UNIVERSITAT
ROVIRA i VIRGILI

URAIS
Unitat de Radioquímica Ambiental i Sanitària

DEVELOPMENT OF A METHOD TO DETERMINE C-14 BY LIQUID SCINTILLATION COUNTING IN WATER SAMPLES

Abril Trullàs Estrem

Supervised by Carme Aguilar Anguera

Bachelor's degree in Chemistry

Tarragona, June 2023

INDEX

1. ACKNOWLEDGEMENTS 5
2. SUMMARY 5
3. INTRODUCTION 6
    - 3.1. C-14 9
    - 3.2. Liquid Scintillation Counting 10
        - 3.2.1. LSC components 11
            - 3.2.1.1. Scintillators 11
            - 3.2.1.2. LSC detector: Photomultiplier 12
            - 3.2.1.3. Scintillation vials 13
        - 3.2.2. Background contribution 13
        - 3.2.3. Counting efficiency 14
        - 3.2.4. Counting energy window 14
        - 3.2.5. Common interferences in LSC 15
    - 3.3. State of the art of C-14 determination in water samples 16
4. GOAL 18
5. EXPERIMENTAL PART 18
    - 5.1. Reagents and standards 18
    - 5.2. Equipment 19
    - 5.3. Analytical method 20
        - 5.3.1. Calibration process 20
        - 5.3.2. Sample measurement 21
6. RESULTS AND DISCUSSION 21
    - 6.1. TriCarb 2900TR 21
        - 6.1.1. Optimization parameters 21
            - 6.1.1.1. Counting time 21
            - 6.1.1.2. Counting energy window 22
            - 6.1.1.3. Vial selection 24
            - 6.1.1.4. Sample-cocktail ratio and cocktail selection 25
        - 6.1.2. Calibration 26
        - 6.1.3. Validation 28
            - 6.1.3.1. Interlaboratory comparison 30
        - 6.1.4. Tritium and C-14 31
    - 6.2. Quantulus 1220 32
        - 6.2.1. Optimization parameters 32

6.2.1.1. Counting energy window 32

6.2.2. Calibration 34

6.2.3. Validation 35

6.2.3.1. Interlaboratory comparison 36

6.2.4. Tritium and C-14 37

7. CONCLUSIONS AND FUTURE WORK 38

8. REFERENCES.. 39

9. ANNEX.. 43

9.1. Scintillation cocktails’ safety data sheets 43

9.2. Radiocarbon standard certificate... 49

1. ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to my supervisors in URAIS laboratory, Dr. Joana Martínez and Dr. Sandra Peñalver, who have supported me during the whole bachelor thesis journey. And also, I would like to thank my professor Dr. Carme Aguilar, who supervised and corrected this project.

2. SUMMARY

The evaluation of environmental radioactivity is a great concern nowadays since the presence of natural and artificial radionuclides in different environmental samples can contribute to a risk if these radionuclides reach human beings. That is why environmental radioactivity laboratories, as URAIS, have developed methods sensitive enough to determine those radionuclides in several environmental matrices.

The main motivation for the realization of this bachelor thesis is the lack of a C-14 method within URAIS working methods. URAIS is a laboratory mainly focused on the control of radiochemical parameters established in the current Spanish legislation in water devoted to human consumption. More specifically, C-14 is an artificial radionuclide that sometimes must be controlled and that does not have a method developed in URAIS laboratory. That is why, this bachelor thesis’ main goal is to develop a method to determine C-14 in water samples using liquid scintillation counting (LSC) technique.

The project starts with an introduction to URAIS laboratory, the current legislations regarding environmental radioactivity control, the main sources of C-14 in water, and the main components of LSC. Following with an insight of important parameters in LSC and the possible interferences encountered during analysis. Additionally, the analytical method is presented with the corresponding equipment and reagents used. Finally, the most extended part of this project discusses all the steps followed during the optimization and validation of the method.

Actualment, l'avaluació de la radioactivitat ambiental és de gran preocupació, ja que la presència de radionúclids naturals i artificials en diferents mostres medi ambientals pot contribuir a un risc si aquests radionúclids arriben a l’ésser humà. És per això que els laboratoris de radioactivitat ambiental, com URAIS, han desenvolupat mètodes prou sensibles per determinar aquests radionúclids en diferents matrius.

La principal motivació per a la realització d'aquesta treball de fi de grau és la manca d’un mètode per determinar C-14 a URAIS. URAIS és un laboratori centrat principalment en el control de paràmetres radioquímics establerts a l’actual legislació espanyola, en aigües de consum humà. Més concretament, el C-14 és un radionúclid artificial que de vegades cal controlar, però que no disposa d’un mètode desenvolupat al laboratori URAIS. És per això que l'objectiu principal d'aquest treball és el desenvolupament d’un mètode per determinar C-14 en mostres d'aigua mitjançant la tècnica de comptatge de centelleig líquid (LSC).

El treball s'inicia amb una introducció al laboratori URAIS, la legislació vigent en el control de la radioactivitat ambiental, les principals fonts de C-14 a l'aigua, i els principals components de LSC. Seguidament, es parla dels paràmetres importants en LSC i les possibles interferències trobades durant l'anàlisi. Tot seguit, es presenta el mètode analític amb els equips i reactius utilitzats. Per acabar, la part més extensa d'aquesta tesi analitza tots els passos seguits durant l'optimització i validació del mètode.

3. INTRODUCTION

The laboratory of *Unitat de Radioquímica Ambiental i Sanitària (URAIS)*, where I have developed my bachelor thesis, is mainly focused on the analysis of environmental radioactivity. It is coordinated by the research group of Chromatography. Environmental Applications of *Universitat Rovira i Virgili (URV)*, and it is located in the installations of *LQAIGUA* laboratory – in Consorci d'Aigües de Tarragona (CAT)'s facilities. URAIS is equipped with the adequate instrumentation to perform radiochemical analysis, for instance gamma spectrometers, and alpha and beta counters.

URAIS was originated in 2001 due a mutual interest between CAT and URV to create a laboratory in which it would be possible to perform the environmental radiological control of water devoted to human consumption. Since 2006, the laboratory has been accredited by ENAC, according to ISO/IEC 17025 - which states the general requirements for the competence of testing and calibration laboratories.[1]

From the very beginning of URAIS activity, different research studies focused on the evaluation of environmental radioactivity have been performed, and as a result four doctoral theses have been developed until now in that field in this laboratory. The first three theses were aimed to different monitoring studies and to the development of methods able to determine different natural and artificial radionuclides in the Ebro River basin[2–4]. On the other hand, the latest thesis was focused on the determination of artificial radionuclides from medical sources in water samples and other matrices.[5]

In addition, URAIS' key pillar consists in providing services to different companies or entities[6]. They are mainly focused on the radiological quality control of drinking water samples, which is ensured by the determination of several radiochemical parameters, stated in Real Decreto 3/2023. In this legislation, technical and sanitary criteria for drinking water's quality control are stablished.[7]

According to current regulations, the radiochemical parameters to be controlled in waters devoted to human consumption are gross alpha, gross beta without K-40 contribution indexes, radon (Rn-222), and tritium (H-3) activities. Table 1 shows in a schematic way the methodology followed depending on the activity obtained of the previously mentioned parameters, and Table 2 shows when each radiochemical parameter must be controlled in drinking water samples.

Table 1. Drinking water parameters to control environmental radioactivity and methodologies stated by Real Decreto 3/2023.

Parametric values (Bq/L)				**Is a further analysis necessary?**
Gross alpha	**Gross beta without K-40**	**H-3**	**Indicative dose (mSv/year)**	
≤ 0.1	≤ 1.0	< 100	≤ 0.1	No
> 0.1	> 1.0	≤ 100	None	Yes, individual radionuclides (first natural, and then artificial if needed) *
≤ 0.1	≤ 1.0	> 100	None	Yes, individual artificial radionuclides *
> 0.1	> 1.0	> 100	None	Yes, individual natural and artificial radionuclides *

* Natural radionuclides are shown in Table 3 and artificial radionuclides are displayed in Table 4.

Table 2. Radiochemical parameters and when they are determined stated by Real Decreto 3/2023.

Radiochemical parameters	**When the legislated parameters must be determined?**
Gross alpha index Gross beta without K-40 contribution index	Always.
Rn-222	When the water source is subterranean.
H-3	When the water source is superficial and exists a nuclear plant in the upstream of the catchment area.
Indicative dose (ID)*	When the other radiochemical parameters surpass their parametric value (see Table 1)

*Effective dose for one year of intake due to all radionuclides detected in drinking water, whether of natural or artificial origin, except H-3, K-40, Rn-222, and short-lived radon decay products.[7]

Overall, when any parametric value does not exceed the parametric value reported in Real Decreto 3/2023, the ID is considered to be below 0.1 mSv/year and it is not necessary to perform further analysis. Nonetheless, when all or one parametric value is surpassed (see Table 1), ID must be calculated from the activity concentrations of individual radionuclides of natural or artificial origin (see Tables 3 and 4) and the dose coefficients listed in Real Decreto 783/2001- which approves the regulation on health protection against ionizing radiation.[8] If the indicative dose, calculated with the individual radionuclides’ activity, is below or equal to 0.1 mSv/year, additional radiological investigations are not required. However, if ID surpasses 0.1 mSv/year, it must be informed to health authorities which will assess whether that value implies a health risk, and also they will evaluate the need of corrective measures to reduce the ID.[7]

Table 3. Natural radionuclides to be determined by legislation in certain situations.

Natural isotopes	
U-238	Ra-228
U-234	Pb-210
Ra-226	Po-210

Table 4. Artificial radionuclides to be determined by legislation in certain situations.

Artificial isotopes	
C-14	Co-60
Sr-90	Cs-134
Pu-239	Cs-137
Pu-240	I-131
Am-241	

The aforementioned legislations focus on drinking water due to population's extensive use of it. It is important to study the presence of radionuclides in this matrix, since drinking water is generally obtained by treating superficial water, which can present natural radionuclides such as Ra-228 – found in rocks and soils and which dissolve in water[9], and artificial radionuclides owing to human activity such as Cs-137 – a fission product from nuclear plants[10]. Although superficial water is treated in drinking water treatment plants, according to several studies[11,12] and particularly to previous URAIS’ studies[2–5,10] it is demonstrated that there is not a total elimination after the treatment processes in these plants of the radionuclides present in the incoming waters. Thus, some radionuclides might reach drinking water and consequently, the population.

The primary purpose of monitoring radioactivity in water samples is to be able to guarantee its radiological quality, so that its consumption does not generate a health risk. This is the reason why, as already mentioned, norms are stablished, and some parameters are measured. Hence, laboratories focused on the analysis of environmental radioactivity have encountered the necessity to develop quick and reproducible methods to determine individual radioisotopes in waters devoted to human consumption, and thus being able to calculate the ID. Moreover, as water sample’s radiological content is usually at environmental levels, low background and sensitive equipment is needed. [3]

As it has already been mentioned, URAIS is located within the drinking water treatment plant laboratory in CAT’s installations – that treats Ebre river’s water –, whose incoming water may be influenced by the nuclear power plant discharges in Ascó (see Figure 1). That is why, URAIS already has working methods to determine legislated radionuclides. However, URAIS laboratory has found the necessity to expand its technical capacity due to the lack of a C-14 determination method.

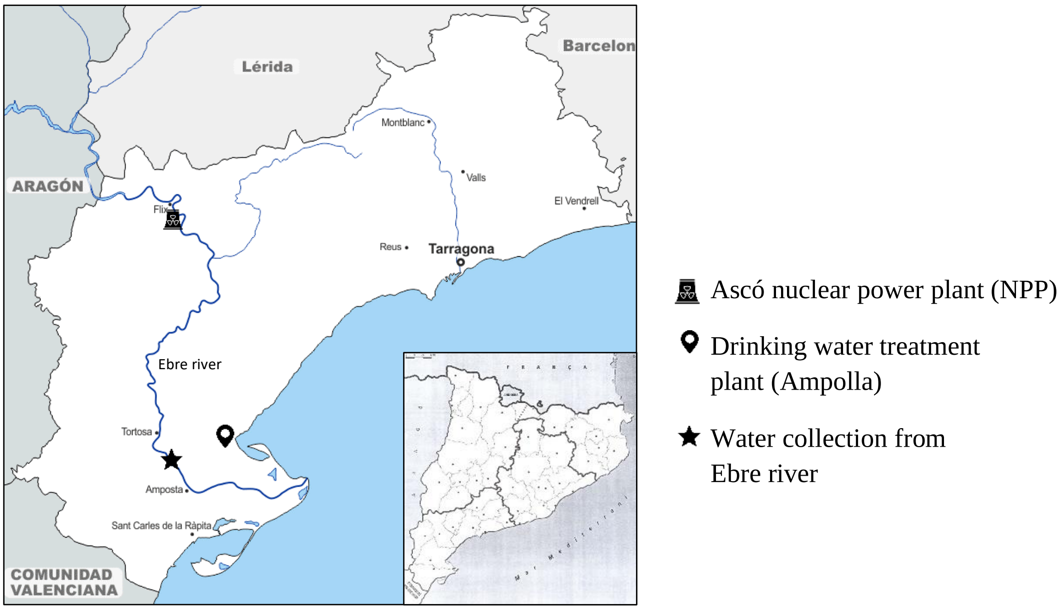


Figure 1. URAIS and Ascó nuclear plant location in Catalonia.

In the following section, more detailed information about C-14 characteristics, origins in environment, and activity values in legislation focused on drinking water is given.

3.1. C-14

The “C” element with atomic number (Z) six, has thirteen known isotopes, of which only C-12 and C-13 are stable, and the rest are all radioactive. The most known radioactive isotope of carbon is C-14 which is very used in geochemical, geological, and archaeological fields. It is an isotope found in nature but has an abundance negligible compared to the other carbon isotopes, thus the high abundance is due to its artificial nature when nuclear power plants or fuel reprocessing plants discharge nuclear fuel, mostly in gas form. C-14 has a half-life of 5730 years, and decays by beta emission to yield the stable N-14 isotope, with a maximum energy of 156 keV.[13,14]

C-14 is a radioisotope which is formed in all nuclear plants due to the absorption of neutrons by carbon, nitrogen, or oxygen present in fuel composition or as fuel impurities in form of carbide, nitride, or oxide. Then, most of the radiocarbon formed is converted to carbon dioxide in its gaseous form at the fuel reprocessing plant – where the used fuel gets separated into fissionable material and waste[15,16]. This gas is released to the environment and, as a consequence, it gets in the troposphere and becomes part of the carbon environmental cycle.[17] Additionally, carbon dioxide or carbonates formed from radiocarbon are deposited in sediments or deep oceanic waters. Due to that deposition, the nuclear discharges, and also the natural production of C-14 by nitrogen nuclei’s reaction with neutrons – generated by nuclear reactions when high-energy particles from space enters Earth’s atmosphere (cosmic rays)[18] – radiocarbon activity concentration is introduced in the atmosphere, water and biota.[16]

Therefore, the introduction of C-14 in human metabolism through air, water or biota, and its long half-life time makes C-14 a hazardous radioisotope which must be controlled.

As it has already been mentioned, in water samples devoted to human consumption, specific radionuclide determination is only required when gross alpha, gross beta without K-40, and/or tritium activity values are surpassed (see Table 1). In the case of radiocarbon and other artificial radioisotopes (see Table 4), they are mainly determined either when tritium activity surpasses 100 Bq/L or, when all the aforementioned parameters overcome their limit value. Furthermore, the maximum legislated C-14 activity concentration in drinking water is 240 Bq/L and its corresponding minimum detectable activity (MDA) must be 20 Bq/L.[7]

3.2. Liquid Scintillation Counting

Liquid scintillation counting (LSC) is used for the quantitative analysis of radionuclides in liquid samples. These include alpha and beta particle-emitting radionuclides.

LSC is based on the properties of some organic molecules which enable to convert the energy absorbed from nuclear radiation into photons of light. These photons are then converted to a beam of electrons by the photomultiplier tube (PMT). A general diagram of the process is shown in Figure 2. In this figure, it can be observed the excitation of the solvent molecule by absorbing the radionuclide's emission energy and then, this energy is transferred to the scintillator molecule which emits light, later captured by the photomultiplier tube.

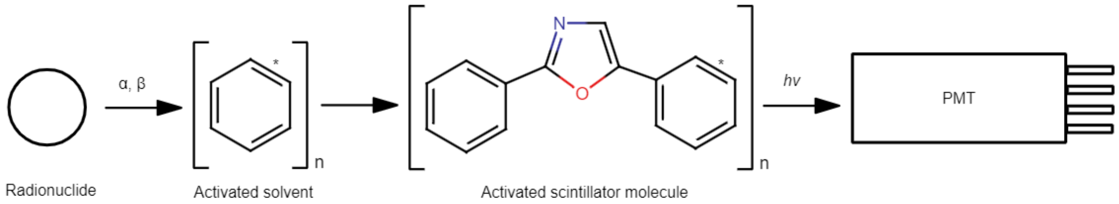


Figure 2. Schematic process of liquid scintillation counting.

This technique involves placing the sample into a container, well-known as scintillation vial which may be made of polyethylene or glass, plus an amount of scintillation cocktail – the latter being the responsible to catch and convert radiation energy to light. Within LSC, the vial is surrounded by two photomultipliers and only coincident electric pulses from both PMTs are accepted as counts, since pulses from only one of them are considered as background or noise contributions. As a result, the technique's sensitivity is enhanced[13]. The use of that scintillation cocktail makes the closest contact with the sample hence, beta particles in the sample are converted to photons, thus improving the detection efficiency, compared to other kind of instruments. That is why, LSC is an adequate technique to determine and quantify individual beta-decayed radionuclides.[19]

Concerning LSC’s equipment characteristics, specific radioisotope’s spectrum – a graphical representation of counting rate versus the emission energy of that radionuclide in keV or channels -, and count rate are obtained. Furthermore, it enables the automation in computer data processing which makes easier the collection of results. Plus, in newer equipment such as Quantulus 1220, the determination of simultaneous alpha and beta emitters is available which allows the differentiation of pulses caused by alpha particles from the beta particles.[3,20]

3.2.1. LSC components

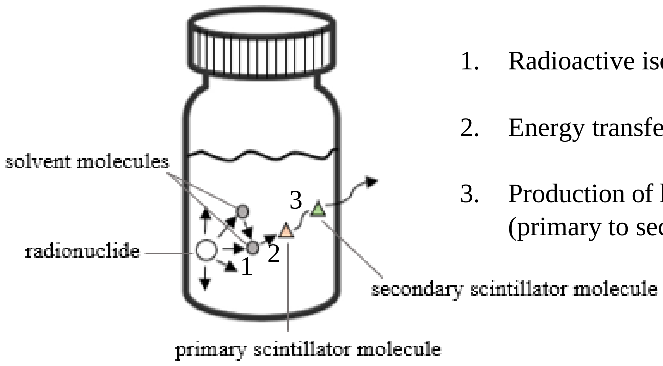
There exist different scintillation techniques – solid and liquid – which differ in the scintillator that absorbs emission energy from radioactive molecules[21]. However, as the determination of C-14 is performed using LSC, the following subsections are only focused on liquid scintillation analysis and on a deeper insight of its different components.

3.2.1.1. Scintillators

Scintillation counting operates with chemical molecules well-known as scintillators. Liquid scintillators – also called scintillation cocktails –, are mainly organic molecules that contain at least two components, the scintillant and the solvent, with the first being dissolved into the latter, independently on which radionuclide is determined. The role of the solvent is to absorb most of the energy emitted by radioisotopes, in other words, it acts as an energy collector. Then, this energy is transferred to the scintillant, who is responsible for emitting light.

The most efficient solvents are aromatic organic such as toluene, pseudocumene, or linear alkyl benzene – the first ones being the traditional solvents which are found in scintillation cocktails such as InstaGel Plus, and the last solvent giving safer cocktails like Ultima Gold[22]. The π cloud of aromatic rings provides a target for β-interactions which enables to catch energy of the emitting particle. This captured energy is usually lost through energy transfer to other solvent molecules which allows the efficient energy capture by the scintillant.[23]

Most of scintillation cocktails are composed by a primary and secondary scintillator. Primary scintillators transfer energy between the scintillant and the excited solvent molecules. Nevertheless, secondary scintillators, also known as wavelength shifters, which moves the wavelength of the scintillation light to longer values to match the optimum wavelength of conventional photomultipliers[24]. Figure 3 shows a general scheme of the role of each component exposed.



1. Radioactive isotope excites the solvent.
2. Energy transfer from solvent to primary scintillator.
3. Production of light followed by change of wavelength (primary to secondary scintillator).

Figure 3. Energy transfer process between the solvent and the scintillators.

Besides, as a major part of radioisotopes are present in aqueous media and the fact that water is not miscible in the organic solvents in scintillation cocktails, emulsifiers (also called surfactants or detergents) are used to bring both phases in a closer contact by forming microemulsions. Those microemulsions are nanosized “water-oil” droplets which facilitate the efficient energy transfer. [24]

In any radionuclide determination using LSC equipment, including C-14, the selected scintillation cocktail should have certain characteristics. The main requirements are that the sample-cocktail mixture becomes homogeneous, in order to efficiently transfer the energy from the sample to the cocktail, and that the solvent in the liquid do not diffuse through the vial’s walls. Plus, the scintillation cocktail should show low toxicity and high biodegradability. And in the specific case of C-14 determination, the cocktail should not contain radiocarbon within its composition so as to guarantee that the C-14 determined is only from the sample. [25]

3.2.1.2. LSC detector: Photomultiplier

Photomultipliers’ main task is to convert light photons emitted by the scintillant into an electronic current, which provides an electric signal to be analysed. A general photomultiplier tube scheme is observed in Figure 4.

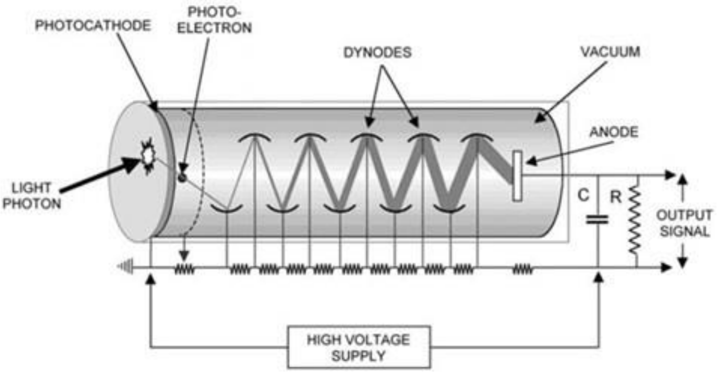


Figure 4. Scheme of a photomultiplier tube. [26]

They consist in two main components, the photocathode, and the dynodes. The photocathode is a photoelectric cell, whereas each of the dynodes is connected to the previous one with an increasing voltage. Within the photocathode, the scintillation light is absorbed, and a certain number of photoelectrons are released. Those photoelectrons are attracted by the first dynode, where they collide and release some electrons by a process known as secondary emission. These electrons are attracted by the next dynode from which more electrons are released after collision. Finally, the total current is collected by the anode, which provides electric signal.

It must be noted that the entire system is under vacuum in order not to affect the electrons’ trajectory.

3.2.1.3. Scintillation vials

There exist several vials that show distinctive characteristics depending on their material composition. Therefore, the selection of the vial, when optimizing a method using LSC, is mandatory. For radiocarbon determination, the most used vials are glass, polyethylene, and polyethylene with Teflon covering ones, usually with sample a capacity of 20 mL.[27–29]

Both polyethylene and glass vials show advantages and disadvantages regarding background, solvent diffusion, and transparency. Polyethylene vials are unbreakable, cheaper and display lower background signal than glass vials. The lower background signal is mainly due to their lower radioactivity content and lower density, which results in a lower probability of particle or photon interactions with the vial walls. Polyethylene vials are permeable when the traditional solvents such as benzene and toluene are used. However, these vials do not show solvent diffusion when environmentally safe cocktails, as Ultima Gold, are used. In some cases, Teflon-covered vials are selected, as the solvent diffusion is reduced. Despite that, the main drawback of Teflon-covered vials is their higher cost compared to traditional polyethylene vials.

On the other hand, glass vials provide the transparency to visualize the sample and scintillation cocktail solution, to enable the examination of undesired properties like colour, residues, or sample heterogeneity. Nonetheless, they contain ^{40}K which is the major contributor to the background measurement.[20]

3.2.2. Background contribution

It is defined as counts appearing from radiation external to the sample, for instance cosmic, environmental radiation or the vial material, or from instrument noise.

Background is an important measure since water samples usually analysed contain trace level activity concentrations, thus background must be measured and subtracted from the sample’s counts to obtain the actual sample’s activity, and not to obtain an overestimated result. It is determined by counting a vial which contains the scintillation cocktail plus water without any radionuclide – or the product of the separation process without any radionuclide. Once the blank vial is prepared, it must be counted for an adequate period of time in order to get accurate results[13], such as two or three cycles of 1000 minutes – cycles being the number of times a measure is repeated within the same vial and sample.

3.2.3. Counting efficiency

Efficiency is the percentage of disintegrations emitted by a radioactive source that are registered by the detector in the optimum energy counting range[30]. It is an important parameter to calculate the activity of a sample as it corrects the photons and particles that are not measured by the detector, thus not underestimating the final results. It is defined as the quotient between the number of particles detected per unit of time, in other words counts per minutes (cpm) and, the number of "real" disintegrations in the sample per unit of time, which is to say, disintegrations per minute (dpm):

$$E\ (\%) = \frac{cpm_{net}}{dpm} \cdot 100 \qquad \text{Equation 1}$$

Where $cpm_{net} = cpm_{sample} - cpm_{background}$, and dpm are calculated from the known sample activity.

To determine the counting efficiency, it is necessary a background sample and a water sample with a known activity measurement. The standard's measurement must be performed under conditions that are identical to those used for an unknown activity sample.

3.2.4. Counting energy window

All beta-emitting isotopes – including C-14 – emit β-particles at different energy ranges which are observed in their individual spectrum. Therefore, the counting energy window it is defined as a lower level (LL) and an upper level (UL) energy within the spectrum of a specific radionuclide, and which must be optimized to obtain the best performance. Indeed, when using any LSC instrument, the equipment's configuration enables to choose different energy windows to count. The energy range is optimized by measuring a known radionuclide activity and a blank sample. Then, the performance with different energy selections is assessed using the figure of merit (FOM) parameter, whose equation (Equation 2) contains the detection efficiency (Equation 1) and background count rate (min^{-1}).

$$FOM = \frac{E^2}{B} \qquad \text{Equation 2}$$

Particularly, radiocarbon emission spectrum is between 0 and 156 keV – or between 1 and 450 when a logarithmic scale of the energy is performed, giving the channel units[25]. Thus, the best energy range must be evaluated between those values when optimising the experimental conditions for the determination of C-14.

3.2.5. Common interferences in LSC

The main counting interferences found in liquid scintillation analysis must be considered before sample measurement, as some of them may end up underestimating the activity of the sample. Those interferences include quenching and luminescence, which are described below.

Quenching consists in the inhibition of light production in the scintillation process. For that reason, when a sample shows quenching, there is an absorption of energy or photons by the quenching agent, losing intensity and hence, moving the spectrum towards lower energies, as it can be observed in Figure 5.

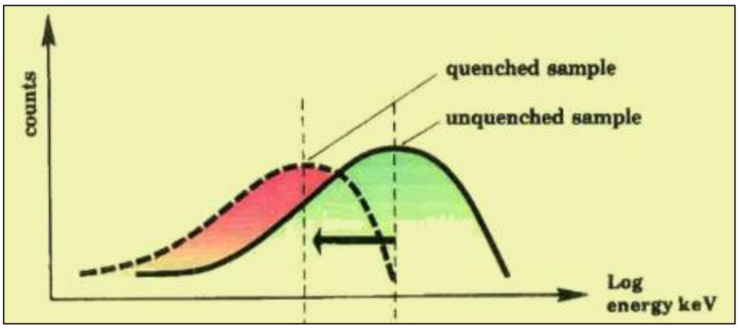


Figure 5. Effect of quenching on energy spectrum. [19]

There exist two types of quenching depending on the agents producing the effect. The first mechanism is chemical quenching which is caused by a chemical substance in the sample that absorbs radioactive emission energy and consequently, blocks the transfer of energy to the scintillation solvent. The second mechanism is colour quenching which occurs when the sample to be counted presents some type of colouring. It acts by absorbing photons of light before they can be detected by the photomultiplier tube.[13]

The most usual method to measure quench is the external standard method. It consists in the sample’ spectrum induction by an external gamma source within the LSC equipment – Ba-133 in TriCarb 2900TR and Eu-152 in Quantulus 1220. And then, with a mathematical spectral transformation, the quench-indicating parameter is obtained – transformed Spectral Index of the External Standard (tSIE) in TriCarb 2900TR and Spectral Quench Parameter External (SQP(E)) for Quantulus 1220. These quench parameters become lower as the quench increases and shifts the spectrum towards lower energies.

When unknown samples are measured, the count rate and quench parameter are obtained. Therefore, to calculate their efficiencies, and later the activity, quenching curves are used – a graphical representation of a quench-indicating parameter versus efficiency (%) of a standard of the radionuclide to be measured [31–33]. These quench curves are prepared within the calibration step, for each individual radionuclide and each LSC equipment used.

As regards to luminescence, it is a type of interference caused by emission of light as a result of energy absorption from a non-nuclear radiation source. There are two phenomena that might generate luminescence, photo- and chemiluminescence. [13]

Photoluminescence is the result of the exposure of the sample-cocktail mixture to ultraviolet or sun light [19]. Its contribution is completely eliminated by letting the sample mixture in the dark after 12 hours, in order to ensure photoluminescence has become negligible[34]. On the other hand, chemiluminescence is the light emission within the sample-cocktail mixture due to a chemical reaction. Its contribution is minimized with several strategies such as avoiding the reaction to happen or changing the sample preparation.[13]

3.3. State of the art of C-14 determination in water samples

The determination of radiocarbon in drinking water samples is mandatory since C-14 is a parameter to control when the evaluation of the ID should be performed, to guarantee population's health. Real Decreto 3/2023 states that the radiocarbon's maximum activity concentration in drinking water should be 240 Bq/L and that the method used must be able to determine C-14 with a MDA of 20 Bq/L.[7]

In the literature, there are several reported strategies focused on the determination of C-14 in water samples. Table 5 summarizes the most relevant features of different methodologies to determine radiocarbon in water samples. In general, all these reported methods involve the use of LSC as measuring technique[27–29,35–37], but there are also some authors who use accelerator mass spectrometry (AMS)[38,39], despite that this last technique is not as usual as LSC.

As it is observed in Table 5, AMS requires a sample pre-treatment in order to isolate isotopic carbon – in form of $^{14}CO_2$ –, similarly to benzene synthesis and direct CO_2 absorption, thus interference with other beta-emitters do not occur. However, that radionuclide separation involves complex and long sample preparation processes, and high costs for the laboratory, compared to the direct measurement using LSC. That is why, the main characteristic of the direct measurement is that sample preparation step is not required, consequently it provides advantages such as rapidity, analysis simplicity and low-cost, features that are very convenient in case of nuclear emergency, even though the main problem might appear with that lack of sample treatment, since interferences with other beta emitting particles may occur [27]. Nevertheless, the main difference between LSC-based methods and AMS, is the counting time which is much less in AMS case – 15 minutes[39] compared to 300-1200 minutes in other radiometric methods –, as it measures isotopic carbon ions instead of counting its emissions.

Concerning the methods using LSC technique – benzene synthesis, direct CO_2 absorption, and direct measurement –, aforementioned bibliography focuses on optimizing the parameters that affect the measurement – energy window, vial material, sample-cocktail ratio, and scintillation cocktail selection –, with the latter application in "real" water samples. All studies have in common that the optimum parameters are stablished with the highest FOM value – calculated with Equation 2. As regards to the direct measurement, it is seen that efficiency values are dependent on the optimum parameter's value fixed. For example, for an energy window of 130-450 channels, using a sample-cocktail ratio of 8:12 and Ultima Gold LLT, the detection efficiency is approximately 80%, whereas an energy window of 150-450 channels, using a sample-cocktail ratio of 9:11 and Ultima Gold AB, the detection efficiency is approximately 60%. In addition, as seen in Table 5, polyethylene vials are preferred in this method, compared to the glass ones in the other methods, which results in a cheaper analysis for the laboratory.

Lastly, most of the mentioned studies applied their methods to "real" water samples, such as ground water, surface water, sea water, or wastewater. Generally, all C-14 activity concentration values are lower than their detection limit, with the exception of wastewater from the nuclear facilities in which the radiocarbon activity are higher than other studies, when using the direct measurement. In the last case, it is concluded that C-14 activity is overestimated due to the presence of other beta-emitters that contribute to the radiocarbon activity[27].

Table 5. C-14 determination in water samples using different methods and techniques with their corresponding features and optimum variables.

Ref.	**Sample preparation**	**Measuring technique**	**Total measuring time (min)**	**Energy window (channels)**	**Detection efficiency (%)**	**Vial material**	**Sample-cocktail ratio**	**Cocktail**
27	None	LSC	300	350-450	~ 24	PE*	9:11	Ultima Gold AB
28	None	LSC	300	130-450	~ 82	PE*	8:12	Ultima Gold LLT
29	None	LSC	540	350-500	~ 13	PE*	10:10	Ultima Gold LLT
35	$^{14}CO_2$ absorption	LSC	1700	100-400	~ 65	Glass	Not specified	Not specified
36	$^{14}CO_2$ absorption	LSC	1000	100-450	~ 64	Glass	Not specified	Not specified
37	Benzene synthesis	LSC	900	219-525	~ 82	Glass	Not specified	Not specified
38	^{14}C to graphite	AMS	~ 15	None	None	None	None	None
39	^{14}C chemical extraction	AMS	~ 15	None	None	None	None	None

*PE stands for polyethylene.

4. GOAL

The main aim in this bachelor thesis is the development of a direct method to determine C-14 in drinking water samples using a liquid scintillation counting (LSC) equipment, since URAIS laboratory do not dispose of one and this is needed in some cases in which artificial radionuclides must be controlled.

In order to achieve this general aim, some particular objectives are established:

- Optimization of sample preparation and counting parameters.
- Method calibration with the optimum conditions.
- Method validation.
- Application to water samples

5. EXPERIMENTAL PART

In the following sections, the experimental part is described. It includes the reagents and equipment used, and the analytical method procedure to be able to determine C-14 in a drinking water sample.

5.1. Reagents and standards

The reactants used in this project are found in Table 6, in which the corresponding toxicity and handling is described.

Table 6. Reagents and standard used with their corresponding hazards and manipulation.

Reagent	Hazards	Handling
C-14 standard	Radioactive hazard	Goggles Gloves Protective clothing
HPLC-quality H_2O	-	-
Ultima Gold LLT* (Scintillation cocktail) (PerkinElmer, the Netherlands)	Health hazard Corrosion Irritant Environmental hazard	Goggles Gloves Protective clothing
Ultima Gold uLLT* (Scintillation cocktail) (PerkinElmer, the Netherlands)	Health hazard Corrosion Irritant Environmental hazard	Goggles Gloves Protective clothing
Optiphase Hisafe 3* (Scintillation cocktail) (PerkinElmer, the Netherlands)	Health hazard Corrosion Irritant Environmental hazard	Goggles Gloves Protective clothing

*The corresponding safety data sheets are found in the Annex (section 9.1)

During the whole experimentation, one stock solution of C-14 was used, whose characteristics and certificate are shown in Figure A.7. From this commercial standard, two working standard solutions were prepared. Both standard solutions contain C-14 at an activity concentration of $7.277 \cdot 10^2$ Bq/L, but one with HPLC-quality water and the other with 0.1M NaOH and 30 µg/g C Na_2CO_3 media.

It must be pointed out that the preparation date of the working standards is 05/04/2023 and 21/04/2023, respectively. That date is an important data in order to consider the decay of C-14 and to correct the measured activity due to the time difference from the standard preparation.

5.2. Equipment

In URAIS laboratory, there are two liquid scintillation instruments, Quantulus 1220 (Figure 6A) from PerkinElmer company (developed by Wallac in Turku, Finland) [33], and TriCarb 2900TR (Figure 6B) from Packard company (latter acquired by PerkinElmer in Illinois, USA).[32]

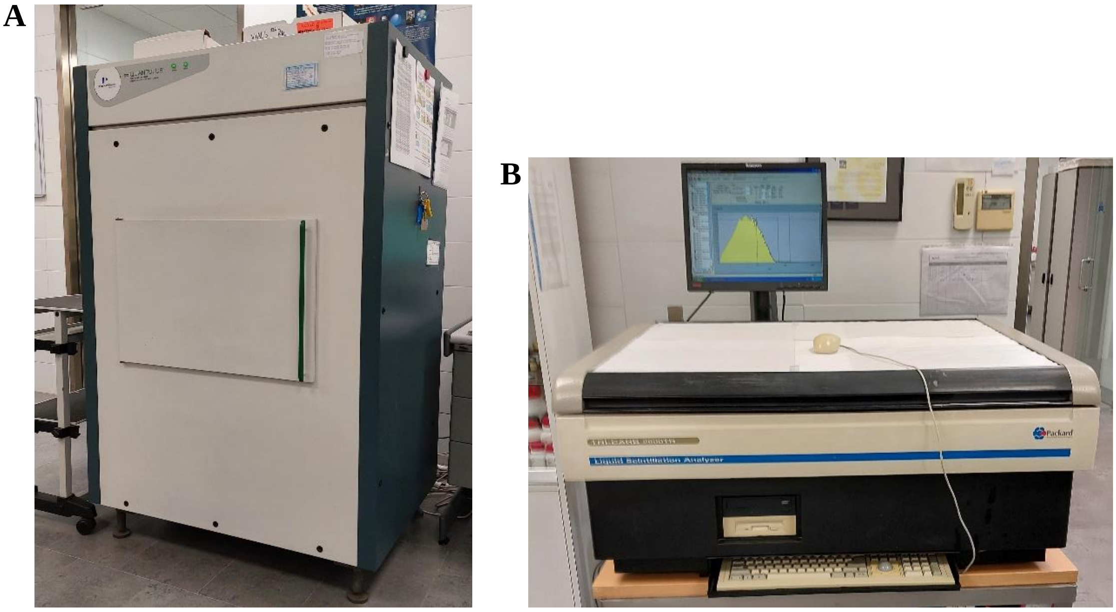


Figure 6. A) Quantulus 1220 low background liquid scintillator counter and B) TriCarb 2900TR liquid scintillator counter.

Both equipment display the spectra of the radionuclides measured, but they differ in the units of the emitted energy within the plot. In TriCarb 2900TR the energy units are kilo electron-volt (keV) and in Quantulus 1220 the energy is found in a logarithmic scale of the energy, the so-called channels. Another distinction between both equipment is the indicative quenching parameter, that is SQP(E) in Quantulus 1220, and tSIE in TriCarb 2900TR instrument.

5.3. Analytical method

In the following sections, the direct method’s procedure for the determination of C-14 in drinking water using a LSC instrument is described, with the corresponding optimum conditions. It includes the calibration process – with the measurement of standards and background – and the sample measurement.

5.3.1. Calibration process

The calibration process consists in performing a quenching curve constructed by plotting the quenching-indicating parameter versus the efficiency (in %).

For this process, a background and known concentration standard samples with different quench levels measurements are required.

To prepare the background sample vial, the subsequent steps are followed. Firstly, 8 mL of HPLC-quality water plus 12 mL of Ultima Gold LLT are added into a polyethylene scintillation vial, and it is mixed vigorously to homogenize the mixture. Similarly, to prepare standard samples the next steps are followed. Firstly, 4 mL of HPLC-quality water, 4 mL of a standard of $7.277 \cdot 10^2$ Bq/L (reference date at 21/04/2023) – prepared in 0.1M NaOH and 30 µg/g C Na_2CO_3 media -, plus 12 mL of Ultima Gold LLT are added within a polyethylene vial. Additionally, each vial contains an increasing amount of quenching agent, CCl_4 – 0, 25, 50, 75, 100 µL. Then, all vials are kept for 24 hours in the dark – better if it is inside the equipment since it will cool down to the equipment’s temperature –, in order to minimize or remove the luminescence contribution. Finally, the background samples are measured for 3 cycles of 1000 minutes whereas the each standard sample is measured for 3 cycles of 180 minutes.

As regards to LSC equipment configuration, each LSC equipment has its own characteristics and measurement configuration.

With respect to TriCarb 2900TR, vials are placed within trays that move around the equipment. In order to measure radiocarbon, a specific protocol must be created. To create the protocol, an energy window between 10 to 60 keV, tSIE as quench indicator, and the count time – 3 repetitions of 180 minutes for the standards or 3 repetitions of 1000 minutes for the background – are selected.

On the other hand, concerning Quantulus 1220, a counting configuration of high coincidence bias – a configuration for high energy isotopes in which the spectrum below channel 100 is cut off [40] – and C-14 is selected – a high energy beta counting setup. Moreover, a counting time of 3 cycles of 180 minutes for the standards and 3 cycles of 1000 minutes for the background, and an energy window between 130 and 450 channels are selected.

Lastly, the efficiency is calculated using Equation 1, and the quenching curve is represented with the quenching parameter – tSIE in TriCarb equipment and SQP(E) in Quantulus - versus the percentage efficiency. Then, the plot’s equation is obtained using the trendline that fits the best, and this equation is used to calculate the efficiency when measuring a sample with unknown radiocarbon activity.

5.3.2. Sample measurement

Firstly, 8 mL of water sample, plus 12 mL of scintillation cocktail is mixed vigorously into a polyethylene vial, in order to obtain a homogeneous mixture. Then, the vial is kept 24 hours in the dark within the equipment. Finally, the sample is measured for 1 cycle of 180 minutes.

6. RESULTS AND DISCUSSION

In the following sections, the results of the optimization of the different parameters affecting the measurement, the calibration and the method validation are discussed for both equipment, TriCarb 2900TR and Quantulus 1220.

6.1. TriCarb 2900TR

6.1.1. Optimization parameters

6.1.1.1. Counting time

Counting time is a parameter which have an important influence on the desired detection limit by the laboratory. In URAIS, one requirement is that their method should present a detection limit lower than the value stated by the current legislation. In the case of C-14, the legislation points out a value of 20 Bq/L, so one of our aims was to reach an MDA of 2 Bq/L.

To optimize the counting time, a radiocarbon standard sample was measured with the same experimental conditions as the reported in similar studies[25,28,41] – sample-cocktail ratio of 8:12, Optiphase Hisafe 3, and polyethylene vials –, and it was measured in all the C-14 energy range – from 0 to 156keV – for three cycles of 180 minutes each standard and three cycles of 1000 minutes each background sample. [25,29,42]

The MDA ($c_A^{\#}$) was calculated using Equation 3, expression found in UNE-EN ISO 13162:2021 in which a direct method to determine C-14 by LSC in water samples is introduced. [42]

$$c_A^{\#} = \frac{2 \cdot \mathrm{c}_\mathrm{A}^{*} + \frac{(\mathrm{w} \cdot \mathrm{k}^2)}{\mathrm{t_g}}}{1 - \mathrm{k}^2 \cdot \mathrm{u}_\mathrm{rel}^2(\mathrm{w})} \qquad \text{Equation 3}$$

The previous equation includes $k = k_{1-\alpha} = 1.65$, $\mathrm{t_g}$ is the sample's total counting time, and $w = \frac{1}{V \cdot E \cdot 60}$ which contains volume (L) and efficiency (Equation 1). The uncertainty of w is expressed as $\mathrm{u}_\mathrm{rel}^2(\mathrm{w}) = \mathrm{u}_\mathrm{rel}^2(\mathrm{V}) + \mathrm{u}_\mathrm{rel}^2(\mathrm{E})$, in which $\mathrm{u}^2_\mathrm{rel}(\mathrm{V})$ is the tolerance of the 8 mL pipette, and the uncertainty of the efficiency ($\mathrm{u}^2_\mathrm{rel}(\mathrm{E})$) is:

$$u_{rel}^2(E) = u_{rel}^2(r_s - r_0) + u_{rel}^2(A) = \frac{\frac{r_s}{t_s} + \frac{r_0}{t_0}}{(r_s - r_0)^2} + u_{rel}^2(A) \qquad \text{Equation 4}$$

where r_s is the standard' sample total count rate (min^{-1}), t_s is the standard' sample total counting time (minutes), r_0 is the background count rate (min^{-1}), t_0 is the background total counting time (minutes), and $u^2_{rel}(A)$ is the uncertainty of the standard's sample activity (Bq) – that is half of the expanded uncertainty shown in Figure A.7.

Moreover, in Equation 3, the decision threshold (c_A^*) is calculated as follows,

$$c_A^* = k_{1-\alpha} \cdot w \cdot \sqrt{\frac{r_0}{t_g} + \frac{r_0}{t_0}} \qquad \text{Equation 5}$$

in which t_g is the sample total counting time (minutes).

The results obtained from the measurement of the standard and background samples are shown in Table 7.

Table 7. Average results of 3 cycles of standard and background samples using the experimental conditions specified in the text above.

Energy window (keV)	**CPM$_{standard}$ (min^{-1})**	**CPM$_{background}$ (min^{-1})**	**MDA (Bq/L)**
0-156	141.52	21.79	3.64

As it can be observed from the results, an MDA of 3.64 Bq/L was obtained when measuring the standard samples for 3 cycles of 180 minutes and the background sample for 3 cycles of 1000 minutes. Despite that a higher counting time would decrease the detection limit, it is considered that the resulting detection limit with the selected counting time is correct for URAIS purpose and since it is lower than 20 Bq/L (stated by legislation).

6.1.1.2. Counting energy window

Radiocarbon emission spectrum has an energy range between 0 and 156 keV. Therefore, to optimize the energy range in our study, a sample-cocktail ratio of 8:12, Optiphase Hisafe 3, and polyethylene vials were selected.

Moreover, as TriCarb 2900TR does not show any configuration to remove low energy beta emission counts, tritium – which emission energy is between 0 and 18.6 keV –, may appear as an interference and overlap in the low energy range of the radiocarbon spectrum. According to literature[27,43], there exist two methods to determine C-14 with H-3 contribution. The first method consists in finding the knee point – point in which the contribution of C-14 in H-3 region, from 0 to 12 keV, is approximately similar to the H-3 contribution within C-14 region, from 12 to 156 keV –, and then the efficiency from each region is calculated. On the other hand, the easiest method is the removal of H-3 contribution by measuring C-14 standard samples in a narrower energy range – such as from 12 to 156 keV.

Therefore, it was chosen to cut off the tritium contribution from the radiocarbon spectrum, since it was the fastest method among the two found in bibliography. That is why, a tritium standard – with an activity of $8.375 \cdot 10^2$ Bq/L (reference date at 22/02/2023) – is measured, with the same conditions as the radiocarbon standard.

After measuring standards of tritium and C-14, both resulting spectra are plotted together in Figure 7.

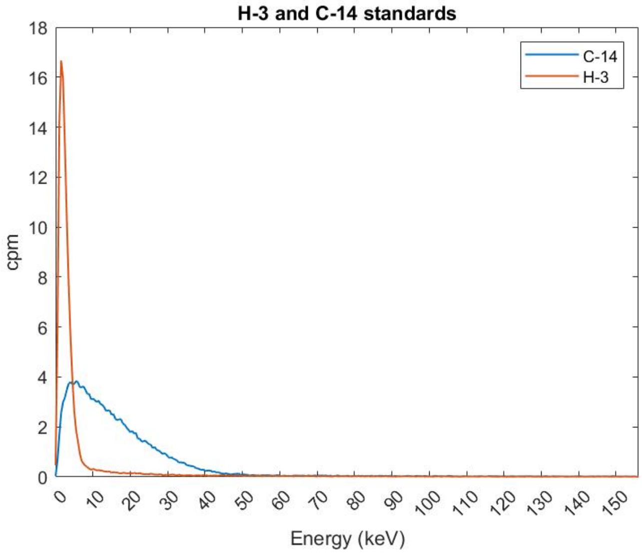


Figure 7. Spectra of C-14 and H-3 standards.

In this Figure it can be observed that tritium spectrum overlaps with C-14 spectrum, mainly in the range between 0 and 10 keV, thus the C-14 optimum energy window was established from 10 keV so that the tritium contribution is cut off even though the C-14 detection efficiency might be reduced.

Then, the final counting energy range was evaluated mainly with the figure of merit (FOM) parameter, using Equation 2. Figure of merit is a value used to characterize a device or method's performance, as a high value of FOM normally means a higher efficiency and a lower background values which is translated in a better performance of that instrument.[44] The results are shown in Table 8.

Table 8. Average of 3 cycles for count rate, detection efficiency and FOM results for different energy window ranges.

Energy window (keV)	$CPM_{standard}$ (min^{-1})	$CPM_{background}$ (min^{-1})	E (%)	FOM (min)
0-156	141.52	21.79	68.6	215.68
10-156	98.64	17.18	46.6	126.63
10-90	95.51	13.96	46.7	156.20
10-60	91.39	11.50	45.7	181.95

It can be observed that as the energy window was reduced, the counting rate also did, thus the detection efficiency diminishes since the counts cut off do not contribute to the final count rate, thus were considered as light photons that do not reach the detector.

Overall, the optimum counting energy was the one that cuts off the significant tritium signal, and the one with highest FOM. Then, that energy range is seen highlighted in Table 8, and it is between 10 and 60 keV.

6.1.1.3. Vial selection

According to bibliography, glass vials show a higher background count rate due to the K-40 presence within glass composition. However, polyethylene vials are normally chosen in direct C-14 measurement methods owing to their lowest cost[28,43]. Moreover, Teflon-covered polyethylene vials also exist, which show lower solvent diffusion over time compared to traditional polyethylene vials, nonetheless they are more expensive. [20,29]

In URAIS laboratory, there are three 20 mL vial material available – the polyethylene, the polyethylene with Teflon covering and the glass vials –, which were evaluated to check their performance and we chose the best one which provides a low background count rate, high detection efficiency, and high FOM value. To perform these experiments, Optiphase Hisafe 3 scintillation cocktail and a sample-cocktail ratio of 8:12 were selected. In addition to this, samples with a known concentration of C-14 and background samples were measured within TriCarb 2900TR equipment, selecting an energy window of 10 to 60 keV, and with counting times of three cycles of 180 minutes for spiked samples and three cycles of 1000 min for background samples.

After measuring the standard samples with all the types of vials with the same experimental conditions, the results are shown in Table 9.

Table 9. Average count rate, detection efficiency and FOM results for different vial material type, measured in TriCarb 2900TR.

Vial material	**$CPM_{standard}$ (min^{-1})**	**$CPM_{background}$ (min^{-1})**	**E (%)**	**FOM (min)**
Polyethylene	91.39	11.50	45.7	181.95
Polyethylene covered by Teflon	84.51	11.07	42.1	159.67
Glass	96.24	11.86	48.3	196.92

The best performance – high efficiency and FOM values – were obtained for the glass vial. However, glass containers are fragile and higher priced than polyethylene ones. Moreover, if glass vials were selected, a reuse and clean-ups would need to be performed in order to increase cost savings and the lifespan of the vials, but most likely leading to contaminations and waste of time.

Therefore, polyethylene vials were selected mainly due to economic and commodity concerns, and also because their performance compared with glass vials was similar.

6.1.1.4. Sample-cocktail ratio and cocktail selection

Sample-cocktail ratio and cocktail selection is different among different research studies focused on the determination of C-14 [27,28,41,45]. In general, there is not much information in bibliography about scintillation cocktails and the authors do not optimize this parameter. However, some studies choose between some scintillation cocktails, according to FOM and efficiency values, even though the most used cocktails are Ultima Gold family scintillation liquids. Regarding sample-cocktail ratio, the usual ratios reported in the literature[27,28,41,45] are 8:12, 9:11 and 10:10.

To evaluate the performance of different scintillation cocktails available in URAIS – Optiphase Hisafe 3, Ultima Gold LLT and Ultima Gold uLLT –, and the effect of changing the sample-cocktail ratios, several experimentations were performed.

All the tests were carried out by measuring samples of known activity concentration and background vials, using polyethylene vials, an energy window of 10 to 60 keV, and with counting times of three cycles of 180 minutes for spiked samples and two cycles of 1000 minutes for background samples. The corresponding results are shown in Table10.

Table 10. Average results of 3 cycles of different spiked samples with the same activity concentration, using different sample-cocktail ratio and scintillation cocktails, measured in TriCarb 2900TR.

Cocktail	**Sample: Cocktail**	**$CPM_{standard}$ (min^{-1})**	**$CPM_{background}$ (min^{-1})**	**E (%)**	**FOM (min)**
Optiphase Hisafe 3	7:13	91.18	11.61	45.6	178.77
	8:12	91.39	11.51	45.7	181.86
	9:11	84.24	11.59	41.6	149.37
	10:10	82.02	11.53	40.4	141.38
Ultima Gold LLT	7:13	85.55	9.78	43.4	192.57
	8:12	87.21	9.56	44.5	206.77
	9:11	83.81	9.92	42.3	180.44
	10:10	80.25	10.13	40.1	159.21
Ultima Gold uLLT	7:13	89.36	9.50	45.7	220.21
	8:12	92.93	9.90	47.5	228.42
	9:11	92.97	10.27	47.4	218.46
	10:10	84.98	9.76	43.1	190.16

To select the optimum scintillation cocktail and sample-cocktail ratio, background count rate, efficiency and FOM values were considered. With respect to background count rate, Optiphase Hisafe 3 showed a higher value, meaning that the scintillation cocktail contained a higher amount of C-14 within the scintillation cocktail composition than the Ultima Gold family scintillation liquids. Regarding efficiency and FOM values, the highest value was obtained for Ultima Gold uLLT. However, the selected scintillation cocktail was Ultima Gold LLT as both Ultima Gold family scintillation liquids showed similar performances and the selected liquid was cheaper.

As regards to sample-cocktail ratio, the same parameters as in the selection of scintillation selection were considered. Overall, independently on the scintillation cocktail, the lowest background count rate, and the highest FOM and efficiency values were observed to be for 8:12 sample-cocktail ratio.

6.1.2. Calibration

One of the main interferences in liquid scintillation analysis is quenching. It inhibits the production of light by absorbing energy that will not reach the detector, thus moving the final signal towards lower energies and consequently, reducing the detection efficiency.[20] That is why, in order to avoid a possible underestimation of results and an undesired reduction of detection efficiency, a quenching curve is constructed.

Before displaying results, some remarks and changes must be commented. After observing the results shown in the previous sections, it was considered to do a study of the standards' stability over time. This experimentation results are shown in Table 11, in which it was observed that different standard samples measured in different dates show distinct results, thus concluding that the radiocarbon working standard with HPLC-quality water was not stable. That is why, it was decided to prepare another C-14 working standard with the same media as the commercial standard – 0.1M NaOH plus 30µg/g C of Na_2CO_3 – and perform a stability study among time. In the last case, the results are shown in Table 12, and it was concluded that the stability was maintained since the count rate results obtained were similar between them. Ideally, in this situation, the previous tests should be repeated to confirm that the results are maintained, but due to a limitation of time, it was decided to follow with the calibration and the next tests with the standard sample that shows stability among time –with 0.1M NaOH plus 30µg/g C of Na_2CO_3 media.

Regarding background samples, some measurements were also performed to see whether the medium affected their count rate. It was observed that the average count rate obtained with HPLC-quality water was 9.56, and the count rate obtained with 0.1M NaOH plus 30µg/g C of Na_2CO_3 was 9.43. This difference between results was concluded to be negligible as the percentage uncertainty (2s%) given by the equipment is 1.35%. Thus, the background samples were decided to keep being prepared with HPLC-quality water.

Table 11. Average results for different prepared vials in different days of measurement in TriCarb 2900TR, using HPLC-quality water media.

Date of measurement	**$CPM_{standard}$ (min^{-1})**
12/04/23	87.21
17/04/23	96.25
24/04/23	95.87

Table 12. Average results for different prepared vials in different days of measurement in TriCarb 2900TR, using 0.1 M NaOH and 30µg/g C of Na_2CO_3 media.

Date of measurement	**$CPM_{standard}$ (min^{-1})**
21/04/23	108.38
28/04/23	108.36
02/05/23	109.09

According to the literature, quenching curves are constructed by measuring a set of C-14 spiked samples and adding an increasing amount of quenching agent. The most used quenching agents in research studies are nitromethane[29,43] and CCl_4 [28,46]. CCl_4 was selected to perform the quench curve in this study due to its availability in the laboratory and its milder quenching ability, compared to nitromethane [47].

To sum up, a quenching curve with the optimum conditions selected in the previous sections, plus a standard solution with 0.1M NaOH and Na_2CO_3 was built. Moreover, each standard vial contained an increasing volume of a quenching agent, CCl_4 – 0, 25, 50, 75, 100 µL. Each standard sample was measured for 3 cycles of 180 minutes and the background counts were selected from the results obtained in the sample-cocktail optimisation – which were counted for 3 cycles of 1000 minutes. The measurement results are shown in the Table 13.

Table 13. Average results of 3 cycles of standard and background count rate, with the corresponding quench-indicating parameter and efficiency.

CCl_4 volume (µL)	**$CPM_{standard}$ (min^{-1})**	**$CPM_{background}$ (min^{-1})**	**E(%)**	**tSIE**
0	108.38	9.56	56.7	307.28
25	94.97	9.56	49.0	249.37
50	80.89	9.56	40.9	202.74
75	61.70	9.56	29.9	159.30
100	46.96	9.56	21.5	131.81

The decrease of efficiency and quench-indicating parameter with different additions of CCl_4 can be observed in Table 13, meaning that the C-14 spectra became quenched as higher volumes of CCl_4 were added, as expected. Then, using Table 13, the corresponding quench curve was plotted, as shown in the next figure.

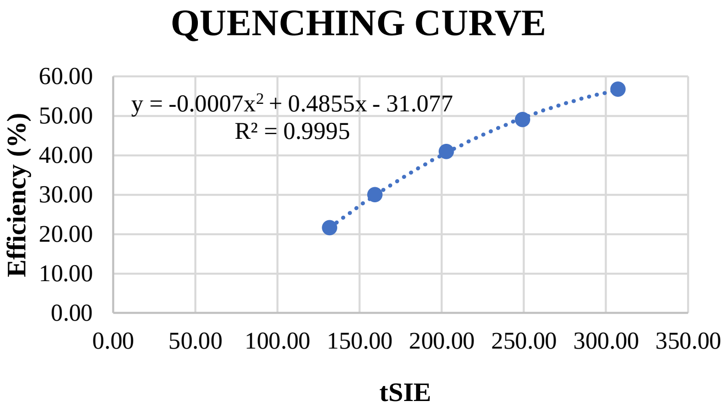


Figure 8. Quenching curve using a polynomial trendline for TriCarb 2900TR.

The quench curve was fitted in a polynomial trendline since the fitting was the best – with a coefficient of determination (R^2) of 0.9995. This curve will be used when an unknown sample is measured, by using the quench-indicating parameter to obtain the detection efficiency, and afterwards its activity.

6.1.3. Validation

The parameters to be determined in an environmental radioactivity method validation are precision, trueness, uncertainty, and detection limit (MDA) (Equation 3). [48–51]

Precision – in this case intermediate precision (analysis of the same sample with the same method within one laboratory and one equipment, but at different days of analysis) [50] – was calculated using the relative standard deviation (RSD) percentage, which expression is the following,

$$\mathrm{RSD}\ (\%) = \frac{\sigma}{\overline{\overline{\mathrm{X}}}} \cdot 100 \qquad \text{Equation 6}$$

where σ is the standard deviation of the calculated activity, and $\overline{\mathrm{X}}$ is the average calculated activity.

To determine trueness, samples with a known concentration were required – reference materials or spiked samples. To calculate it, the relative bias (RB) percentage was used, and it was defined as,

$$\mathrm{RB}\ (\%) = \frac{|\overline{\mathrm{X}} - \mathrm{X}_{\mathrm{ref}}|}{\mathrm{X}_{\mathrm{ref}}} \cdot 100 \qquad \text{Equation 7}$$

in which $\overline{\mathrm{X}}$ is the average activity measured by the laboratory and X_{ref} is the activity of the reference material or spiked sample.

Lastly, the activity (Equation 8) and its combined uncertainty were assessed using the expression in UNE-EN ISO 13162:2021.

$$\mathrm{c}_A\ (\mathrm{Bq/L}) = \frac{r_g - r_0}{60 \cdot \mathrm{V} \cdot \mathrm{E}} = (r_g - r_0) \cdot w \qquad \text{Equation 8}$$

$$\mathrm{u}(\mathrm{c}_A)\ (\mathrm{Bq/L}) = \sqrt{w^2 \cdot \left(\frac{r_g}{t_g} + \frac{r_0}{t_0}\right) + c_A^2 \cdot u_{rel}^2(w)} \qquad \text{Equation 9}$$

where r_g is the sample's total count rate (min^{-1}), r_0 is the background's count rate (min^{-1}), t_0 is the background's total counting time, t_g is the sample's total counting time, and volume (L) and efficiency (Equation 1) are expressed as $w = \frac{1}{V \cdot E \cdot 60}$. The uncertainty is expressed as $\mathrm{u}_{\mathrm{rel}}^2(\mathrm{w}) = \mathrm{u}_{\mathrm{rel}}^2(\mathrm{V}) + \mathrm{u}_{\mathrm{rel}}^2(\mathrm{E})$, in which $u^2_{rel}(V)$ is $2 \cdot 10^{-5}$ L – the tolerance of the 8 mL pipette –, and the uncertainty of the efficiency ($u^2_{rel}(E)$) is found in Equation 4.

Moreover, the aforementioned literature introduces the concentration levels in which the validation process is performed, these concentration levels are between the MDA and ten times MDA, between ten times MDA and one hundred times the MDA values, and also activities with higher than one hundred time the MDA. Furthermore, they also present the number of replicas to be measured during the validation steps. In the case of this study, the validation process corresponds to methods that is newly applied in the laboratory and that is based on norms. That is why, the number of replicas to be performed during the validation was five – five replicas for the three levels of activity concentrations.

To proceed with the validation, it was decided to measure a set of spiked samples, knowing that the MDA of an unquenched sample found in Table 13, first row, was 3.20 Bq/L (Equation 3). Therefore, five replicas of activity concentrations between 3 and 30 Bq/L, 30 and 300Bq/L, and higher than 300 Bq/L were measured, with the optimum conditions – energy window between 10 and 60 keV, Ultima Gold LLT cocktail, 8:12 sample-cocktail ratio, and polyethylene vials –, and with a counting time of 1 cycle of 180 minutes. The background was not measured again, and its count rate was taken as 9.56 cpm – the one obtained in the sample-cocktail ratio and cocktail optimisation.

The results of the measurements may be observed in Table 14.

Table 14. Average validation results obtained for five replicas in three levels of concentration after measuring 1 cycle of 180 minutes, for TriCarb 2900TR equipment.

Concentration level (Bq/L)	**$CPM_{spiked\ sample}$ (min^{-1})**	**tSIE**	**E (%) ***	**C_A (Bq/L)**	**$C_{A,theoretical}$ (Bq/L) ****
3-30	12.99	306.69	52.0	13.76	15.01
30-300	30.63	307.60	52.0	84.38	90.96
> 300	109.33	302.79	51.7	401.65	363.86

*Efficiency is calculated from the quenching curve obtained in the previous section, with the polynomial equation $-0.0007x^2 + 0.4855x - 31.077$.

**The theoretical activity of the spiked samples has a reference date at 05/05/2023.

With these results, it was observed that efficiency and quenching was maintained in all concentration levels. Using the data in Table 14, precision (Equation 6), trueness (Equation 7), uncertainty (Equation 9), and MDA (Equation 3) were calculated and their average in each concentration level is shown in the following table.

Table 15. Average results of the validation parameters for five replicas of three concentration levels, in TriCarb 2900TR instrument.

Concentration level (Bq/L)	**Precision (%) ****	**Trueness (%)**	**Uncertainty (%) ***	**MDA (Bq/L)**
3-30	6.5	8.4	21.3	3.20
30-300	3.3	7.2	11.2	3.20
> 300	0.8	10.4	10.3	3.21

*Expanded uncertainty with k = 2, percentage calculated with respect to the activity measured (see Table 14).

**Percentage precision in terms of intermediate precision.

According to bibliography[48,51], the acceptance criteria for precision and trueness are 30% in the concentration level between the 3 and 30 Bq/L, and 25% between 30 and 300 Bq/L as well as for concentrations higher than 300 Bq/L. That is why, with the results displayed in Table 15, it can be observed that precision and trueness values were below the ones stated by the literature, meaning that the validation is correct. Regarding precision values, a decreasing tendency was observed, meaning that at higher concentration levels the intermediate precision was the highest. With respect to trueness, as not expected, at high concentration activities the trueness became the lowest. Overall, it was concluded that at high activities the method was more precise and less accurate than between 3 and 30 Bq/L.

Regarding the percentage uncertainty, it can be observed that at lower concentration levels the percentage uncertainty was doubled in comparison to higher activity concentrations. The explanation may be that at lower activities the quantification was highly influenced by the background count rate – the average spiked sample's count rate was approximately 13 cpm and the background count rate was 9.56 cpm –, thus giving rise to higher errors.

Lastly, MDA was always maintained at 3.20 Bq/L which confirmed the detection limit fixed initially during the counting time selection.

The following subsection addresses an interlaboratory comparison between two laboratories to finish the validation process and to ensure the validity of the results.

6.1.3.1. Interlaboratory comparison

To verify the validity of the results obtained in the previous tests, participation in proficiency tests is ideal. However, since in the period in which this research was performed proficiency tests focused on C-14 were not available. For that reason, it was decided to exchange spiked samples with another laboratory, in this case with the *Universitat Politècnica de València (UPV)*. UPV sent a C-14 spiked sample with 292.54 Bq/L with an associated uncertainty of 11.78 Bq/L – reference date 19/05/2023 –, and URAIS prepared a C-14 spiked sample with 218.31 Bq/L with an associated uncertainty of 15.31 Bq/L – reference date 22/05/2023.

Both spiked samples were measured with the same conditions as if they were unknown samples. The results were evaluated with trueness (Equation 7) and ζ-score (Equation 10). ζ-score was useful to assess the laboratory ability to obtain results close to the assigned value.[49]

$$\zeta\text{−}score = \frac{x_{lab} - x_{ref}}{\sqrt{u_{lab}^2 + u_{ref}^2}} \qquad \text{Equation 10}$$

Where x_{lab} is the activity obtained in the laboratory, x_{ref} is the theoretical activity of the spiked sample, u^2_{lab} is the associated uncertainty to the activity obtained in the laboratory, and u^2_{ref} is the uncertainty associated to the spiked sample's theoretical activity.

The uncertainty, trueness and ζ-score results are shown in Table 16.

Table 16. Results of the UPV sample, with TriCarb 2900TR.

C_A (Bq/L)	Uncertainty (Bq/L) *	Trueness (%)	ζ-score
328.59	34.58	12.3	1.97

* Expanded uncertainty with k = 2 and using Equation 9.

As regards to trueness, the spiked sample showed a percentage value of 12%. This value is higher than the one obtained during the validation within the last two activity concentration levels (30-300 and >300 Bq/L). That is why, even though bibliography established an acceptance criterion for trueness and precision, the laboratory established its own depending on the results obtained during the validation process and interlaboratory comparison. That acceptance criterium for trueness and precision was decided to be fixed at 15% for the two last activity concentrations (according to the results obtained in the interlaboratory comparison), and 10% for the lower activity concentration level (according to the results obtained during the validation).

Concerning the ζ-score, and according to the literature [49,52], a result that gives the absolute value of the ζ-score ≤ 2 is considered to be a satisfactory performance, a ζ-score absolute values between 2 and 3 is a warning signal, and a ζ-score absolute value ≥ 3 is an unsatisfactory result which error must be checked by the laboratory. In this study, the zeta-score value was below 2, meaning that our results were satisfactory and close to the assigned value.

6.1.4. Tritium and C-14

As it has already been mentioned, one of the main limitations of the direct determination of C-14 in water samples is the interference with other beta-emitters, whose spectra may overlap with that of C-14. An example of a possible radionuclide interferent is H-3 which emission energy is between 0 and 18.6keV [43] – or 1 and 240 channels [28]. For this reason, it decided to evaluate whether tritium influenced the C-14 quantification, by preparing spiked samples including both H-3 – with an activity concentration of $8.375 \cdot 10^2$ Bq/L (reference date at 22/02/2023) – and C-14 in different H-3:C-14 ratios. After measuring them for 1 cycle of 180 minutes, the C-14 results are displayed in the following table.

Table 17. C-14 results of spiked samples with different H-3:C-14 ratio, with TriCarb 2900TR.

Ratio H-3:C-14	$C_{A,theoretical}$ (Bq/L)	C_A (Bq/L)	Uncertainty (Bq/L) *	Trueness (%)
2:1	182.36	205.00	21.58	12.4
1:1	364.18	401.70	41.56	10.3
1:2	182.32	198.83	20.95	9.0

* Expanded uncertainty with k = 2 and using Equation 9.

It was observed that the presence of tritium in the spiked sample do not interfere with the C-14 measurement, since the trueness in all cases was within the acceptance criteria established before (15%). Despite that, it can be commented that when the concentration activity of tritium was the double of the radiocarbon one (H-3:C-14 ratio of 2:1), the trueness decreased as well as the calculated activity increased due to the tritium contribution – which generated a slightly overestimation of C-14 concentration activity.

6.2. Quantulus 1220

6.2.1. Optimization parameters

It must be highlighted that all the preparation parameters were only optimized in TriCarb 2900TR. Quantulus 1220 is used in daily analysis measurements in URAIS – to determine H-3, Rn-222, and other radionuclides –, thus due to that availability concerns, TriCarb 2900TR was used for optimization. Thus, in this section, only the counting energy window was studied.

6.2.1.1. Counting energy window

Radiocarbon emission spectrum appears between 1 and 450 channels. Thus, the optimization of the energy window was evaluated among those values. Equally to TriCarb 2900TR, this experimentation was performed using a sample-cocktail ratio of 8:12, Optiphase Hisafe 3, and polyethylene vials.

In the same way as TriCarb 2900TR, a tritium standard was measured in order to see its contribution within C-14 spectrum. Nonetheless, in this case, Quantulus 1220's counting program includes a configuration to measure C-14 and other beta emitters – C14 configuration and high coincidence bias configuration. High coincidence bias is used to cut off spectra below 100 channels and some counts above it, hence lowering the background noise and removing possible luminescence contribution[40]. The difference between using high and low coincidence bias configuration when measuring a tritium standard with C-14 counting conditions is shown in Figure 9.

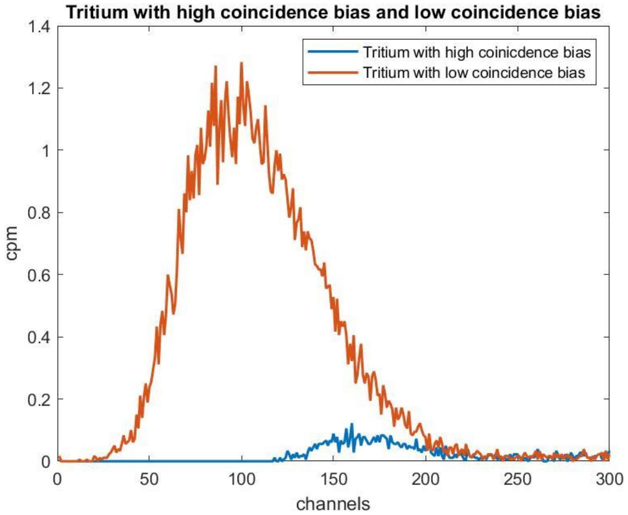


Figure 9. Tritium spectra using low and high coincidence bias configuration in Quantulus 1220.

As it can be observed, the use of high coincidence bias highly reduced most of the tritium influence. Consequently, the contribution of tritium within C-14 spectrum was considered as negligible, as it is observed in Figure 10. That is why, using high coincidence bias configuration it was not necessary to remove the tritium spectra by narrowing the energy window range of C-14, therefore not sacrificing efficiency in the process.

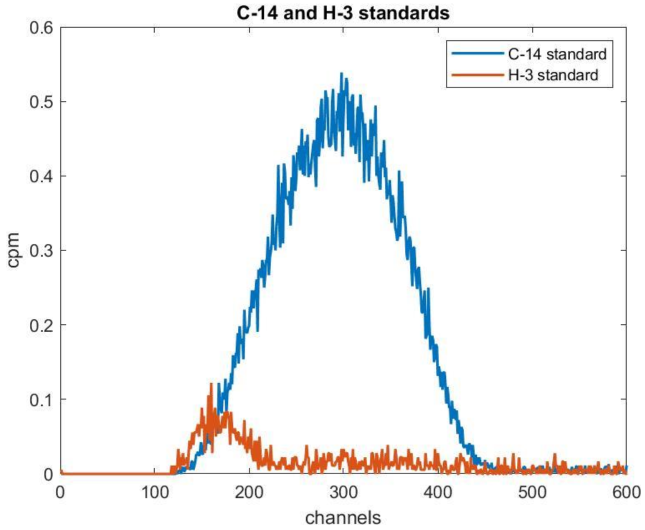


Figure 10. Tritium and radiocarbon spectra using C-14 and high bias configuration in Quantulus 1220.

As a result, the counting energy window in Quantulus 1220 was studied starting where the C-14 spectrum appears – at 120 channels – and using the figure of merit and efficiency parameters. The different results are presented in Table 18.

Table 18. Average results in polyethylene vials using Optiphase Hisafe 3 and a sample-cocktail ratio of 8:12 in different counting energies (channels).

Energy window (channels)	**CPM$_{standard}$ (min^{-1})**	**CPM$_{background}$ (min^{-1})**	**E (%)**	**FOM (min)**
120-450	84.34	3.33	46.4	645.98
120-500	84.61	3.28	46.6	661.00
130-450	84.32	3.07	46.5	705.08
130-500	84.61	3.28	46.6	661.32
150-450	84.01	3.31	46.2	645.34
150-500	84.28	3.58	46.2	597.08

By far, the best energy range - with the highest FOM value and high efficiency - was between 130 and 450 channels, which was the same as another research study[28] had chosen using the same direct method. Therefore, it was confirmed that this was the best energy window to work with Quantulus 1220 to determine C-14 by direct measurement.

6.2.2. Calibration

A quenching curve with the optimum conditions – 8:12 sample-cocktail ratio, Ultima Gold LLT as the scintillation cocktail, and polyethylene vials – selected in TriCarb 2900TR equipment, plus a standard solution with 0.1M NaOH and Na_2CO_3 was built. Moreover, each standard vial contained an increasing volume of a quenching agent, CCl_4. Each standard sample was measured for 3 cycles of 180 minutes and the background samples were measured for 3 cycles of 1000 minutes. The average measurement results are shown in Table 19.

Table 19. Average results of standard and background count rate, with the corresponding quench-indicating parameter and efficiency.

CCl_4 volume (µL)	**$CPM_{standard}$ (min^{-1})**	**$CPM_{background}$ (min^{-1})**	**E(%)**	**SQP(E)**
0	117.81	1.63	66.5	745.63
25	109.14	1.63	61.6	718.00
50	98.69	1.63	55.6	685.63
75	83.91	1.63	47.1	649.52
100	70.14	1.63	39.2	619.92

Using the data in Table 19, the corresponding quench curve is plotted.

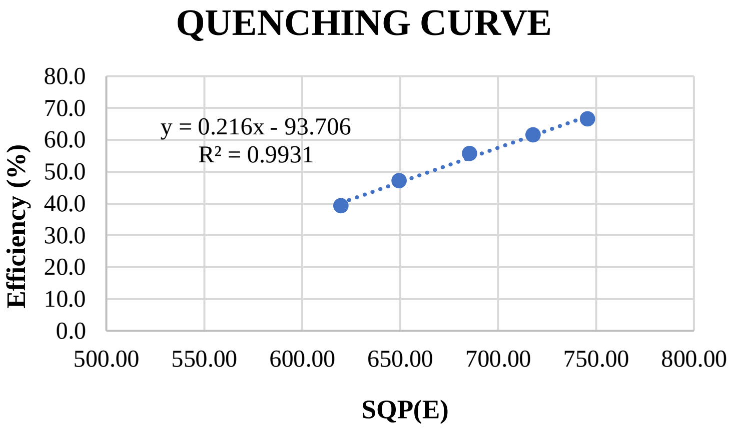


Figure 11. Quenching curve using a lineal trendline for Quantulus 1220.

In Quantulus 1220's quench curve, the trendline was lineal as the fitting was the best – with a determination coefficient (R^2) of 0.9931. Moreover, the quench parameter without quenching was 745 whereas with the highest quenching, the SQP(E) was 610. In this case, the sample's quench parameter was not highly affected by CCl_4 additions, whereas the detection efficiency was almost halved when comparing the unquenched and quenched sample.

6.2.3. Validation

The validation in Quantulus 1220 was performed in the same way as in TriCarb 2900TR equipment. Knowing that the MDA of an unquenched sample shown in Table 19 (first row) was 1.05 Bq/L, five replicas of activity concentrations between 1 and 10 Bq/L, 10 and 100Bq/L, and higher than 100 Bq/L were measured, with the selected optimum conditions and with a counting time of 1 cycle of 180 minutes. The background was not measured again, and its count rate was taken as 1.63 cpm – the one obtained during the quenching curve assessment. The results of the measurements may be observed in Table 20.

Table 20. Average validation results obtained for five replicas in three levels of concentration, for Quantulus 1220 equipment.

Concentration level (Bq/L)	$CPM_{spiked\ sample}$ (min^{-1})	SQP(E)	E (%) *	C_A (Bq/L)	$C_{A,theoretical}$ (Bq/L) **
1-10	3.91	748.97	68.1	6.98	9.10
10-100	24.83	750.05	68.3	70.77	90.96
> 100	89.66	748.68	68.0	269.67	272.89

*Efficiency is calculated from the quenching curve obtained in the previous section, with the lineal equation 0.216x – 93.706.

*The theoretical activity of the spiked samples has a reference date at 05/05/2023.

With these results, it was observed that efficiency and quenching was maintained in all concentration levels, a similarity with TriCarb 29000TR. Using the data in Table 20, precision (Equation 6), trueness (Equation 7), uncertainty (Equation 9), and MDA (Equation 3) were calculated and their average in each concentration level are shown in the following table.

Table 21. Average results of the validation parameters for five replicas of three concentration levels, in Quantulus 1220 instrument.

Concentration level (Bq/L)	Precision (%)	Trueness (%)	Uncertainty (%) *	MDA (Bq/L)
1-10	4.2	23.3	18.3	1.04
10-100	1.8	22.2	10.8	1.03
> 100	0.9	10.4	10.4	1.04

* Expanded uncertainty with k = 2, percentage calculated with respect to the activity measured (see Table 20)

In the results displayed in Table 21, it can be observed that precision and trueness values were below the ones stated by the literature[48], meaning that the validation was satisfactory [48]. Regarding the precision values, the same decreasing tendency was observed as in the other equipment, concluding that both instruments show intermediate precision in all concentration levels but a higher precision at high concentration activities. With respect to trueness, also a decreasing tendency was observed. In this parameter, a clear distinction was observed between both instruments, as Quantulus 1220 showed the highest trueness at high concentration levels whereas TriCarb 2900TR showed a high trueness at a concentration level between 3 and 300 Bq/L. Summarizing, it was concluded that at high activities Quantulus 1220 shows high precision and trueness.

Regarding the percentage uncertainty, it can be observed the same tendency as TriCarb 2900TR which is that at lower concentration levels the percentage uncertainty was almost doubled in comparison to higher activity concentrations. The explanation may be that at lower activities the quantification was highly influenced by the background count rate – the average spiked sample’s count rate was approximately 4 cpm and the background count rate was 1.63 cpm –, thus giving rise to higher associated uncertainties.

Finally, MDA was always maintained at 1.0 Bq/L which confirms that Quantulus 1220 can detect lower activity concentrations than TriCarb 2900TR. Moreover, comparing background samples count rates – 1.63 cpm for Quantulus 1220 9.56 cpm for TriCarb 2900TR – it was also confirmed that Quantulus 1220 is a low background and low-level instrument as it claims to be. [33]

The following subsection addresses interlaboratory comparison between two laboratories to finish the validation process and to ensure the validity of the results.

6.2.3.1. Interlaboratory comparison

Similar to TriCarb 2900TR, both spiked samples exchanged between UPV and URAIS were measured in Quantulus 1220. Both spiked samples were measured with the same conditions as if they were unknown samples. The results were studied with trueness (Equation 7) and ζ-score (Equation 10) and the corresponding results are displayed in Table 22.

Table 22. Results of the UPV spiked sample, with Quantulus 1220.

C_A (Bq/L)	Uncertainty (Bq/L) *	Trueness (%)	ζ-score
289.85	29.97	0.9	0.17

* Expanded uncertainty with k = 2 and using Equation 9.

In relation to trueness, UPV spiked sample showed a percentage value of 0.9%. That value was lower than the one obtained during the validation within the highest activity concentration level (>100 Bq/L). That is why the acceptance criteria in URAIS laboratory for trueness and precision in the last two concentration levels was decided to be fixed to the values obtained during the validation, which was 25% for the two lowest concentration levels (1-10 and 10-100 Bq/L) and 10% for activity concentrations higher than 100 Bq/L.

In this study, zeta-score value was very low and below 2, meaning that the results were satisfactory and were very close to the assigned value.

6.2.4. Tritium and C-14

Same to TriCarb 2900TR, spiked samples with both H-3 and C-14 radionuclides in different H-3:C-14 ratios were prepared. After measuring them for 1 cycle of 180 minutes, the C-14 results are displayed in the following table.

Table 23. C-14 results of spiked samples with different H-3:C-14 ratio, with Quantulus 1220.

Ratio H-3:C-14	**$C_{A,theoretical}$ (Bq/L)**	**C_A (Bq/L)**	**Uncertainty (Bq/L) ***	**Trueness (%)**
2:1	182.36	191.79	19.99	5.2
1:1	364.18	365.19	37.65	0.3
1:2	182.32	180.43	18.83	1.0

* Expanded uncertainty with k = 2 and using Equation 9.

In this equipment it was better observed how with a H-3:C-14 ratio of 2:1 the trueness become lower since the contribution of tritium within C-14 spectra is higher, thus giving a higher calculated activity concentration value. In the same way as TriCarb 2900TR, it was concluded that tritium does not interfere within C-14 activity calculation, even though a slight overestimation was observed.

7. CONCLUSIONS AND FUTURE WORK

The most relevant conclusions of the whole project are displayed in this section, and are presented as follows:

- The optimum conditions for the direct measurement of C-14 in water samples are polyethylene vials, a sample-cocktail ratio of 8:12, Ultima Gold LLT, and a counting time of 3 cycles of 180 minutes for standard samples, 3 cycles of 1000 minutes for background samples and 1 cycle of 180 minutes for unknown samples.
- Both equipment are able to determine C-14 with an MDA lower than the legislated (20Bq/L).
- Quantulus 1220 is indeed a low background and low-level equipment, compared to TriCarb 2900TR whose background count rate and MDA is higher.
- Quenching curves of both equipment fitted differently, TriCarb 2900TR needed a second order polynomial whereas Quantulus 1220 fitted into a lineal trendline. However, both quenching curves are valid and are used to obtain efficiency, and later the activity of an unknown sample.
- Even though during the validation Quantulus 1220 gave a lower precision and trueness than TriCarb 2900TR, the verification with an interlaboratory comparison ended up being better using Quantulus 1220.
- An acceptance criteria, for both trueness and precision, of 10% for the lowest concentration levels (3-30Bq/L) and 15% for the other concentration levels (between 30-300 and >300Bq/L) for TriCarb 2900TR. And, for Quantulus 1220, an acceptance criterion of 25% for the lowest concentration levels (between 1-10 and 10-100Bq/L) and 10% for activities of higher than 100Bq/L were selected.
- Tritium (H-3) contribution do not affect the C-14 direct measurement, in any LSC equipment.

A future work in this project is the participation in a proficiency test as well as the performance of the validation process with "real" water samples – to end the validation process – and the application of the method to that water samples.

8. REFERENCES

(1) Asociación Española de Normalización (UNE). *General requirements for the competence of testing and calibration laboratories (UNE-EN ISO/IEC 17025:2017)*; 2017.

(2) Arques, M. M. Desenvolupament de tècniques analítiques per a la determinació de radionúclids. Ph.D. Thesis, Universitat Rovira i Virgili, 2013.

(3) Gálvez, M. P. Determinació de radionúclids en mostres ambientals. Ph.D. Thesis, Universitat Rovira i Virgili, 2010.

(4) García, E. F. Impacte Radiològic ambiental causat per la industrialització al tram baix del riu Ebre. Ph.D. Thesis, Universitat Rovira i Virgili, 2016.

(5) Ratia, J. M. Presència de Radionúclids d’origen mèdic en el medi. Ph.D. Thesis, Universitat Rovira i Virgili, 2019.

(6) *El laboratori*. URAIS - URV. https://www.urais.urv.cat/ca/laboratori/ (accessed 2023-04-06).

(7) Boletín Oficial del Estado (BOE). *Real Decreto 3/2023, de 10 de Enero, por el que se establecen los criterios técnico-sanitarios de la calidad del agua de consumo, su control y suministro*; España, 2023; pp 4253–4354.

(8) Boletín Oficial del Estado (BOE). *Real Decreto 783/2001, de 6 de Julio, por el que se aprueba el reglamento sobre protección sanitaria contra radiaciones ionizantes*; 2001; pp 1–130.

(9) *Natural radionuclides in public drinking water*. US EPA . https://www.epa.gov/radtown/natural-radionuclides-public-drinking-water (accessed 2023-06-08).

(10) Martínez, J.; Peñalver, A.; Baciu, T.; Artigues, M.; Danús, M.; Aguilar, C.; Borrull, F. Presence of artificial radionuclides in samples from potable water and wastewater treatment plants. *J. Environ. Radioact.* **2018**, *192*, 187–193. https://doi.org/10.1016/j.jenvrad.2018.06.024.

(11) Manu, A.; Santhanakrishnan, V.; Rajaram, S.; Ravi, P. M. Concentration of natural radionuclides in raw water and packaged drinking water and the effect of water treatment. *J. Environ. Radioact.* **2014**, *138*, 456–459. https://doi.org/10.1016/j.jenvrad.2014.08.013.

(12) Gäfvert, T.; Ellmark, C.; Holm, E. Removal of radionuclides at a waterworks. *J. Environ. Radioact.* **2002**, *63* (2), 105–115. https://doi.org/10.1016/S0265-931X(02)00020-6.

(13) L’Annunziata, M. F. *Handbook of radioactivity analysis*; Academic Press, 1998.

(14) *Isotopes of carbon*. Global Monitoring Laboratory (GML) of the National Oceanic and Atmospheric Administration (NOAA). https://gml.noaa.gov/ccgg/isotopes/chemistry.html (accessed 2023-05-05).

(15) Edmonds, H. N. Nuclear fuel reprocessing and related discharges. *Encycl. Ocean Sci.* **2001**, 1921–1928. https://doi.org/10.1006/RWOS.2001.0169.

(16) Svetlík, I.; Tomeš, M.; Brabcová, K.; Michálek, V.; Tomášková, L. Carbon-14 in the vicinity of nuclear power plants. In *International Symposium on In situ nuclear metrology as a tool of radioecology (INSINUME)*; 2004.

(17) Davis, W. J. *Carbon-14 production in nuclear reactors*; ORNL/BREG/TM-12; Oak Ridge National Laboratory, U.S, 1977. https://www.osti.gov/biblio/7114972 (accessed 2023-04-20).

(18) *Cosmic ray-induced neutron*. Hitachi. https://www.hitachi.com/rd/glossary/c/cosmic_ray_induced_neutron.html (accessed 2023-05-08).

(19) Hou, X. Liquid scintillation counting for the determination of beta emitter — Principle and application. In *NKS-B RadWorkshop, Risø*-DTU, Denmark, November 16-20, 2009.

(20) García-León, M. *Detecting environmental radioactivity*; Springer, 2022.

(21) Benito, C.; Jiménez, A.; Sánchez, C.; Navarro-Sabaté, A. Liquid and solid scintillation: principles and applications. In *Handbook of instrumental techniques for materials, chemical and biosciences research*; Scientific and Technological Centers of the University of Barcelona (CCiTUB), 2012.

(22) Edler, R. *Cocktails for liquid scintillation counting. PerkinElmer*, 2015. https://resources.perkinelmer.com/lab-solutions/resources/docs/app_cocktails-for-liquid-scintillation-counting-011940_01.pdf (accessed 2023-04-13).

(23) Principles and applications of liquid scintillation counting- A primer for orientation. *National Diagnostics*. 2004, pp 4–5. https://ehs.psu.edu/sites/ehs/files/lsc_theory_of_operation_part_1.pdf (accessed 2023-04-11).

(24) *Liquid scintillation cocktails*. PerkinElmer, Inc. https://www.perkinelmer.com/es/lab-products-and-services/application-support-knowledgebase/radiometric/liquid-scintillation-cocktails.html (accessed 2023-04-12).

(25) Lanuza, M. P. Aplicació de l'escintil·lació líquida al control de la radioactivitat ambiental. M.S. Thesis, Universitat Rovira i Vigili, 2004.

(26) *Photomultiplier tubes (PMT)*. SENSE. https://www.sense-pro.org/lll-sensors/pmt (accessed 2023-04-14).

(27) Stojković, I.; Todorović, N.; Nikolov, J.; Krajcar Bronić, I.; Bátor, G.; Kovács, T. Investigation of fast screening lsc method for monitoring 14c activity in wastewater samples. *Radiat. Meas.* **2019**, *121*, 1–9. https://doi.org/10.1016/j.radmeas.2018.12.004.

(28) Muñoz, M. S.; Ortiz, J.; Martorell, S. Desarrollo de procedimientos rápidos para la determinación de tritio y carbono-14 en muestras de agua mediante centelleo líquido. In *46º Reunión Anual de la Sociedad Nuclear Española*; 2021; pp 1–6.

(29) Gudelis, A.; Gaigalaitė, L.; Gvozdaitė, R. Application of the absolute method for determination of tritium and radiocarbon in groundwater from radioactive waste facility. *J. Radioanal. Nucl. Chem.* **2019**, *322* (3), 1391–1396. https://doi.org/10.1007/s10967-019-06897-9.

(30) Sáez-Muñoz, M. Desarrollo de procedimientos rápidos de ensayo para la vigilancia radiológica ambiental en situaciones de emergencia, Universitat Politècnica de València, 2018. https://doi.org/10.4995/Thesis/10251/118653.

(31) Thomson, J. Use and preparation of quench curves in liquid scintillation counting. *PerkinElmer, Inc.* 2004, pp 1–7.

(32) PerkinElmer. *Manual de referencia de quantasmart*; 2011.

(33) Perkin Elmer. 1220 Quantulus instrument manual. **2002**, 210.

(34) Asociación Española de Normalización (UNE). *Medición de la radiactividad. Determinación de las actividades de los emisores beta. Método de ensayo mediante recuento de centelleo de líquidos (UNE-EN ISO 19361:2017) (Ratificada por la Asociación Española de Normalización en Abril de 2020)*; 2020.

(35) Varlam, C.; Stefanescu, I.; Varlam, M.; Popescu, I.; Faurescu, I. Applying the direct absorption method and LSC for 14C concentration measurement in aqueous samples. *Radiocarbon* **2007**, *49* (2), 281–289. https://doi.org/10.1017/S003382220004220X.

(36) Varlam, C.; Stefanescu, I.; Varlam, M.; Bucur, C.; Popescu, I.; Faurescu, I. Optimization of 14C concentration measurement in aqueous samples using the direct absorption method and LSC. In *International conference on advances in liquid scintillation spectrometry*; 2005; pp 423–428.

(37) Horvatinčić, N.; Barešić, J.; Bronić, I. K.; Obelić, B. Measurement of low 14C activities in a liquid scintillation counter in the Zagreb radiocarbon laboratory. *Radiocarbon* **2004**, *46* (1), 105–116. https://doi.org/10.1017/S0033822200039412.

(38) Molnár, A.; Molnár, M.; Veres, M.; Czébely, A.; Rinyu, L.; Rozmanitz, P. J.; Janovics, R. Determination of the total 14C concentration of water samples using the COD method and AMS. *Radiocarbon* **2022**, *64* (5), 1065–1074. https://doi.org/10.1017/RDC.2022.42.

(39) Lawson, E. M. AMS measurements. In *Second quaternary dating workshop*, Australian Nuclear Science and Technology Organisation (ANSTO), April 8-9 1999; pp 24–28.

(40) PerkinElmer. *Windows software for controlling 1220 Quantulus (WinQ)*; 2012.

(41) Firman, N. F. A.; Noor, A.; Zakir, M.; Maming; Fathurrahman, A. F. Optimization of cocktail AquaLight LLT Volume for estimation of 14C activity in aqueous sample using LSC Hidex 300 SL. *Egypt. J. Chem.* **2021**, *64* (10), 5443–5448. https://doi.org/10.21608/ejchem.2021.69931.3541.

(42) Asociación Española de Normalización (UNE). *Calidad del agua. Carbono 14. Método de ensayo utilizando el recuento por centelleo líquido (UNE-EN ISO 13162:2021) (Ratificada por la Asociación Española de Normalización en Julio de 2021)*; 2021.

(43) Reddy, P. J.; Bhade, S. P. D.; Narayan, K. K.; Narayanan, A.; Babu, D. A. R.; Sharma, D. N. Comparative study of different methods for the activity quantification of 3H and 14C radionuclides in dual labeled samples using liquid scintillation analyzer. *Appl. Radiat. Isot.* **2009**, *67* (10), 1945–1951.

https://doi.org/10.1016/j.apradiso.2009.07.006.

(44) Olivieri, A. C.; Escandar, G. M. Chapter 6- Analytical figures of merit. In *Practical Three-Way Calibration*; Elsevier, 2014; pp 93–107.

(45) Ge, X.; Yan, L.; Xiong, Q.; Chen, F.; Liang, J.; Zhang, Z.; Yang, H.; Long, D. Direct analysis method of 14C radioactivity concentration in urine samples. *Radiat. Med. Prot.* **2022**, *3* (1), 26–30. https://doi.org/10.1016/j.radmp.2021.12.001.

(46) Verrezen, F.; Loots, H.; Hurtgen, C. A Performance comparison of nine selected liquid scintillation cocktails. *Appl. Radiat. Isot.* **2008**, *66* (6–7), 1038–1042. https://doi.org/10.1016/j.apradiso.2008.02.050.

(47) Varlam, C.; Faurescu, I.; Vagner, I.; Faurescu, D.; Duliu, O. G. Nitromethane and other quenching agents used to determine the tritium activity concentration by liquid scintillation spectroscopy. *J. Radioanal. Nucl. Chem.* **2015**, *303* (1), 789–795. https://doi.org/10.1007/s10967-014-3364-y.

(48) Terés, L. P. Actividades de validación de ensayos y aseguramiento de la calidad en la medida por centelleo en fase líquida. Quality assurance in liquid scintillation counting. *Ingeniería Civil*. 2018, pp 41–52.

(49) Pujol, L. Validation of a method for measuring Radon-222 in water using two-Phase liquid scintillation counting according to the ISO/IEC 17025 criteria. *J. Radioanal. Nucl. Chem.* **2022**, *331* (8), 3259–3268. https://doi.org/10.1007/s10967-022-08313-1.

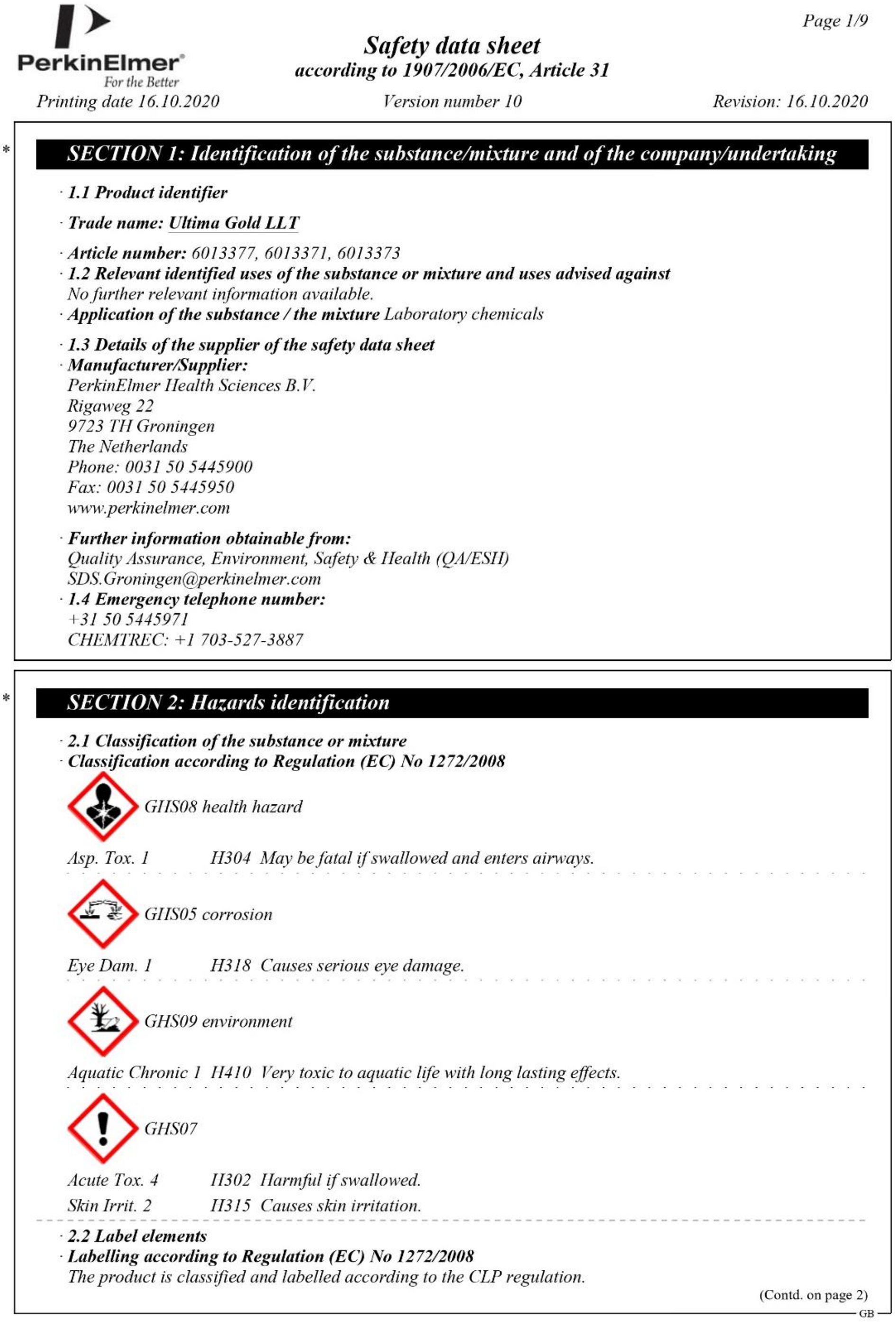
(50) *Requisitos mínimos para la validación de métodos de ensayo en el campo de la radiactividad. Rev 18/11/2011*. Internal Document.

(51) Moragón, J. O. Criterios desarrollados para la validación de procedimientos. In *VII Jornadas sobre Calidad en el Control de la Radiactividad Ambiental*, Tarragona, Spain, 2012; pp 135–144.

(52) International Standardisation Organisation (ISO). *Statistical methods for use in proficiency testing by interlaboratory comparison (ISO 13528:2015)*; 2015.

9. ANNEX

9.1. Scintillation cocktails' safety data sheets



PerkinElmer
For the Better

Page 1/9

Safety data sheet
according to 1907/2006/EC, Article 31

Printing date 16.10.2020 Version number 10 Revision: 16.10.2020

* **SECTION 1: Identification of the substance/mixture and of the company/undertaking**

· **1.1 Product identifier**

· **Trade name:** **Ultima Gold LLT**

· **Article number:** 6013377, 6013371, 6013373
· **1.2 Relevant identified uses of the substance or mixture and uses advised against**
No further relevant information available.
· **Application of the substance / the mixture** Laboratory chemicals

· **1.3 Details of the supplier of the safety data sheet**
· **Manufacturer/Supplier:**
PerkinElmer Health Sciences B.V.
Rigaweg 22
9723 TH Groningen
The Netherlands
Phone: 0031 50 5445900
Fax: 0031 50 5445950
www.perkinelmer.com

· **Further information obtainable from:**
Quality Assurance, Environment, Safety & Health (QA/ESH)
SDS.Groningen@perkinelmer.com
· **1.4 Emergency telephone number:**
+31 50 5445971
CHEMTREC: +1 703-527-3887

* **SECTION 2: Hazards identification**

· **2.1 Classification of the substance or mixture**
· **Classification according to Regulation (EC) No 1272/2008**

GHS08 health hazard

Asp. Tox. 1	H304 May be fatal if swallowed and enters airways.

GHS05 corrosion

Eye Dam. 1	H318 Causes serious eye damage.

GHS09 environment

Aquatic Chronic 1	H410 Very toxic to aquatic life with long lasting effects.

GHS07

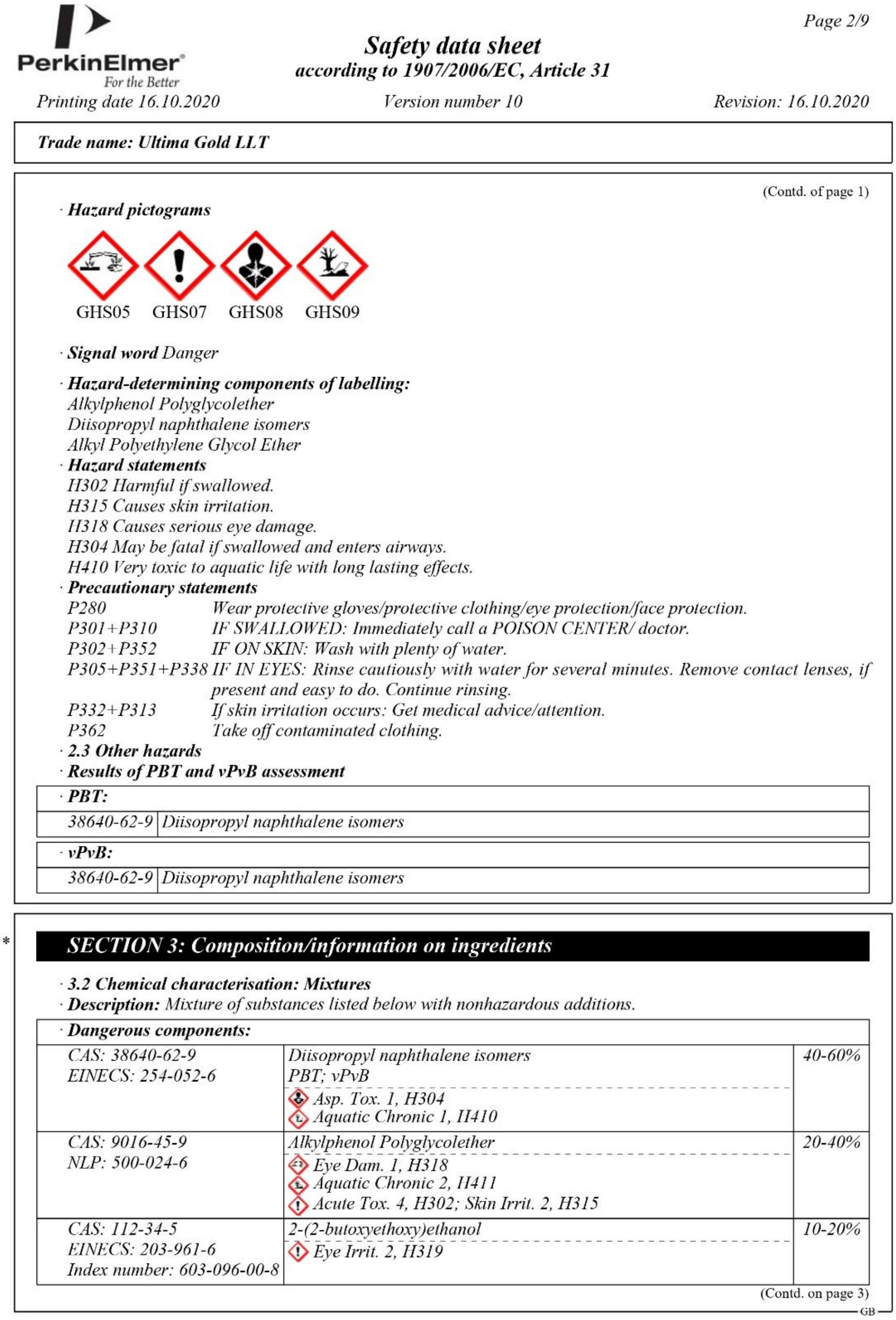
Acute Tox. 4	H302 Harmful if swallowed.
Skin Irrit. 2	H315 Causes skin irritation.

· **2.2 Label elements**
· **Labelling according to Regulation (EC) No 1272/2008**
The product is classified and labelled according to the CLP regulation.

(Contd. on page 2)

GB

Figure A.1.Ultima Gold LLT's safety data sheet, first page.



PerkinElmer®
For the Better

Page 2/9

Safety data sheet
according to 1907/2006/EC, Article 31

Printing date 16.10.2020 | Version number 10 | Revision: 16.10.2020

Trade name: Ultima Gold LLT

(Contd. of page 1)

· **Hazard pictograms**

GHS05 GHS07 GHS08 GHS09

· **Signal word** Danger

· **Hazard-determining components of labelling:**
Alkylphenol Polyglycolether
Diisopropyl naphthalene isomers
Alkyl Polyethylene Glycol Ether

· **Hazard statements**
H302 Harmful if swallowed.
H315 Causes skin irritation.
H318 Causes serious eye damage.
H304 May be fatal if swallowed and enters airways.
H410 Very toxic to aquatic life with long lasting effects.

· **Precautionary statements**

P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/ doctor.
P302+P352	IF ON SKIN: Wash with plenty of water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P362	Take off contaminated clothing.

· **2.3 Other hazards**

· **Results of PBT and vPvB assessment**

· **PBT:**	
38640-62-9	Diisopropyl naphthalene isomers

· **vPvB:**	
38640-62-9	Diisopropyl naphthalene isomers

*

SECTION 3: Composition/information on ingredients

· **3.2 Chemical characterisation: Mixtures**

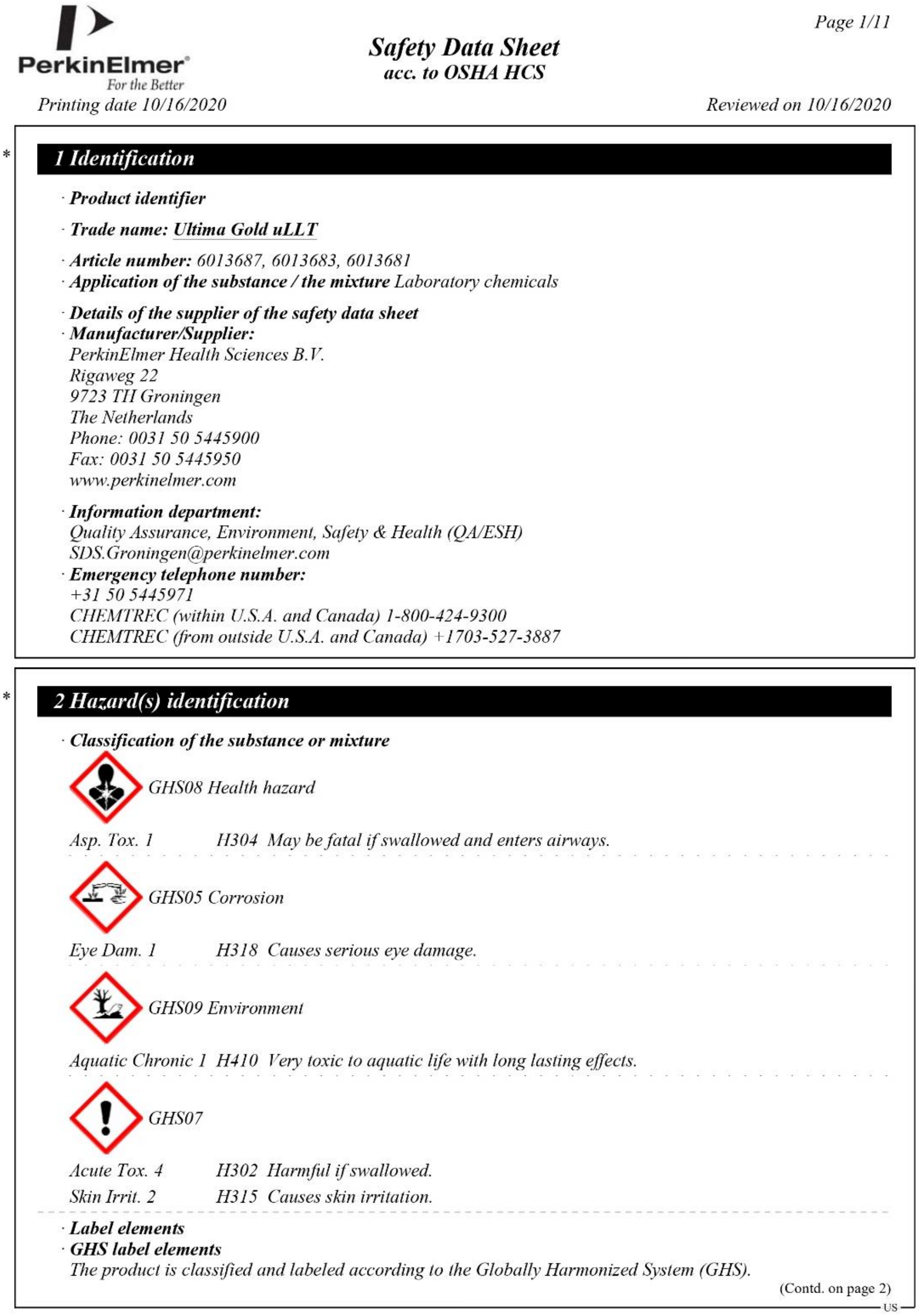
· **Description:** Mixture of substances listed below with nonhazardous additions.

· **Dangerous components:**		
CAS: 38640-62-9 EINECS: 254-052-6	Diisopropyl naphthalene isomers PBT; vPvB Asp. Tox. 1, H304 Aquatic Chronic 1, H410	40-60%
CAS: 9016-45-9 NLP: 500-024-6	Alkylphenol Polyglycolether Eye Dam. 1, H318 Aquatic Chronic 2, H411 Acute Tox. 4, H302; Skin Irrit. 2, H315	20-40%
CAS: 112-34-5 EINECS: 203-961-6 Index number: 603-096-00-8	2-(2-butoxyethoxy)ethanol Eye Irrit. 2, H319	10-20%

(Contd. on page 3)

GB

Figure A.2. Ultima Gold LLT's safety data sheet, second page.



PerkinElmer
For the Better

Printing date 10/16/2020

Safety Data Sheet
acc. to OSHA HCS

Page 1/11

Reviewed on 10/16/2020

* ***1 Identification***

· ***Product identifier***

· ***Trade name: Ultima Gold uLLT***

· ***Article number:*** *6013687, 6013683, 6013681*
· ***Application of the substance / the mixture*** *Laboratory chemicals*

· ***Details of the supplier of the safety data sheet***
· ***Manufacturer/Supplier:***
PerkinElmer Health Sciences B.V.
Rigaweg 22
9723 TH Groningen
The Netherlands
Phone: 0031 50 5445900
Fax: 0031 50 5445950
www.perkinelmer.com

· ***Information department:***
Quality Assurance, Environment, Safety & Health (QA/ESH)
SDS.Groningen@perkinelmer.com
· ***Emergency telephone number:***
+31 50 5445971
CHEMTREC (within U.S.A. and Canada) 1-800-424-9300
CHEMTREC (from outside U.S.A. and Canada) +1703-527-3887

* ***2 Hazard(s) identification***

· ***Classification of the substance or mixture***

GHS08 Health hazard

Asp. Tox. 1 *H304 May be fatal if swallowed and enters airways.*

GHS05 Corrosion

Eye Dam. 1 *H318 Causes serious eye damage.*

GHS09 Environment

Aquatic Chronic 1 *H410 Very toxic to aquatic life with long lasting effects.*

GHS07

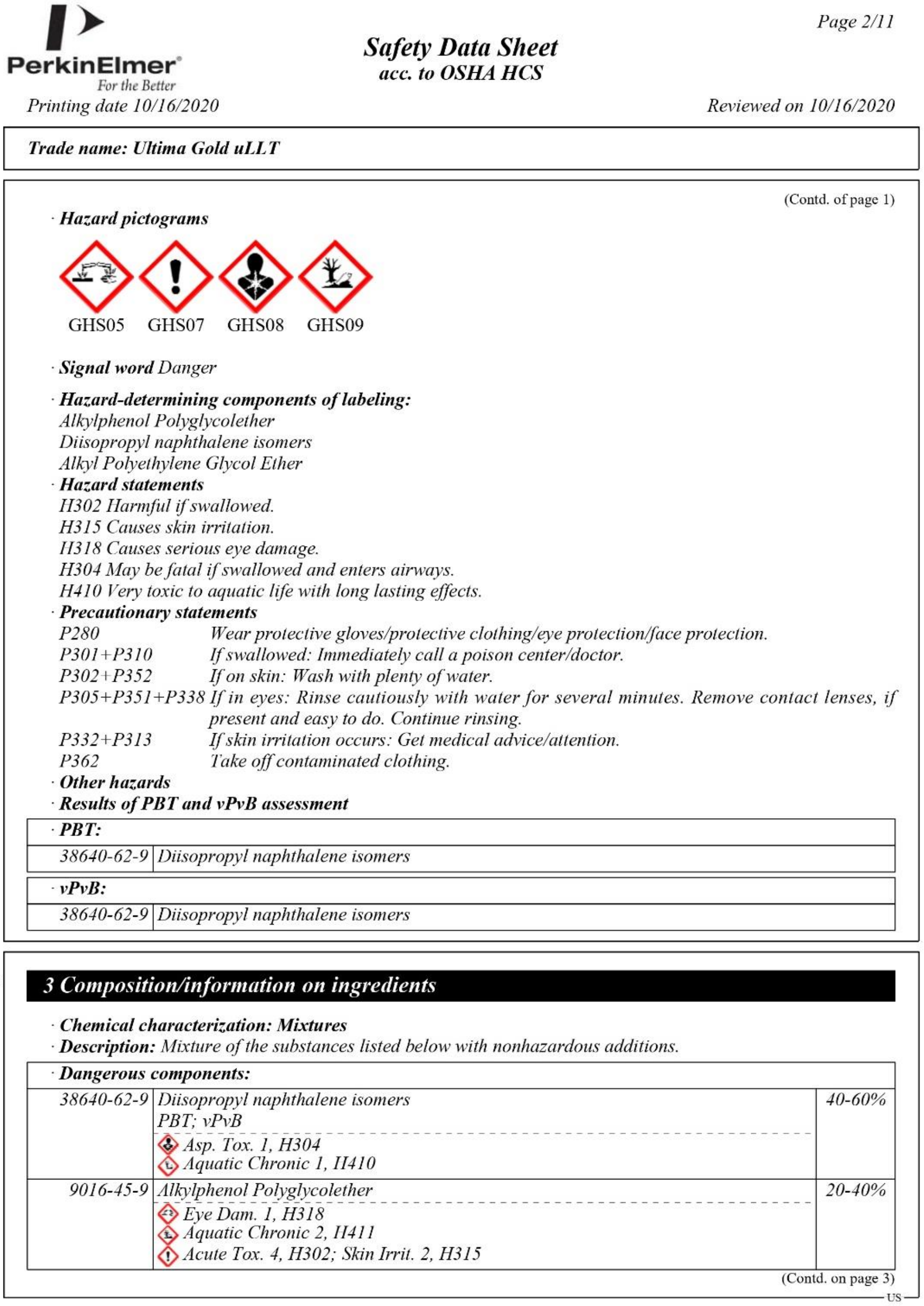
Acute Tox. 4 *H302 Harmful if swallowed.*
Skin Irrit. 2 *H315 Causes skin irritation.*

· ***Label elements***
· ***GHS label elements***
The product is classified and labeled according to the Globally Harmonized System (GHS).

(Contd. on page 2)

US

Figure A.3. Ultima Gold uLLT's safety data sheet, first page.



Page 2/11

PerkinElmer
For the Better

Safety Data Sheet
acc. to OSHA HCS

Printing date 10/16/2020

Reviewed on 10/16/2020

Trade name: Ultima Gold uLLT

(Contd. of page 1)

· **Hazard pictograms**

GHS05 GHS07 GHS08 GHS09

· **Signal word** *Danger*

· **Hazard-determining components of labeling:**
Alkylphenol Polyglycolether
Diisopropyl naphthalene isomers
Alkyl Polyethylene Glycol Ether

· **Hazard statements**
H302 Harmful if swallowed.
H315 Causes skin irritation.
H318 Causes serious eye damage.
H304 May be fatal if swallowed and enters airways.
H410 Very toxic to aquatic life with long lasting effects.

· **Precautionary statements**

P280	*Wear protective gloves/protective clothing/eye protection/face protection.*
P301+P310	*If swallowed: Immediately call a poison center/doctor.*
P302+P352	*If on skin: Wash with plenty of water.*
P305+P351+P338	*If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.*
P332+P313	*If skin irritation occurs: Get medical advice/attention.*
P362	*Take off contaminated clothing.*

· **Other hazards**

· **Results of PBT and vPvB assessment**

· **PBT:**	
38640-62-9	*Diisopropyl naphthalene isomers*

· **vPvB:**	
38640-62-9	*Diisopropyl naphthalene isomers*

*

3 Composition/information on ingredients

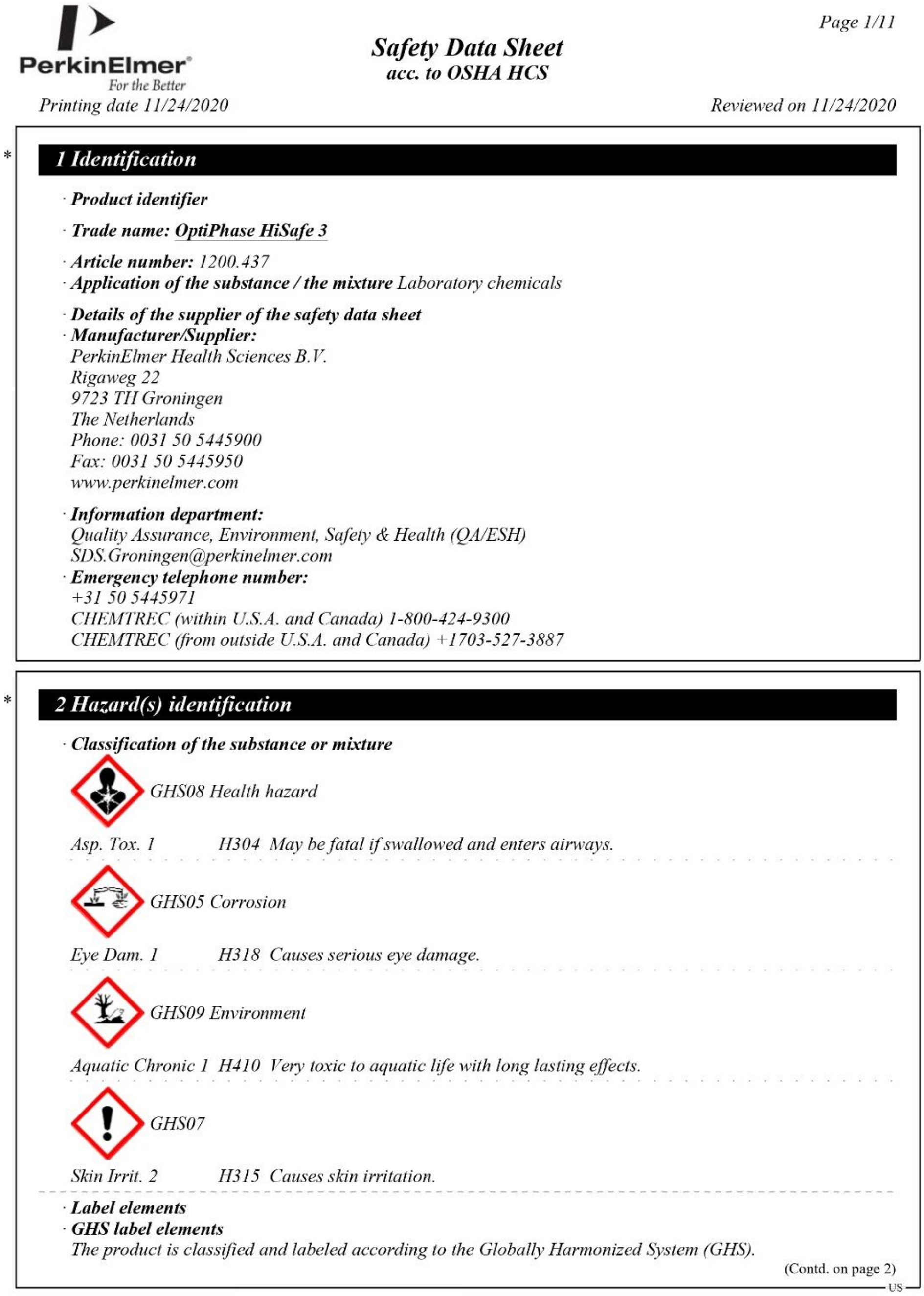
· **Chemical characterization: Mixtures**
· **Description:** *Mixture of the substances listed below with nonhazardous additions.*

· **Dangerous components:**		
38640-62-9	*Diisopropyl naphthalene isomers* *PBT; vPvB* *Asp. Tox. 1, H304* *Aquatic Chronic 1, H410*	*40-60%*
9016-45-9	*Alkylphenol Polyglycolether* *Eye Dam. 1, H318* *Aquatic Chronic 2, H411* *Acute Tox. 4, H302; Skin Irrit. 2, H315*	*20-40%*

(Contd. on page 3)

US

Figure A.4. Ultima Gold uLLT’s safety data sheet, second page.



PerkinElmer®
For the Better

Page 1/11

Safety Data Sheet
acc. to OSHA HCS

Printing date 11/24/2020 *Reviewed on 11/24/2020*

*

1 Identification

· ***Product identifier***

· ***Trade name: OptiPhase HiSafe 3***

· ***Article number:*** *1200.437*
· ***Application of the substance / the mixture*** *Laboratory chemicals*

· ***Details of the supplier of the safety data sheet***
· ***Manufacturer/Supplier:***
PerkinElmer Health Sciences B.V.
Rigaweg 22
9723 TH Groningen
The Netherlands
Phone: 0031 50 5445900
Fax: 0031 50 5445950
www.perkinelmer.com

· ***Information department:***
Quality Assurance, Environment, Safety & Health (QA/ESH)
SDS.Groningen@perkinelmer.com
· ***Emergency telephone number:***
+31 50 5445971
CHEMTREC (within U.S.A. and Canada) 1-800-424-9300
CHEMTREC (from outside U.S.A. and Canada) +1703-527-3887

*

2 Hazard(s) identification

· ***Classification of the substance or mixture***

GHS08 Health hazard

Asp. Tox. 1 *H304 May be fatal if swallowed and enters airways.*

GHS05 Corrosion

Eye Dam. 1 *H318 Causes serious eye damage.*

GHS09 Environment

Aquatic Chronic 1 H410 Very toxic to aquatic life with long lasting effects.

GHS07

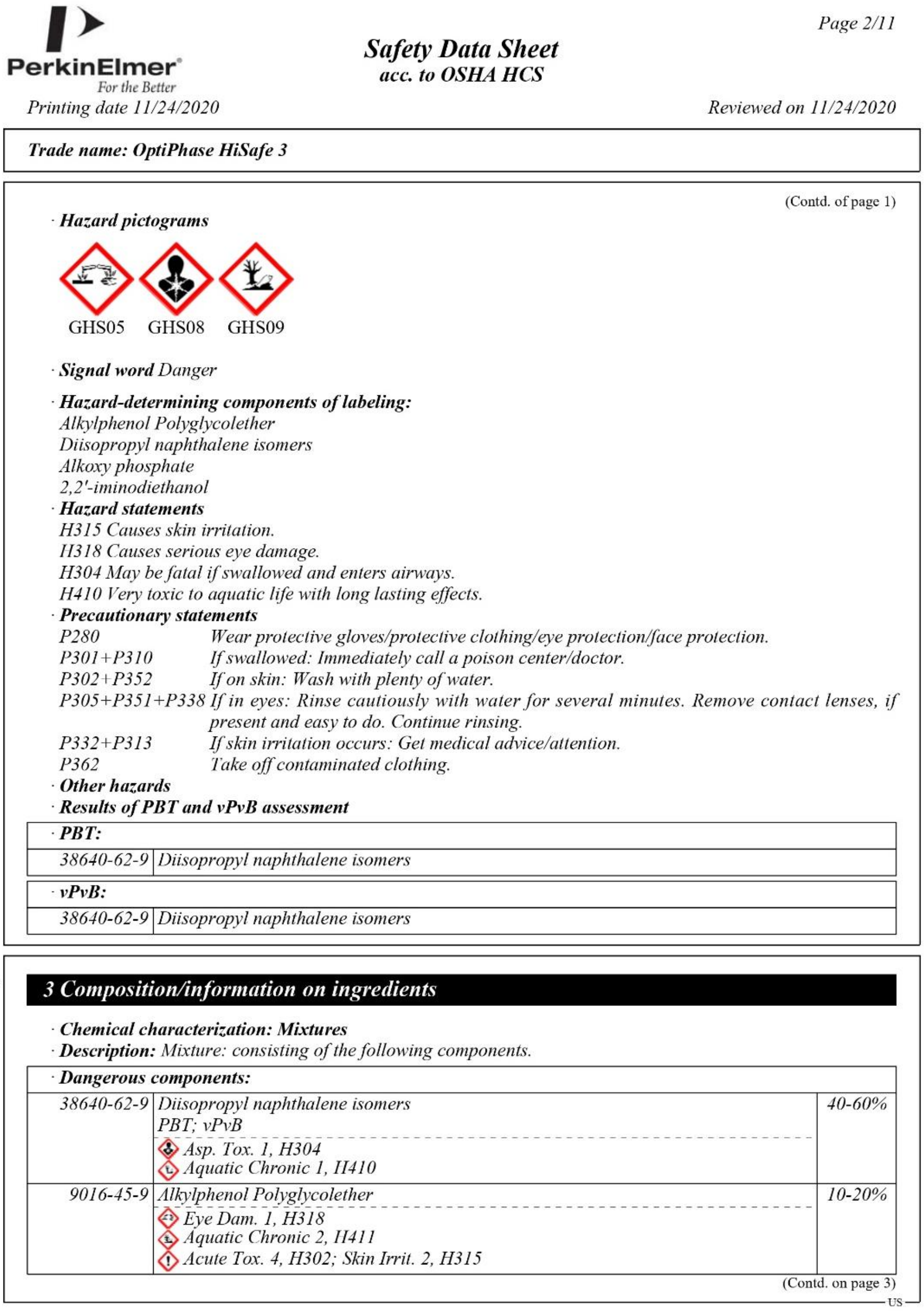
Skin Irrit. 2 *H315 Causes skin irritation.*

· ***Label elements***
· ***GHS label elements***
The product is classified and labeled according to the Globally Harmonized System (GHS).

(Contd. on page 2)

US

Figure A.5. Optiphase Hisafe 3's safety data sheet, first page.



Page 2/11

Safety Data Sheet
acc. to OSHA HCS

PerkinElmer
For the Better

Printing date 11/24/2020 *Reviewed on 11/24/2020*

Trade name: OptiPhase HiSafe 3

(Contd. of page 1)

· ***Hazard pictograms***

GHS05 GHS08 GHS09

· ***Signal word*** *Danger*

· ***Hazard-determining components of labeling:***
Alkylphenol Polyglycolether
Diisopropyl naphthalene isomers
Alkoxy phosphate
2,2'-iminodiethanol

· ***Hazard statements***
H315 Causes skin irritation.
H318 Causes serious eye damage.
H304 May be fatal if swallowed and enters airways.
H410 Very toxic to aquatic life with long lasting effects.

· ***Precautionary statements***

P280	*Wear protective gloves/protective clothing/eye protection/face protection.*
P301+P310	*If swallowed: Immediately call a poison center/doctor.*
P302+P352	*If on skin: Wash with plenty of water.*
P305+P351+P338	*If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.*
P332+P313	*If skin irritation occurs: Get medical advice/attention.*
P362	*Take off contaminated clothing.*

· ***Other hazards***
· ***Results of PBT and vPvB assessment***

· ***PBT:***	
38640-62-9	*Diisopropyl naphthalene isomers*

· ***vPvB:***	
38640-62-9	*Diisopropyl naphthalene isomers*

*

3 Composition/information on ingredients

· ***Chemical characterization: Mixtures***
· ***Description:*** *Mixture: consisting of the following components.*

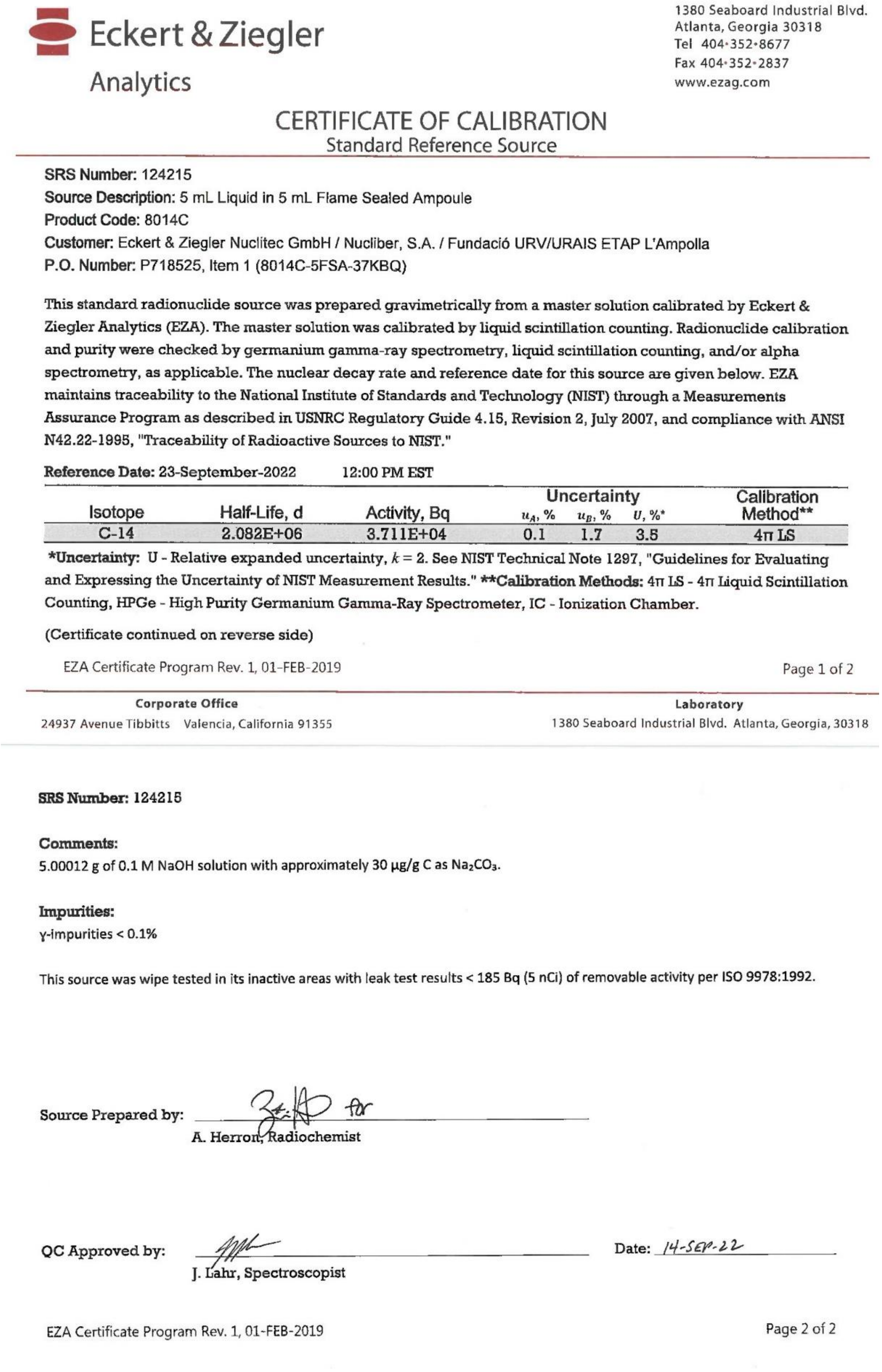
· ***Dangerous components:***		
38640-62-9	*Diisopropyl naphthalene isomers* *PBT; vPvB* *Asp. Tox. 1, H304* *Aquatic Chronic 1, H410*	*40-60%*
9016-45-9	*Alkylphenol Polyglycolether* *Eye Dam. 1, H318* *Aquatic Chronic 2, H411* *Acute Tox. 4, H302; Skin Irrit. 2, H315*	*10-20%*

(Contd. on page 3)

US

Figure A.6. Optiphase Hisafe 3's safety data sheet, second page.

9.2. Radiocarbon standard certificate



Eckert & Ziegler
Analytics

1380 Seaboard Industrial Blvd.
Atlanta, Georgia 30318
Tel 404-352-8677
Fax 404-352-2837
www.ezag.com

CERTIFICATE OF CALIBRATION
Standard Reference Source

SRS Number: 124215
Source Description: 5 mL Liquid in 5 mL Flame Sealed Ampoule
Product Code: 8014C
Customer: Eckert & Ziegler Nuclitec GmbH / Nucliber, S.A. / Fundació URV/URAIS ETAP L'Ampolla
P.O. Number: P718525, Item 1 (8014C-5FSA-37KBQ)

This standard radionuclide source was prepared gravimetrically from a master solution calibrated by Eckert & Ziegler Analytics (EZA). The master solution was calibrated by liquid scintillation counting. Radionuclide calibration and purity were checked by germanium gamma-ray spectrometry, liquid scintillation counting, and/or alpha spectrometry, as applicable. The nuclear decay rate and reference date for this source are given below. EZA maintains traceability to the National Institute of Standards and Technology (NIST) through a Measurements Assurance Program as described in USNRC Regulatory Guide 4.15, Revision 2, July 2007, and compliance with ANSI N42.22-1995, "Traceability of Radioactive Sources to NIST."

Reference Date: 23-September-2022 12:00 PM EST

Isotope	Half-Life, d	Activity, Bq	Uncertainty			Calibration Method**
			u_A, %	u_B, %	U, %*	
C-14	2.082E+06	3.711E+04	0.1	1.7	3.5	4π LS

***Uncertainty:** U - Relative expanded uncertainty, $k = 2$. See NIST Technical Note 1297, "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results." ****Calibration Methods:** 4π LS - 4π Liquid Scintillation Counting, HPGe - High Purity Germanium Gamma-Ray Spectrometer, IC - Ionization Chamber.

(Certificate continued on reverse side)

EZA Certificate Program Rev. 1, 01-FEB-2019 Page 1 of 2

Corporate Office
24937 Avenue Tibbitts Valencia, California 91355

Laboratory
1380 Seaboard Industrial Blvd. Atlanta, Georgia, 30318

SRS Number: 124215

Comments:
5.00012 g of 0.1 M NaOH solution with approximately 30 µg/g C as Na_2CO_3.

Impurities:
γ-impurities < 0.1%

This source was wipe tested in its inactive areas with leak test results < 185 Bq (5 nCi) of removable activity per ISO 9978:1992.

Source Prepared by: for
A. Herron, Radiochemist

QC Approved by:
J. Lahr, Spectroscopist

Date: 14-SEP-22

EZA Certificate Program Rev. 1, 01-FEB-2019 Page 2 of 2

Figure A.7. Eckert & Ziegler's C-14 standard certificate