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Liquid Scintillation Spectrometry as a Tool of Biofuel Quantification

Romana Krištof and Jasmina Kožar Logar

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Abstract

Biofuels are common addition or substitute for fossil fuels, applied as an attempt to decrease the impact of transport on the environment. Because of a large variety of already known biofuels and intensive research in the field, there is a high demand for analytical techniques for their quantification in fuels. Liquid scintillation counting (LSC) is one of the ideal candidates for this kind of measurements because the measured substance is radiocarbon found in all biofuels. This chapter describes the fundamental feature of LSC measurements and possible sample preparation steps. One of the methods (direct LSC method) is highlighted. One of the method's advantages is simple sample preparation, thus suitability for every LSC laboratory. Calibration and validation results of three types of biocomponents, i.e., bioethanol, synthetic biodiesel [hydrogenated vegetable oil (HVO)], and conventional biodiesel [fatty acid methyl esters (FAME)], are presented. All results show that the described method is suitable for routine analysis of various biocomponents.

Keywords: LSC, liquid scintillation spectrometry, ^{14}C, diesel, bioethanol, synthetic diesel, fossil fuels

1. Introduction

Recently observed environmental changes and increased exploitation of fuels lead to a concern and emphasis of substituting fossil fuels by biofuels. With numerous types of renewables with very different characteristics, from bioethanol, ETBE (ethyl tetra butyl ether), biodiesel or FAME (fatty acid methyl esters), synthetic diesel or HVO (hydrogenated vegetable oil), Fischer-Tropsch products, etc., the need for several methods for biofuel quantification has aroused [1]. Several analytical techniques are in use, some of them have a status of standar-

dized methods. For instance, in EN 14078 [2], near-infrared spectrometry with trans-reflectance fiber optics for determination of methyl esters is mentioned while oxygenates, i.e., ethanol and ETBE are determined using an oxygenate flame ionization detector (EN 1601) or gas chromatography using column switching (EN 13132) [3, 4].

For differentiation between ethanol and bioethanol, common analytical techniques cannot be used since both components are chemically identical. Measurement of ^{14}C activity of such fuel mixture presents a solution because radiocarbon is only present in recently grown components (biocomponent) while in fossil component all ^{14}C has already decayed with radionuclide's half-life (5760 years). Radiocarbon or ^{14}C is a cosmogenic radionuclide produced in the atmosphere by neutron capture from ^{14}N. Since production and decay of ^{14}C in the atmosphere is in equilibrium, living organisms who uptake ^{14}C via photosynthesis, ingestion or inhalation have closely related activity as its environment [5]. The principle of radiocarbon dating is therefore usable also for quantification of ^{14}C in fuels. Genuine standard ASTM D6866 describes the accelerator mass spectrometry (AMS) and liquid scintillation counting (LSC) methods. Sample treatment is needed for these two methods. Additionally, a so-called direct LSC method that does not require special sample preparation before measurement is included in standard DIN 51637 [6]. It is only mentioned for limited types of biocomponents.

The following section explains the detection principle of the LSC system. Possible sample preparation techniques and their differences will be explained. Finally, the chapter shows some biofuel measurements using the direct LSC method. Results of ethanol, synthetic biofuel, and FAME measurements as well as validation parameters are presented and discussed.

## 2. Liquid scintillation counting (LSC)

Liquid scintillation spectrometry is one of the techniques for radioactivity measurements; especially suitable for the detection of β-emitting isotopes such as ^{14}C. As previously stated, isotope ^{14}C is naturally produced in the atmosphere by neutron capture. When radiocarbon decays it releases electron and antinevtrino (see Eq. (1)), produced energy is distributed among created particles what makes distinguishing continuous spectra of the β particle.

$$^{14}\text{C} \xrightarrow{\text{decay}} {}^{14}\text{N} + \beta^- + \overline{v} \tag{1}$$

where: $\overline{v}$ is Antinevtrino.

In order to detect β decay by means of LSC, a scintillation cocktail consisted of three types of chemicals: solvent, emulsifier, and scintillator (fluorescent material) has to be mixed with sample. Decay energy is absorbed in the emulsifier and via solvent transferred to the scintillator. The scintillator then emits energy in the form of light and so produced scintillations are detected by the photomultiplier tubes (PMT) in which conversion to an electrical pulse occurs (see **Figure 1**). If measurement is conducted without interferences, the measured count rate is

directly proportional to the activity of the sample. In the case of biofuels, counting efficiency should be taken into account for proper transformation of counts to activity because of substantial effects of quenching.
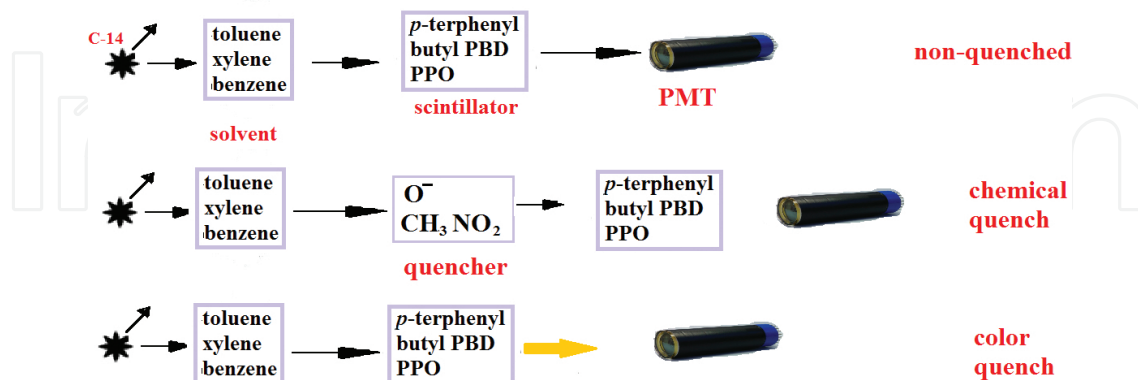
Figure 1. Quenching processes [7].

The quenching occurs when energy transfer between the radioactive isotope and the scintillation cocktail is disturbed. Reduced photon production results in a reduced number of counts as well as shifted spectrum toward lower energies. There are several types of quenching but two the most important ones have also an effect on biofuel measurements, i.e., chemical and color quench. As shown in **Figure 1**, chemical quench disturbs energy transmission between solvent and the scintillator while color quench affects transmission of energy between scintillator and PMT by attenuation of produced light in the sample.

A level of quenching can be evaluated through quench indication parameters describing the path by which they were obtained, i.e., the spectral quench parameter of the external standard (SQP(E)), spectral index of the sample (SIS), and spectral quench parameter of the internal standard (SQP(I)). A calibration curve describes the relation between the quenching parameter and counting efficiency. The most common approach to obtain the calibration curve goes through the preparation of a spiked set of samples in which various quantities of quenchers such as nitromethane is added. Efficiency is calculated by easy division of a count rate with known activity of the sample. There is also a possibility of quench set made from samples which by their nature have a variety of quenching; biodiesel blends have various quench levels (depending on feedstock and quantity of the biofuel) what can be useful in quench curve sample preparation.

2.1. Sample preparation

There are several sample preparation methods in combination with the LSC technique. In the so-called LSC-A method, also known as the CO_2 or carbamate method, organic carbon in the sample has to be converted to CO_2. This process is conducted in a special apparatus where sample is combusted to the form of CO_2 under a controlled environment. The gas is trapped or absorbed on an absorbent or on one of the components of specially designed scintillation

cocktails. In the LSC community, various mixtures are known [8, 9]. In the LSC-B method, the combusted sample in the form of CO_2 is further carbonized to benzene [10]. Benzene synthesis consists of three steps: forming of carbide (usually lithium carbide), hydrolyzation to acetylene and trimerization into benzene [8]. The LSC-A and LSC-B methods are used for environmental sample measurements for the purposes of age determination, monitoring of nuclear site, etc. The third option for the sample preparation is the direct LSC-C method that does not need any sample pretreatment. The liquid scintillation (LS) sample is prepared by simple mixing of the sample with a suitable scintillation cocktail. Since there is a lack of sample pre-treatment, various matrixes and thus rather more complicated calibration with careful quench correction are needed. In the case of biofuels, extensive quench is induced by samples' yellow color. Namely, yellowish samples are actually very unfavorable for LSC due to complementarity to blue, which is the typical scintillation color. Some authors have reported attempts to degrade and limit the color of the LS sample [11, 12]. Biodegradation and low oxidative stability of biocomponent presents another difficulty in calibration process. The most important features of all three mentioned LSC methods are summarized in **Table 1**.

	Advantages	Disadvantages
LSC-A (CO_2 method)	- Uniform matrix of LS sample - Colorless LS sample - Suitable for liquid, gaseous, and solid samples	- Complicated sample preparation with several possibilities for error - Repeatability of CO_2 absorption - Higher limits of detection
LSC-B (Benzene method)	- Uniform matrix of LS sample - Colorless LS sample - Suitable for liquid, gaseous, and solid samples - Low detection limits	- Long and expensive sample preparation with several possibilities for errors - Demands highly trained personnel - Preparation time
LSC-C (direct method)	- Fast sample preparation - Repeatability, accuracy - General laboratory practice trained personnel	- Complicated calibration - Various matrices

**Table 1.** Comparisons of different LSC methods for quantification of biocomponents in fuels.

3. Biofuel measurements with direct LSC

3.1. Bioethanol measurements

Bioethanol is used as an additive to gasoline or substitution of ethanol. Several authors have already reported measurements of bioethanol using various LSC methods [8, 13–17]. Since ethanol/bioethanol matrices are colorless, the calibration can easily be made also directly from

a count rate. This is a so-called one-step calibration curve, conducted as a correlation between the count rate and the biofuel content. In blends of ethanol/bioethanol/gasoline, a two-step calibration is advised. The two-step calibration consists of efficiency correction due to matrix variation and expected chemical quench.

Calibration samples can easily be made by blending certified fossil ethanol (in our case Fisher Scientific) or gasoline (provided by local petroleum industry) with certified bioethanol (like Carbo Elba ethanol). In each matrix, at least 10 LS samples were made and analysis was conducted. The obtained spectra of the sample can be seen in **Figure 2**. We found that it is useful if blends were made in accordance with market demands; that is several blends in the ranges from 0–to 10% and 80 to 100% and the rest of LS samples in blends in between. Measurements show that the difference in counting efficiency among ethanol/bioethanol Blends does not exceed 1% what is within uncertainty of a typical quench curve. However, in ethanol/bioethanol/gasoline blends, the difference among counting efficiencies can be up to 10% (between 82 and 72%); therefore, quench affects the counting efficiency and it has to be taken into account.
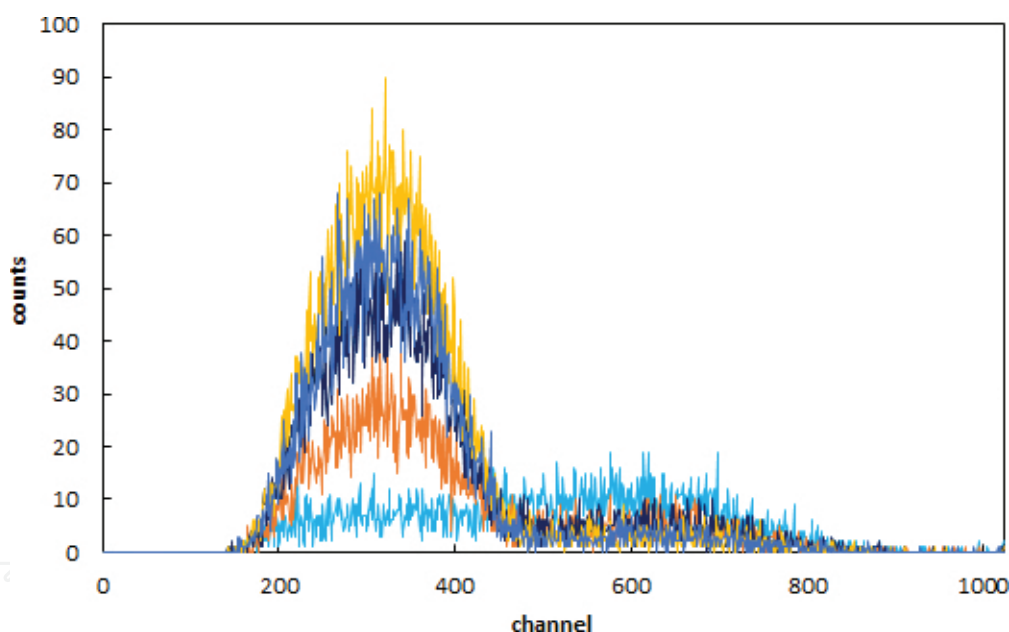
**Figure 2.** The set of bioethanol/ethanol blend spectra.

3.2. Synthetic biodiesel (HVO) measurements

Synthetic biodiesel or hydrotreated vegetable oil has recently been introduced into fuel market as a substitution of classic biodiesel (FAME) due to its oxidative stability and similarity to fossil diesel. The fuel itself can be mixed with diesel in various quantities or can be even substitution of fossil fuel. The latter is a problem with FAME since changes in automotive engine has to be made, while HVO can be used without any consequences to engine. Several authors have reported measurements of HVO blends in the range currently reasonable for fuel market,

which is up to 20% [14, 18, 19]. In the same range, also a standardized method with direct LSC and FT-IR methods is available [6].

The HVO is colorless so one can expect only chemical quench when blends with diesel are made. Furthermore, our measurements have shown that HVO can work as a slight reducer of quench (see **Figure 3**) and counting efficiencies were higher (from 74 to 83%). As in the case of bioethanol blends, blends varying from fossil diesel up to 100% HVO were made and analyzed. Since there is significant difference among counting efficiencies of various blends, a two-step calibration is advised. Counting efficiency can be evaluated with the same quench curve as bioethanol blends in the case of the same range. HVO can be produced from various feedstocks, but according to our results and experiences, the activity of various blends is similar regardless of the initial feedstock or preparation procedure of HVO.
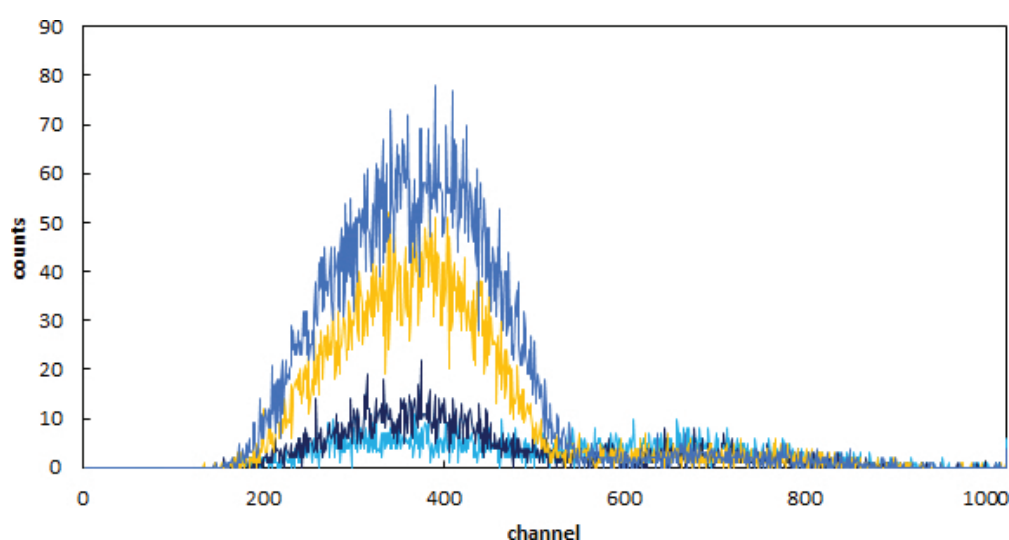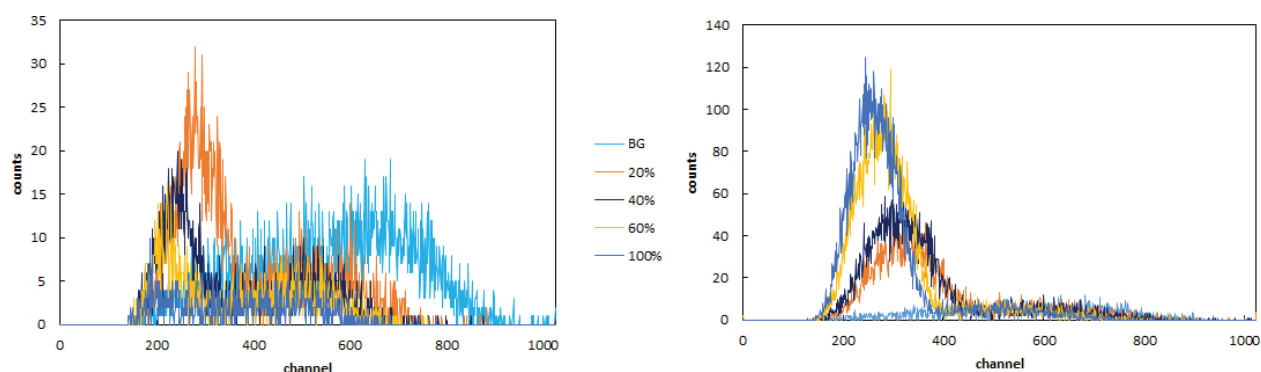

Figure 3. The set of HVO blend spectra.

3.3. Fatty acid methyl ester (FAME) measurements

As one of the most important parts of diesel's biofuels, FAME is referred to as biodiesel. Characteristics such as chemical form, color and oxidative stability depends on feedstock oils, what can affect analysis by the direct LSC method. As was explained in the sample preparation (Section 2.1.), the color of biodiesel has an effect on counting and thus besides chemical also color quench can be observed. Biodiesel aging or oxidative instability is one of the drawbacks that affect the use of biocomponent in fuel market, but as research had shown, it has positive effects on the LSC measurement [12]. That occurs due to decomposition of fatty acid esters while radiocarbon is still present in the sample (see **Figure 4**). However, forced oxidation was not shown as a promising step in sample treatment because of big differences among biodiesels from various feedstocks.

Several authors have reported measurements of biodiesel in various blends but mainly in level up to 20% of biodiesel what is limit of current fuel market [19–21]. However, our research

shows that reliable analyzes of biodiesel in quantities up to 100% of biocomponent can be conducted as well. Some changes of preprepared setups of LS counter Quantulus™ (Perki-nElmer) and counting protocol gave promising results, together with careful and precise calibration [14]. Hence, changes in coincidence circuit enabled analysis of various feedstock biodiesels in the whole range (from 0 to 100% biodiesel). **Figure 5** presents spectra that were obtained measuring various feedstock biodiesel ranging in quench levels from 360 up to 850, where the FAME from waste oil presents the sample with the lowest quenching number and thus the highest observed quenching while sample with the lowest observed quenching was made from sunflower oil.

Figure 4. Biodiesel blend spectra. The spectra set of the first measurement (left) belongs to the fresh biocomponent, while the set of second measurement (right) represents the spectra of already oxidized biocomponent.
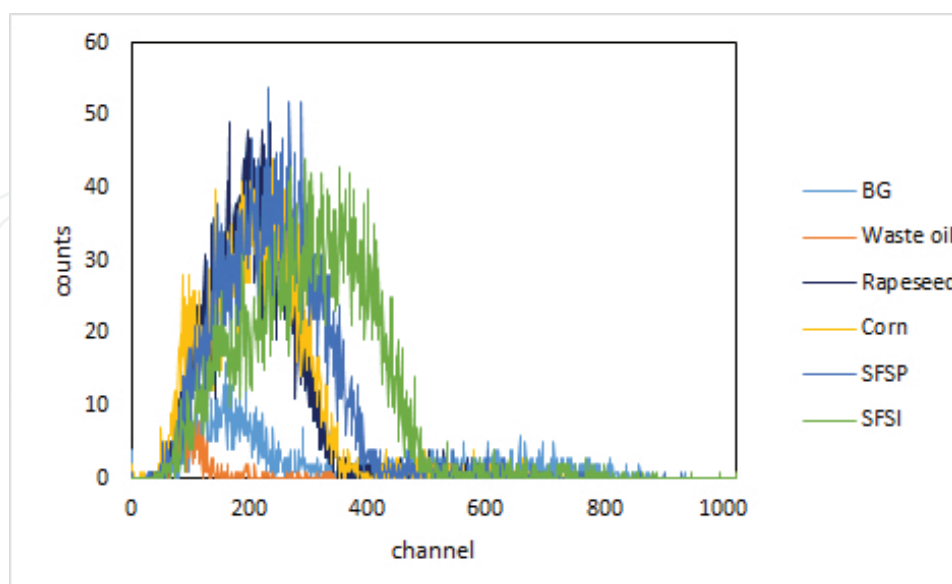
Figure 5. Biodiesel spectra after protocol changes. Legend: BG: background; SFE: sunflower (Spain), SFSI: sunflower (Slovenia).

3.4. Validation parameters

All analytical methods need to go through validation in order to use them in routine analysis. Uncertainty, detection limit, linearity, repeatability, and sensitivity were evaluated. Law of uncertainty propagation was followed while contributions of variable parameters were evaluated using GUM guidelines [22].

It was found that the uncertainty results are directly affected by the uncertainty of the balance, sample and background count rate, counting efficiency, and uncertainty of calibration. The indirect contributions are represented with uncertainty of pipette, temperature variation, and luminescence of the LS samples. It was found that uncertainties of sample and background count rate represent the largest contribution in the measurements near detection limits. In analysis of blends with biocomponents quantity higher than 10%, the largest contribution of uncertainty causes counting efficiency determination. In both cases, the uncertainty of the balance presents negligible part of the uncertainty budget.

In recent years, a new approach in determination of limits of detection is taken; the standard ISO 11929 applies a null measurements uncertainty [23]. Besides background count rate, the background and sample counting time present variables of limit of detection calculation. Although a long-term average of background is taken in our routine analysis, the detection limits were evaluated conservatively, and thus 1000 min of background and 500 min of sample counting time were taken. Obtained limits of detection (see **Table 2**) are comparable to those obtained by other laboratories and methods [2–4, 6, 8, 13, 15–21].

Parameter\component	bioEtOH	HVO	FAME	
Range	DL—100%	DL—100%	DL—10%	DL—100%
Limit of detection	0.63%	1.13%	0.66%	1.67%
Linearity	0.997	0.998	0.999	0.998
Repeatability	0.54%	0.40%	0.5%	0.44%
Sensitivity	17.5	9.156	12.546	10.882

Table 2. Validation parameters summary.

Linearity of calibration curves is demonstrated using the least-square method and the correlation coefficient R^2 while uncertainties of individual calibration curve parameters were used for calculation of calibration uncertainty. As can be seen in **Table 2**, excellent linearity of calibration curves was obtained in all biocomponents measurements. It has to be noted that the individual calibration curve is a result of at least 20 blends analysis.

Repeatability was tested by several measurements (at least 10) of the same sample; standard deviation of analysis results was calculated. A test was conducted on various blends in the full

range of the calibration. As can be seen in **Table 2**, results of the analysis with the direct LSC method are repeatable within 1 standard deviation of results; furthermore, it never exceeds 0.54% that was achieved with bioethanol calibration.

Steepness of the calibration curve slope was taken as a parameter for determination of sensitivity; factor *k* from linear regression line was compared. The most sensitive calibration was shown to be for bioethanol analysis (17.5) while the least sensitive was the HVO calibration curve with 9.156, respectively.

4. Conclusions

Biocomponents in world fuel market differ in their chemical characteristics. As a consequence, several analytical techniques have to be applied for their quantification in fuel blends. Their maintenance needs a lot of human and financial sources, especially in the form of equipment and knowledge. Liquid scintillation spectrometry is a good alternative. Namely, the measured quantity is always the same. Radiocarbon, ^{14}C is found in all biocomponents regardless the type of biofuel.

Three approaches can be applied as the sample preparation step before LSC analysis. Two of them, LSC-A and LSC-B consist of several sample preparation phases, from raw fuel to CO_2 production and benzene synthesis. The final result of sample preparation is the same matrix regardless the initial biocomponent. On the other hand, the LS sample in the LSC-C method can appear in many different forms. The matrix is not constant and predictable. Calibration and validation processes of the method are therefore extended and need expert knowledge. But, it should be done only once.

The maintenance of the already calibrated and validated LSC-C direct method is simple. It demands only periodical and simple check-ups of calibration parameters. The method does not need any special sample preparation steps. Routine analysis with this method is therefore very fast, cheap and does not need highly trained experts.

Author details

Romana Krištof[1,2*] and Jasmina Kožar Logar[1,2]

*Address all correspondence to: romana.kristof@ijs.si

1 Jožef Stefan Institute, Department for Low and Medium Energy Physics, Ljubljana, Slovenia

2 University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia

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