value of the Figure of Merit ($FOM = \varepsilon^2/R_b$, where ε denotes detection efficiency, and R_b count rate of blank sample) and the lowest Minimal Detectable Activity (*MDA*) achievements.

However, during the analysis of the mentioned intercomparison samples, it has been noticed that the sample spectra were generated in a somewhat broader channel range. Therefore, the complete calibration procedure has been carried out on the same calibration sample spectra, but the spectral window was selected in the channel range 150–550. All data obtained for the two biogenic and fossil matrices in a wider spectral window together with one unified calibration curve (quadratic fit), bio-mass percentage dependence on the count rate, is presented in Fig. 1. The background count rate in a broader channel range (150–550) has been obtained as 1.052(33) cpm.

From the confidence and prediction intervals displayed in Fig. 1 some conclusions about the uncertainty of the one-step method could be derived. The narrow confidence intervals (from 0 to 40% of bio-component in fuel mixture approximately) suggest that the method provides results of biogenic mass content in fuel samples with deviations up to 1–2%. However, if a sample contains $\gtrsim 70\%$ of bio-component in fuel mixture, the result of the analysis could have deviations up to roughly 5–10%. The relatively large uncertainty of the method was caused by fitting data from the analysis of diverse biodiesel calibration samples. This demonstrates that the accuracy and precision of the method would be much better if all calibration samples contained the same chemical composition, while the obtained calibration curve would be adequate only for the analysis of fuel blends with the same biogenic/fossil content.

Since the conventional *MDA* calculation according to Currie expression [26] could not be applied to the one-step

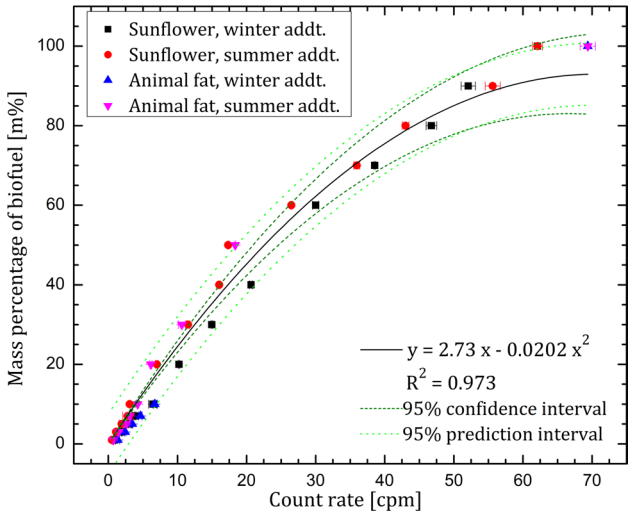

Fig. 1 One-step method calibration curve (spectral window: channels 150–550)

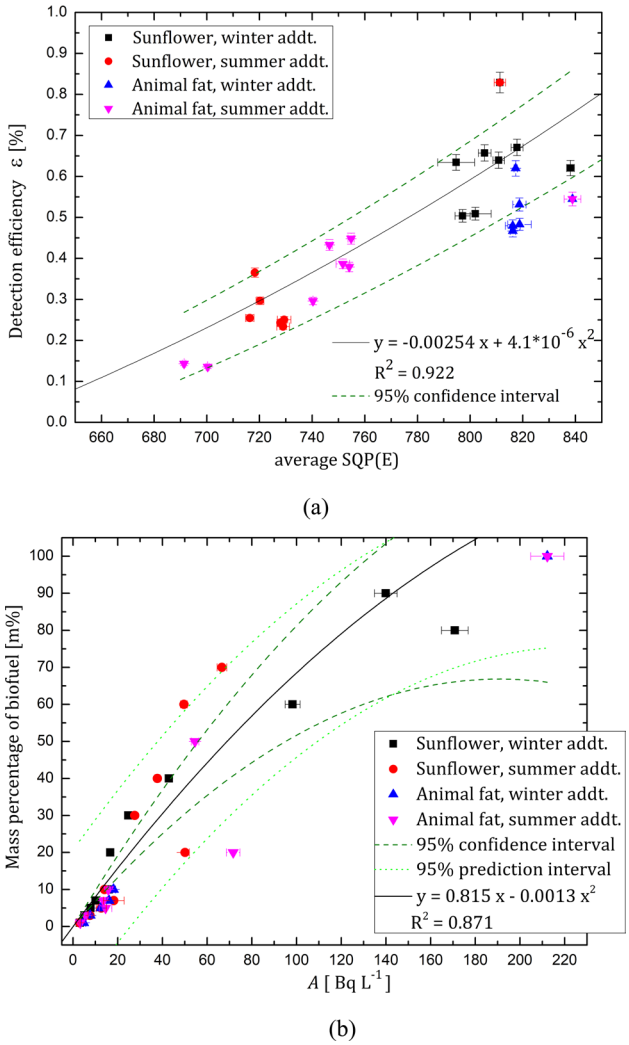

Fig. 2 Two-step method calibration (spectral window: channels 150–550): **a** unified fit quench curve **b** biogenic fraction versus activity concentration curve

method, the minimal detectable biogenic fraction in fuel mixtures has been roughly estimated when the background value was inserted into the equation presented in Fig. 1. This has led to a value of 2.8% as the minimal bio-component that can be detected.

The two-step method has a lot more potential, since it accounts for variations in the quench level of the unknown samples, which should enable more reliable determination of detection efficiency for ^{14}C content via the calibration curve displayed in Fig. 2a. The efficiency was calculated based on the count rates of calibration samples that were spiked with the known ^{14}C activity [20]. Clearly, the ε versus SQP(E) equation (quench curve in the following text) will not necessarily provide precise results for the unknown samples since it has been obtained as the unified fit of data from calibration samples with different chemical composition and various quench levels. Additionally, the quench curve implicitly defines a method's applicability limit, i.e. the SQP(E) value that corresponds to zero detection efficiency. Below SQP(E) = 620, the method is not possible to use since the sample is too quenched for any ^{14}C activity to be detected. Secondly, one can calculate the activity concentration of radiocarbon in a biodiesel sample based on the determined detection efficiency [20]. Its value leads to quantification of bio-component in fuel mixtures by the usage of a dependence shown in Fig. 2b. Here, the unified fit of data generated by the counting of calibration samples with different chemical composition in a wider spectral window (channels 150–550) is presented.

However, the confidence and prediction intervals displayed in Fig. 2b should be interpreted as further limitations of the obtained calibration for the two-step method. The uncertainty of the method increases with the biogenic fraction in the fuel sample. In the samples with biogenic fraction less than 40%, maximal deviations of the obtained results would be up to ~5%, however, if a sample contains ~70% of bio-component in fuel mixture, the result of the analysis could have deviations 10–15%. The presented calibration for the two-step method would not provide reliable results for the samples with $\gtrsim$70% of biogenic component in fuel samples since the obtained results would be derived with unacceptable uncertainty or possibly large deviation. This finding is in accordance with the known fact that direct LSC methods are challenging in the region around 100% in the case of diesel because of strong color quenching [12].

The large uncertainty of the presented method with "generalized" calibration curves based on the measurements of diverse biodiesel calibration samples can be caused by

Table 1 Results of internal intercomparison

Reference biomass of a sample [%]	SQP (E)	One-step method	Two-step method, unified ε versus SQP(E) fit	Two-step method, internal standard ε versus SQP(E) fit
		Obtained biomass of a sample [%]		
100.0 ± 2.0	544.6 ± 1.7	< *MDA*	Below SQP(E) limit (method is not applicable)	Below SQP(E) limit (method is not applicable)
30 ± 4	592.4 ± 0.8	< *MDA*		11.4 ± 2.3 z = − 8.2
21 ± 3	634.3 ± 0.9	4.9 ± 0.4	49 ± 6 z = 4.5	18.3 ± 1.6 z = − 1.7
7.0 ± 1.7	684.1 ± 2.0	4.7 ± 0.3	11.4 ± 1.3 z = 3.4	8.2 ± 0.4 z = 2.9
3.5 ± 1.1	721.2 ± 2.9	3.60 ± 0.27	5.1 ± 0.7 z = 2.3	3.94 ± 0.24 z = 1.8
0.0 ± 0.9	796 ± 5	< *MDA*	0.70 ± 0.28 z = 2.5	0.64 ± 0.20 z = 3.2
7.6 ± 1.1	873.4 ± 2.8	17.5 ± 0.9	9.5 ± 0.8 z = 2.5	10.9 ± 1.0 z = 3.2

the assumption that all those samples have similar, average density and carbon fraction. Namely, the ^{14}C content of modern plants is expressed in specific activity, i.e., the decays per minute per unit mass of carbon [9], while the two-step method refers to ^{14}C activity concentration (decays per second per unit volume) and does not account for variability in sample density and carbon fraction. The importance of carbon fraction measurement in a sample has been demonstrated in recent publications [5, 6, 14, 27], and its determination should improve the accuracy and precision of presented method, which is a matter of future investigation. Other remark to the presented "generalized" modern calibration curve is the assumption that the sample's density and diverse chemical composition are all unequivocally reflected in a quench level of a sample. Further investigation should be conducted in order to test weather the accounting for density variations, beside SQP(E) value, could improve the two-step calibration and diminish its uncertainty in the case of samples that contain biogenic fraction higher than 70%.

Evaluation of methods on intercomparison samples

Results of measurements of the seven intercomparison samples are presented in Table 1, where samples have been ordered by the increasing SQP(E) value, i.e., from the most quenched one to the least quenched one.

Although the one-step method's reliability is restricted to the samples with chemically identical biogenic and fossil components as the calibration samples, application of the calibration obtained in Fig. 1 with biofuel samples with several unknown different compositions has been tested on intercomparison samples to demonstrate its constraints and limited applicability. As shown in Table 1, the one-step method gave results with diverse absolute deviations, ranging from 0.1 to 16.1% of biomass fraction. Intercomparison samples have been selected within the wide range of a quench indicator SQP(E), and the chemical composition of most of them differs from the mentioned calibration samples. Moreover, it can be concluded that it is impossible to define the quenching properties (i.e. SQP(E) limit) above which the one-step method would give reliable results. Namely, for the samples with 7%, 3.5%, and 0% biomass percentage, the one-step method provided results with absolute deviations < 3% of biomass fraction, but for the least quenched sample (the highest SQP(E) parameter) with 7.6% of biocomponent, the method gave inaccurate result with 10% of absolute deviation. All these results demonstrate that the one-step method is strictly dependent on the chemistry of the analyzed fuel blends. It is not possible to define the SQP(E) limit above which this method would be applicable and reliable in the case of analysis of biofuels with unknown bio/fossil matrices. Therefore, the usage of one-step method is reasonable and meaningful only for the samples with the same chemical composition as the calibration samples, in which case the quench level would not differ and it would not alter the obtained detection efficiency.

The calibration curve presented in Fig. 1 is still highly relevant since it is based on the measurement of significant number of calibration samples (40 in total, containing two bio-based feedstock materials that are frequent on the market in the region), but its applicability and validity is limited to samples with the same bio-content (fossil matrix is not the critical or crucial parameter that affects biogenic mass determination [4]).

Application of the described two-step method for the measurement of biomass percentage in intercomparison samples, with explicitly defined limit of method's applicability, was expected to provide more reliable results. The

first two samples were quenched below the SQP(E) limit (SQP(E) < 620), so determination of a biobased component has not been possible in these samples. Furthermore, the biomass percentage of other samples is presented together with a z-score for the obtained values. During intercomparison studies, an indicator of accuracy was z-score value, where acceptable results have $|z| \leq 2$, while results obtained with $|z| > 3$ are regarded as unacceptable. For the third sample that was strongly quenched (SQP(E) = 634.3 ± 0.9, which is near the limit of the method's applicability), the result of the obtained biomass deviated > 20% of the true value, having z = 4.5. It is interesting to notice that in a sample with 0% biomass, the measured count rate was somewhat above the background, thus some "false" biomass percentage has been detected but with significant uncertainty. This is a consequence of the usage of the average value of the background count rate obtained for various types of fossil fuels. Clearly, the blank value for this particular fossil fuel sample was higher than the average value that has been obtained during the development of a method. The general conclusion is that the two-step method provides results with relatively satisfying accuracy (absolute deviations are < 5%, $|z| \lesssim 3$) if the SQP(E) parameter of the analyzed fuels is at least 50 channels higher than the value denoted as the limit of its applicability. For the two-step method, it was proved that it can be successfully applied to unknown samples that are based on different feedstock biomaterials than calibration samples.

In addition, the presented calibration (Fig. 2) involved measurements of biodiesel with two feedstock materials, while the generalized calibration curve should involve samples with more diverse biogenic/fossil blends in order to increase accuracy/precision of the method and its suitability in the case of measurement of the samples with the unknown chemical composition. The two-step method is certainly more reliable and relevant in comparison with one-step method, with strictly determined limit of applicability.

Adaptation of two-step method via internal standard method

One further improvement that was investigated for the two-step method was the usage of the exact detection efficiency parameter determined for each of the analyzed samples via the Internal Standard method. This method has been known as a very effective tool during various LSC measurements [25], and its successful application to radiocarbon analysis in fuel samples has been reported recently [28]. Namely, all intercomparison samples have been spiked with the known activity of ^{14}C standard solution and were recounted to establish the quench curve. This correlation comprised different chemical compositions of all analyzed samples. For the re-calibration purpose, the activity of 91,900 dpm of the standard radioactive aqueous source ^{14}C was spiked in intercomparison samples which had been recounted for 100 min in 5 cycles. The obtained fit of data is presented in Fig. 3, and one can notice very consistent results among intercomparison samples, i.e., consistent ε versus SQP(E) dependence behavior for all analyzed samples. The second step remains the same as it was in the previous, original two-step method, and we use the same calibration curve shown in Fig. 2b.

The described modification of the first part of the method for majority of samples provided more realistic values (better accuracy and lower z-values) of the detection efficiency and, therefore, a more precisely quantified biomass fraction as well, since both the calibration and analyzed samples had the same quench level. It also expanded the applicability of the method itself: the quench level that corresponded to zero detection efficiency was SQP(E) = 568 (Fig. 3), which is a significant improvement to the previously established two-step method presented in Fig. 2a.

Finally, the results of measurements using a modified two-step method can also be seen in Table 1. The accuracy has been improved for most of the samples, especially for the third sample, the highly quenched one certified with 21% of bio-component. The limit of the method's applicability has been shifted towards a lower SQP(E) value, which enabled the detection of ^{14}C content in the second sample with SQP(E) = 592.4 ± 0.8 as well, but with poor accuracy as expected. Results of analysis of samples with SQP(E) parameter roughly 50 channels above the method's applicability limit were obtained with maximal absolute deviations 3.5%, and $|z| \lesssim 3$.

The minimal detectable biogenic fraction in fuel mixtures has been re-calculated according to Currie expression

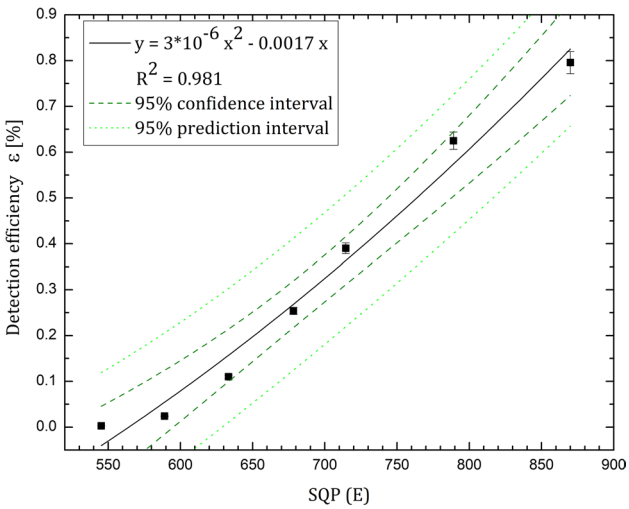

Fig. 3 Two-step method's adaptation, quench curve obtained by the Internal Standard method (spectral window: channels 150–550)

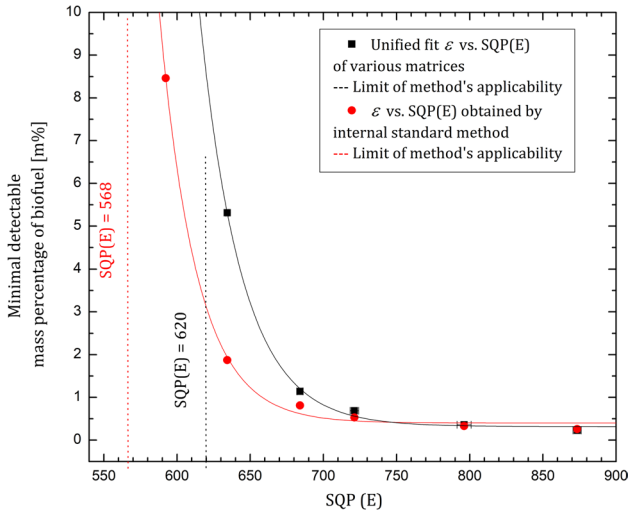

Fig. 4 Limits of the biomass percentage determination in biofuels in two-step method for 1000 min of measurement

[26], taking into account the established average background value but operating with the efficiency dependent on the analyzed samples' quench level. Minimal detectable biomass dependence on the quench level of a sample during 1000 min of counting is presented in Fig. 4 for both variations of the two-step method: unified fit of data obtained with several biogenic/fossil fuel blends, and the Internal Standard method with intercomparison samples. From Fig. 4, one can also conclude that the two-step method for 1000 min of counting enables minimal detectable biomass percentage ~ 1% for samples with quench level SQP(E) ≥ 680, while two-step method coupled with Internal Standard technique provided that minimal detectable biomass percentage ~ 1% is possible to be determined in samples with SQP(E) ≥ 650.

This research confirmed that the Internal Standard method for the detection efficiency determination offers better accuracy and additionally lowers the SQP(E) limit of the method's applicability (from SQP(E) = 620 to SQP(E) = 568). On the other hand, it is a destructive technique that increases the complexity of the method itself and demands more time for the analysis.

Testing of the modified two-step method

The modified two-step LSC method (a combination of calibration curves presented in Figs. 2b and 3) has been tested on several samples made with sunflower seed feedstock. The results are displayed in Table 2.

Despite the fact that calibration samples (i.e. intercomparison samples with unknown composition and a wide range of quench levels) and tested samples do not contain the same fossil fuel/bio fuel materials, results have been obtained with satisfactory accuracy. From 11 analyzed samples, results for 10 samples had acceptable value $|z| < 2$, while for one $|z| \approx 3$

Table 2 The performance of the modified two-step method

Fossil matrix	Reference biomass of a sample [%]	SQP (E)	Obtained biomass of a sample [%]
Commercial fossil fuel with winter additives	4.0	799.7 ± 1.4	5.6 ± 1.5 z = 1.11
	6.0	808 ± 4	7.6 ± 1.3 z = 1.24
	15.0	794.0 ± 2.3	19 ± 3 z = 1.53
	35.0	785 ± 10	42 ± 4 z = 1.73
	65.0	803.1 ± 2.3	68 ± 5 z = 0.66
Commercial fossil fuel with summer additives	4.0	728.6 ± 1.0	5.0 ± 1.1 z = 0.90
	6.0	724 ± 5	8.4 ± 1.6 z = 1.52
	8.5	718.8 ± 0.7	11.2 ± 0.9 z = 3.03
	15.0	723.6 ± 2.4	18.7 ± 2.9 z = 1.30
	45.0	745.7 ± 2.6	50 ± 3 z = 1.53
	85.0	819.8 ± 1.7	84.7 ± 7.0 z = − 0.04

was obtained. All results, the ones obtained during the internal intercomparison and during this testing, have proven that the two-step method generally provides reliable and valid results for the biomass quantification in samples with different fossil/bio matrices.

Conclusion

Liquid biofuels are currently the most viable means of achieving the European Union's transition to renewable and sustainable energy sources. Direct LSC methods enable reliable and effective radiocarbon analysis of fuel blends if the quench correction procedure is adequately carried out. The purpose of the presented research was to evaluate direct LSC method for biogenic fraction determination in biodiesel during intercomparison measurements of the samples with the unknown chemical composition. The one-step method cannot provide meaningful results in cases where the chemical composition of calibration samples and analyzed samples differs since the only parameter that influences biomass percentage is the count rate of samples, neglecting their quench level, which can significantly vary in biodiesel samples. The two-step method has more general application. It enables lower minimal detectable biomass in fuel samples and provides relatively reliable results with satisfying accuracy (the obtained z- values were $2 < |z| \lesssim 3$) for samples with the quench level of roughly 50 channels above the SQP(E) value that is considered the limit of the method's applicability. One way to adapt the method's performance is to use an Internal Standard method for the detection efficiency determination in each analyzed sample, but this approach is destructive, somewhat increases the complexity of the method itself, and demands more time for the analysis. Besides better accuracy (with $|z| < 2$ values), it was demonstrated that this modification of the method additionally lowers the SQP(E) limit of the method's applicability.

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Declarations

Conflict of interest The authors have no financial or proprietary interests in any material discussed in this article.

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