^{14}C Measurements of samples containing biogenic materials with the help of liquid scintillation spectrometry.

Author

Dr. Ronald Edler

Revvity

Introduction

The use of biogenic materials is widespread and no longer limited to biofuels nowadays. The addition of biogenic materials to fuels had at least two reasons. On one hand, the limited fossil sources should be spared, on the other hand, the emission of CO_2 into the atmosphere from fossil sources should be reduced. The Kyoto protocol refers to the reduction of gas emissions, which result in global warming through the green-house effect. The reduction can be achieved by the increased use of alternative energy sources (wind energy, solar energy, etc.) but also by the addition of biogenic materials to fuels. The addition of biogenic materials to fuels is covered by the European Directive 2003/30/EC. The target of this Directive was to have at least 5.75% biogenic materials in fuels. This European Directive was replaced by the European Directive 2009/28/EC from 23rd April 2009. It sets a target of covering at least 20% of the European Community energy needs through renewable sources by 2020 and covering at least 10% of energy consumption from renewable sources in the transport sector. This does not only include bio-fuels but all renewable energy sources.

A particularly important point of the new European Directive is the avoidance of the production of bio-fuels from foods such as rapeseed, maize, sugar beet, palm oil or grain (1st generation bio-fuels) towards the use of organic waste and plant residues for the production of biofuels (2nd generation bio-fuels). Therefore biofuels will continue to play a very important role. For this reason, the German DIN organization (Deutsches Institut für Normung) prepared a new regulation DIN 51637[1]) in 2014 in order to offer a basis for the measurement of biofuels. In addition to fuels, bio-components are now playing an important role in all areas of daily life, and products are increasingly being marketed with the “Bio”-label. In many cases, consumers are willing to pay more when it comes to bio-products. This has led to increased development of such products and increased need for accurate bio-content measurements of products with liquid scintillation counting.

In addition to the fuels that still make up the majority of bio-products for LSC applications, the determination of the ^{14}C content is important in other products such as bio-plastics, bio-perfume, bio-waste, etc.

But where does the ^{14}C activity in bio-products come from? The following figure shows the formation and incorporation of naturally formed ^{14}C into organic matter.

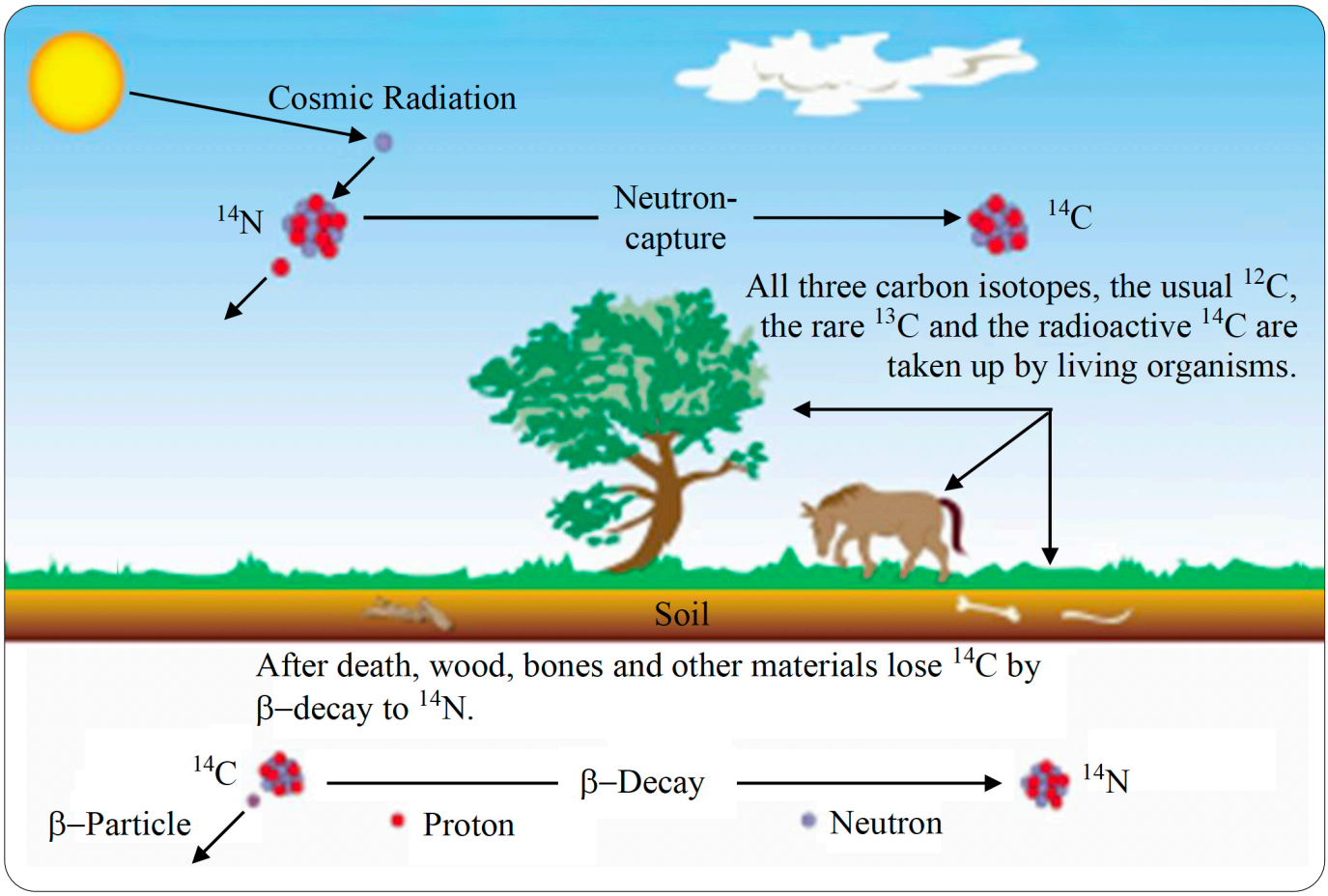


Figure 1: Formation and Uptake of ^{14}C into Organic Matter[2]

The formation of ^{14}C in the atmosphere and its incorporation into plants via photosynthesis is thus a natural process, leading to a possible detection of ^{14}C in biogenic substances in sensitive liquid scintillation counters. The incorporation of ^{14}C into organic matter is fairly constant, at least within a calendar year, but the ^{14}C level in the atmosphere is still slightly elevated and is only slowly decreasing. The reason is mainly based on surface nuclear bomb tests, which were carried out mainly from the second half of the 1960s. Since the ^{14}C level is still falling, corrections have to be made every year.

The following table from DIN 51637:2014-02 shows the activity of one gram pure carbon for the time frame 2004 to 2011. The reduction of the activity is obvious. Values after the year 2011 are based on extrapolations.[2)]

In contrast to the below table the ^{14}C activity of 1 g carbon was only 13.56 +/- 0.70 DPM/g carbon before the nuclear bomb tests.

This application note is intended to show how well various biogenic materials can be measured with very sensitive liquid scintillation counters, as is now the case in many laboratories and described in correspondingly many publications.[3-13)] This application note illustrates some examples for the measurement of bio-ethanol, bio-diesel (HVO and FAME), bio-thymol and bio-acetic acid.

Table 1: Activity of natural carbon

Year	DPM/g Carbon
2004	14.40
2005	14.34
2006	14.33
2007	14.24
2008	14.20
2009	14.19
2010	14.10
2011	14.09
2012	14.04
2013	13.96
2014	13.86
2015	13.83
2016	13.76
2017	13.73
2018	13.69

1. Bio-ethanol

$$CH_3 — CH_2OH$$

The measurement of bio-ethanol in spirits and fuels is meanwhile a routine application in many customers' laboratories. The following values for the measurement of bio-ethanol are averages from 5 measurements with a counting time of 60 minutes each for sample and background. All measurements were performed on a Quantulus GCT 6220 at 15°C.

Table 2: Ethanol measured in the window 0–156 keV, GCT = LOW

Sample	CPM	Net CPM	Efficiency
BKG	0.87	0.0	-
10 ml EtOH	52.89	52.02	89.3%
5 ml EtOH	28.34	27.47	94.2%
1 ml EtOH	6.08	5.21	94.1%
0,5 ml EtOH	3.54	2.67	93.9%
0,25 ml EtOH	2.25	1.38	92.4%

With the help of the SpectraWorks^2 software all energy windows have been optimized for maximum sensitivity to reach the highest E^2/B value. The following table shows the activity found in bio-ethanol in every gram of carbon. The calculation is based on a density of ethanol of 0.789 g/ml. The optimized energy windows varied between 2.5 – 48.5 keV for the sample with 10 ml Ethanol and 3.5 – 110 keV for the sample containing only 0.25 ml ethanol. All ethanol samples were previously measured with the Tri-Carb™ 3170TR/SL. The ethanol in these samples originates from the year 2006 and consequently contains more ^{14}C activity compared to ethanol from today's production. According to table 1 from DIN 51637 samples from 2006 should contain 14.33 DPM/g carbon.

10 ml ethanol contain 7.89 g ethanol and with a carbon content of 51.45% this results in a carbon mass of 4.06 g carbon. All below sample volumes were filled in the Quantulus™ GCT 6220 with Ultima Gold F to a total volume of 20 ml for measurement. For further information about cocktails please also read the corresponding application notes.[14] All DPM-values were determined with quench curves[15,16]

Table 3: Activity per gram carbon measured in bio-ethanol in the optimized window, GCT = Low

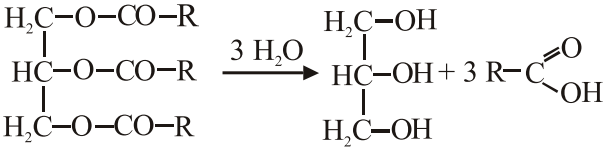
Sample	BKG	Net CPM	DPM/g C	Efficiency
10ml EtOH	0.73	50.00	14.35	85.8%
5ml EtOH	0.77	26.36	14.36	90.4%
1ml EtOH	0.78	5.10	14.16	88.7%
0,5ml EtOH	0.79	2.59	14.29	89.3%
0,25ml EtOH	0.80	1.33	14.51	90.3%

The activities obtained correspond very well to the value of 14.33 DPM/g of carbon from DIN 51637, the largest deviation was found in the sample with only 0.25 ml of ethanol, since the poor counting statistics also leads to a higher error for sensitive liquid scintillation counters. The activity of 97.1 Bq/l for the 10 ml sample had an uncertainty according to ISO 11929 of 2.7 Bq/l (or +/- 2.8%).

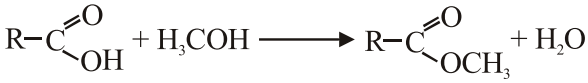
As expected, the best detection limit was also obtained for the 10 ml sample. The detection limit was 0.5 Bq/L with a measurement time of 300 minutes. This corresponds to a detection limit of 0.3 DPM / sample or 0.022 g bio-carbon in the form of ethanol. For $k_1-\alpha = k_1-\beta$ a value of 1.645 was used.

2. FAME and HVO

The biogenic additives for petrol can be obtained from bio-ethanol, e. g. ETBE (ethyl-tert-butyl ether) from isobutene and bio-ethanol, or directly from the addition of bio-ethanol. An important source is also biodiesel, which is obtained from natural fats and oils. Various oils are converted to biodiesel by esterification to make the natural oils useful for diesel engines. To produce biodiesel, transesterification is required in which the triglycerides are converted to fatty acid methyl esters in the presence of potassium hydroxide. As a byproduct, glycerin and potassium sulfate are obtained. The abbreviation FAME stands for Fatty Acid Methyl Ester and is an important component of many diesel fuels.

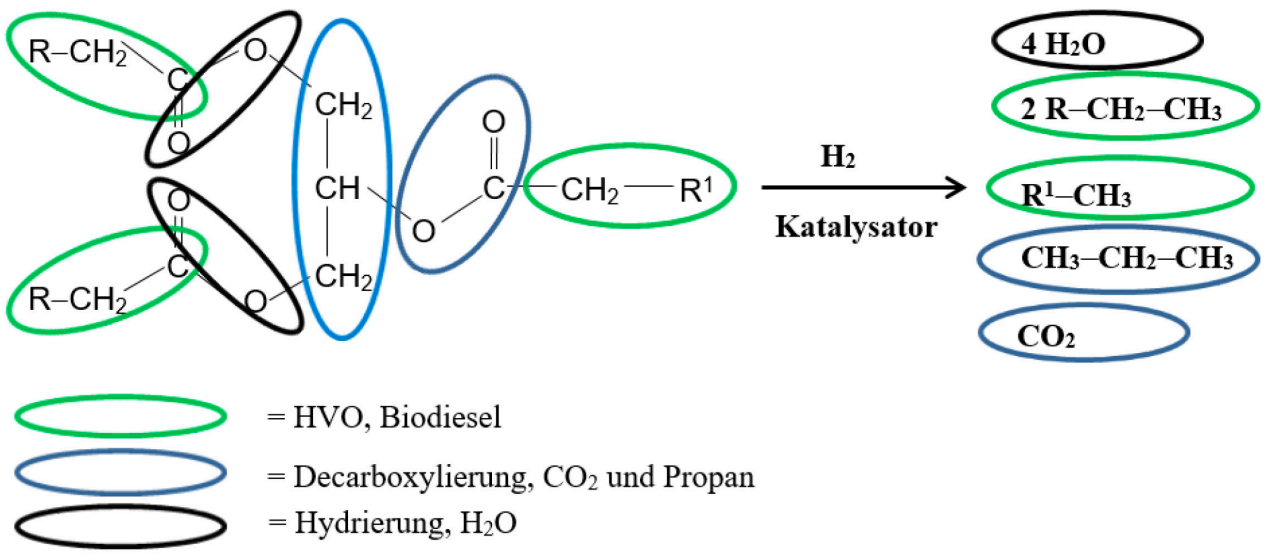


Triglyceride ⟶ Glycerine + Fatty acid



Fatty acid + Methanol ⟶ FAME

Another important additive for diesel fuels is HVO (Hydrogenated or Hydrotreated Vegetable Oil). It can be obtained from plant oils, but also from waste oils and other organic waste sources via catalytic hydration. Heteroatoms such as sulfur and nitrogen are removed as far as possible. The product forms almost colorless saturated hydrocarbons and as by-products H_2S, H_2O and NH_3. Waste can also be used for this process resulting in 2nd generation bio-fuels. The following diagram shows the principle production of HVO.



Production of HVO from Triglycerides in Vegetable Oils

Again Ultima Gold F was added to the HVO samples until a total volume of 20ml was reached. A fossil fuel was used as background source. The advantage of the measurement of HVO compared to bioethanol is the higher carbon content of HVO. In a first approximation an average formula of C_8H_{18} with a carbon content of 84.12% can be used for the measurements. The higher carbon content results in higher count rates even in small samples and consequently better counting statistics.

As mentioned before again the SpectraWorks[2] software was used to calculate the optimum counting window. As the quench determined for the HVO samples varied only minimally using the tSIE quench parameter, it was possible to assume an almost constant quench.

Table 4: HVO measurement in the window from 0–156 keV, GCT=Low

Sample	CPM	Net CPM	Efficiency
BKG	0.67	0.00	–
7.7014g HVO	83.98	83.31	94.50%
3.9039g HVO	43.24	42.57	94.41%
1.5714g HVO	17.84	17.17	94.24%
0.7576g HVO	8.77	8.10	94.20%
0.3884g HVO	5.07	4.40	94.24%

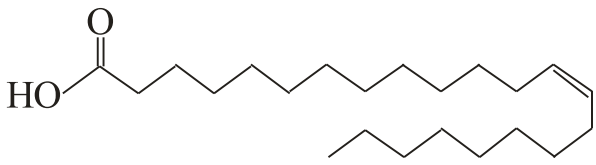
This results in identical counting windows for all samples. The optimum energy window was in the energy range from 20.5 – 113.5 keV. All values are averages from 5 measurements with 60 minutes counting time each.

Table 5: Activity per gram carbon in HVO in the optimized window, GCT = Low.

Sample	Net CPM	DPM/g C	Efficiency
BKG	0.35	0.00	–
7.7014g HVO	56.63	13.89	62.94%
3.9039g HVO	30.08	13.91	65.87%
1.5714g HVO	12.32	13.95	66.78%
0.7576g HVO	5.85	13.73	66.88%
0.3884g HVO	2.99	13.69	66.86%

Noticeable is the reduced activity per gram of sample compared to the bio-ethanol measurements. However, the HVO samples are from the year 2018 with an expected activity of approx. 13.69 DPM/g carbon, which again agrees very well with the obtained data. In the sample containing 10ml HVO (7.7014g) with an activity of 150 Bq/l +/- 4.1 Bq/l (+/– 2.7%), a detection limit of 0.45 Bq/l was achieved in a measurement counting time of 300 minutes. This corresponds to a detection limit of 0.27 DPM/sample or 0.02 g biocarbon.

For k_1-α = k_1-β a value of 1.645 was used. While HVO samples are almost colorless or only very slightly yellow in color, biodiesel from vegetable oils often show an intense yellow color which disturbs the quantification by color quenching. The presence of color reduces the counting efficiency and a correction is required. Frequently dilute solutions are measured to minimize the color quench, but this reduces the sensitivity of the measurement. In the Revvity laboratory in Groningen, successful attempts have been made to decolorize FAME samples and these investigations are described in detail in another application note.[13] The removal of color significantly reduced color quench and measurements gave very good results. With the new Quantulus GCT we now measured diluted samples of FAME, to find out, whether you can get accurate results without the elaborate decolorization. In the past, it has been shown that erucic acid methyl ester is a good model compound to describe the average composition of FAME.[13]



Erucic acid

Erucic acid is a component of many oils. Esterification with Methanol results in FAME. Erucic acid methyl ester has the empirical formula $C_{23}H_{44}O_2$ and thus a molecular weight of 352.603 g and a carbon content of 78.35%.

The amounts of FAME given in the following tables were measured in 20 ml Ultima Gold F. Each sample was measured with five repetitions of 60 minutes each. The measured values given in the tables are mean values from the five measurements.

Table 6: FAME measurement in the window 0-156 keV, GCT = Low

Sample	CPM	Net CPM	Efficiency	tSIE
BKG	0.87	0.0		
0.8639g FAME	8.52	7.65	88.11%	276.2
0.4303g FAME	4.92	4.05	92.31%	483.0
0.2241g FAME	3.03	2.16	94.98%	680.6

Table 7: FAME measurements in the optimized window, GCT = Low

Sample	CPM	Net CPM	Efficiency	tSIE
0.8639g FAME	0.78	7.28	12.8	83.85%
0.4303g FAME	0.75	3.65	13.0	83.19%
0.2241g FAME	0.78	1.92	13.0	84.43%

It is noticeable for FAME measurements, whose sample originated from 2018, that the measured activity per gram of carbon is lower than the expected value assuming that erucic acid methyl ester is a good model compound. However, these different values are quite constant for different dilutions. The deviation is approximately 5% to lower values. A strong influence of color quench can be excluded at least for the two samples with tSIE values of 483 and 680.6. However, it is conceivable that the model compound erucic acid methyl ester is not ideal for these FAME samples and can lead to an error of this magnitude. Indeed, erucic acid was a major component mainly in older rape seed varieties, which are barely grown today. Newer rape seed varieties do not contain much erucic acid anymore.

For example, the European rapeseed methyl ester used in Europe is more likely to have a carbon chain length of C16-C20 which, depending on the degree of saturation of the carbon chain, may account for only 75% of carbon. Table 8 shows DPM-values per gram carbon under this assumption.

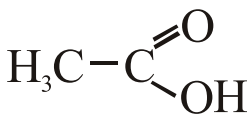
Table 8: Data as Table 7 but with 75% carbon content in FAME

Sample	CPM	Net CPM	Efficiency	tSIE
0.8639g FAME	0.78	7.28	12.8	83.85%
0.4303g FAME	0.75	3.65	13.0	83.19%
0.2241g FAME	0.78	1.92	13.0	84.43%

The measured activity of 167.5 Bq/kg +/- 20.8 Bq/kg (+/- 12.4%) had a detection limit of 3.1 DPM/sample or 0.22 g bio-carbon. Also mixtures of FAME and HVO can be analyzed with the help of LSC in combination with FT-IR, as described in DIN 51637. The LSC can determine the total ^{14}C activity in the mixture and FT-IR allows to determine the amount of FAME based on the quantitation of the ester carbonyl function. Once the concentration of FAME is known, the resulting ^{14}C-activity for FAME can be subtracted from the total activity to obtain the activity for the HVO content.

3. Acetic Acid

The determination of the acetic acid content can also be determined by the LSC measurement, if the vinegar was obtained from bio-production, for example from grapes. Acetic acid has the formula $C_2H_4O_2$ and a molecular mass of 60.052 g/Mol and a carbon content of 40.0%.



Acetic acid

All acetic acid samples were counted in Ultima Gold LLT liquid scintillation cocktail. The sample volume in the vial was made up to 20 ml each with above cocktail. The values in table 9 were obtained in the optimized windows which were determined again with the Spectraworks[2] software. The energy windows for the samples vary significantly due to the quench of acetic acid.

The used energy windows for the samples in table 9 from top to bottom are 2.0-31.5 keV, 7.0-57.0 keV and 10.5-81.5 keV. All acetic acid samples came from vinegar essence samples from 2018 and were bought in a supermarket. The 7.7815g acetic acid essence sample was measured in 14 ml Ultima Gold LLT, the other samples in 16 ml Ultima Gold LLT.

Table 9: Acetic acid measurement in the optimized window, GCT = Low

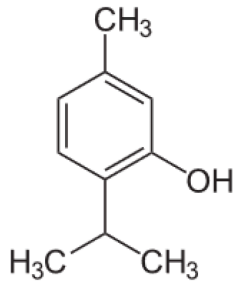
AcOH	BKG CPM	DPM	% AcOH	Efficiency
7.7815g	1.23	11.47	26.7	81.5%
5.1905g	0.89	8.35	28.3	72.5%
3.1088g	0.64	4.58	26.6	66.3%

For the calculation of the acetic acid content a value of 13.69 DPM/g carbon from table 1 was used.

According to the manufacturer, the acetic acid essence should contain 25% acetic acid. The precision and accuracy of this information from the manufacturer could not be determined, but the measured values confirm that this vinegar contains a concentration of this magnitude. The deviation is still within the uncertainty of the measurement. The counting time was 60 minutes and measurements were done in triplicates. The tables contains the averages. For the sample containing 7.7815g acetic acid the determined activity was 11.47 DPM with an uncertainty of 0.55 DPM (4.8%). The detection limit was 1.1 Bq/kg corresponding to 0.5 DPM/sample or 1.2% acetic acid content. Longer counting times allow for detection limits with 1% acetic acid or slightly below. The calculation is based on coefficients for errors of first and second kind $k_1-\alpha = k_1-\beta = 1.645$.

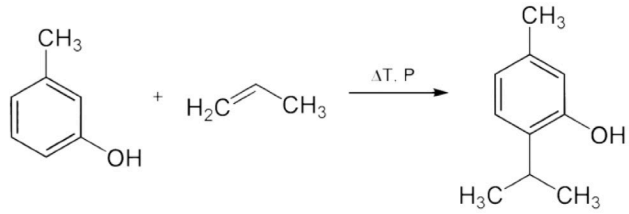
4. Thymol

Thymol is an aromatic compound with intense odor whose scent reminds of thyme and therefore finds application in the perfume industry. Thymol has the formula $C_{10}H_{14}O$ with a molecular mass of 150.22 g. The carbon content is 79.96%.



Thymol

Thymol can also be considered as a di-alkyl substituted phenol. The official IUPAC (International Union of Pure and Applied Chemistry) name is 2-(1-Methylethyl)-5-methyl-phenol. The substance can be obtained relatively simply and inexpensively, for example, by reaction of m-cresol (3-methylphenol) and propene:



The isolation of thymol from plants is time consuming and the cost of obtaining bio-thymol is many times higher than the synthesis from fossil petroleum products. Therefore, there is a great interest in being able to confirm the biogenic nature of the substance by a measurement. Unfortunately, the extraction of thymol from the plants produces an intense yellow-brown colored oil with strong color quench. Added to this is a certain chemical quench of the phenol. Even 1.2 g of completely pure, white crystalline, fossil thymol, which was used as a background sample, already showed a tSIE of 160 in 20 ml of cocktail and an open window of 0-156 keV and only resulted in 81.7% counting efficiency. Therefore, as with the FAME samples, attempts were made to measure smaller sample quantities in order to reduce color quench.

Helpful was that the strongly colored thymol oil formed at longer storage in the refrigerator thymol crystals with markedly weaker color. These crystals were used for the measurements. With a second recrystallization almost colorless crystals can be obtained. The measurements were done with the mentioned amounts of Thymol dissolved in 16 ml Ultima Gold F. The counting time was 60 minutes and all samples were measured in triplicates. All mentioned values are averages from triplicates.

Table 10: Thymol measurement in the open window 0-156 keV

Thymol	CPM	Net CPM	Efficiency	DPM/g C
1.2148g fossil	0.85	0.0	82.14 %	0.0
1.5776g biogenic	14.76	13.91	80.71 %	13.7

The tSIE-value of the fossil background sample was 160 and the tSIE-value of the biogenic sample 137.6. The Thymol sample originates from 2018 and the determined activity of 13.7 DPM per gram carbon is very close to the expected value of 13.69 DPM per gram carbon. Due to the strong quench the determined optimum energy window using the SpectraWorks^2 software was only from 3.0-26.5 keV. In this window the counting efficiency for the sample was 70.76% and the background was 0.56 CPM.

Table 11: Thymol measurement in the optimized window 3.0-26.5 keV

Thymol	CPM	Net CPM	Efficiency	DPM/g C
1.2148g fossil	0.56	0.0	71.8 %	0.0
1.5776g biogenic	12.94	12.38	70.76%	13.9

The activity of the biogenic Thymol sample was 17.5 +/- 0.3 DPM (1.7%) in the optimized window and again results in an activity per gram carbon very close to the expected value of 13.69 DPM per gram carbon.

The detection limit for 180 minutes counting time was 0.4 DPM for a sample containing 1.5776 g of substance or 0.03 g biogenic carbon. For k_1-α = k_1-β a value of 1.645 was used.

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