Supplementary Information

to the article

Comparison of derivatization methods for the quantitative gas chromatographic analysis of oils

Eliise Tammekivi[1,*], Signe Vahur[1], Ott Kekišev[1], Inez D. van der Werf[2], Lauri Toom[1], Koit Herodes[1], Ivo Leito[1]

[1]University of Tartu, Institute of Chemistry, Ravila 14a, 50411 Tartu, Estonia

[2]Cultural Heritage Agency of the Netherlands, Cultural Heritage Laboratory, Hobbemastraat 22, 1071 ZC, Amsterdam, The Netherlands

*Corresponding author: e-mail: eliise.tammekivi@ut.ee; tel: +372 562 34 473.

Contents

Study of the NaOEt quantity

Analyzing the chromatograms that were obtained with NaOEt and BSTFA derivatization confirmed that with this procedure every fatty acid yields two derivatives: the corresponding ethyl and trimethylsilyl esters. It has been tested how the amount of BSTFA affects the degree of derivatization [1]. However, to the best of our knowledge, the impact of the quantity of 0.01 M NaOEt in this two-step derivatization method has not yet been verified. Therefore, this was undertaken in this work. Ten aliquots of the triglyceride's mixture solution (450 µl each time) were derivatized the same way, just varying the quantities of the added reagents, and analyzed on the same day. Table 1 shows how the quantities of the added derivatization reagents affected the intensities of the peaks of the two derivatives.

Table 1. NaOEt followed by BSTFA derivatization of the solution of TAGs mixture with different reagent contents and analysis with GC-MS. [a]

Number of the solution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0.01 M NaOEt (µl) [b]	40	40	60	60	90	90	150	150	200	200
BSTFA (µl)	15	30	15	30	15	30	15	30	15	30
$(A_{EE} + A_{TMSE}) / A_{IS}$ [c]	6.58	6.59	5.55	5.63	5.31	5.97	5.18	5.21	5.42	5.95
$A_{EE} / (A_{EE} + A_{TMSE}) \times 100$ [c]	95.6	95.0	86.5	87.1	58.8	55.4	36.9	35.0	28.9	25.5

[a] 450 µl aliquots of the solution of tripalmitin, triolein, tristearin and trilinolein mixture were used. [b] The volume of used 0.01 M NaOEt corresponds to the volume of added saturated NH_4Cl solution. [c] A_{EE}: sum of peak areas of ethyl esters; A_{TMSE}: sum of peak areas of TMSEs; A_{IS}: peak area of the internal standard.

It was found that the content of BSTFA reagent does not have a noticeable effect on the results. However, the quantity of the 0.01 M NaOEt reagent has a significant impact. When using smaller quantities (40 µl), ethyl esters are mostly produced (approx. 95%), but with higher amounts of 0.01 M NaOEt (200 µl) mainly TMSEs are produced (approx. 74%). In addition, the overall area of ester peaks was higher with smaller amounts of NaOEt, indicating a higher derivatization efficiency. In comparison the original published procedure [2] added 250 µl of NaOEt for paint sample with various masses (0.25–2 mg), we used only 60 µl for the 1.0 mg of oil samples (the oil samples contained higher concentrations of analytes in the prepared stock solutions than TAG mixture).

Parameters of the GC and NMR instruments

An Agilent 5975C inert XL MSD with Triple-Axis Detector, connected to Agilent 7890A GC system with G4513A autosampler (Agilent) was used with Agilent DB-225MS capillary column (50% cyanopropylphenyl-50% dimethyl-polysiloxane). This column is 30 m long with a diameter of 0.25 mm and film thickness of 0.25 μm. The injection volume was 1 μl. The temperatures of the ion source and the mass spectrometer transfer line were 230 °C and 280 °C, respectively. The initial oven temperature was 80 °C, isothermal hold for 2 min, then increased at 10 °C/min to 200 °C with 4 min hold. Then increased once again at 5 °C/min to 220 °C, isothermal for 5 min and finally 10 °C/min to 230 °C. Total run time was 28 min, the mass spectrometer operated in the scan mode of 50–800 *m/z* mass range, the solvent delay was 5 min and electron ionization (EI) with 70 eV electrons was used. The GC inlet temperature was 300 °C and it was operated in splitless mode, split opened after 2 min. Helium 6.0 was used as carrier gas with flow of 3 ml/min. FID temperature was 300 °C, the H_2 flow 30 ml/min and air flow 400 ml/min. This method enabled the separation of all derivatized fatty acids, except for oleic acid and elaidic acid that were determined as a sum. This has also been done in previous studies [3–5] that have shown that in some cases, it is acceptable to report the quantity of octadecenoic acid as a sum of its *E* and *Z* isomers. For the quantitative analysis both the MS detector and FID were used. The obtained chromatograms were analyzed with Agilent MSD ChemStation and mass-spectra with NIST MS Search 2.0 database.

^{1}H NMR spectra were recorded on a Bruker Avance 700 spectrometer working at 700.1 MHz. NMR spectra were measured at 25.0 °C in CDCl3 (99.8% D + 0.03% TMS) and referenced to internal TMS (δ=0.00 ppm). The qNMR experiments were carried out with the following parameters: 90° pulse, prescan delay of 6.5 μs, 80k data points (corresponding to an acquisition time of 3.2 s at a sweep width of 12626 Hz), relaxation delay of 30 s, and a total of 32 scans. Fourier transformation was done after zero filling the data to 256k time domain points and exponential weighting of 0.1 Hz. Phase and baseline corrections were done manually. This manual mode was used also for the signal integration (generally without the 13C satellites).

Synthesis of fatty acid trimethylsilyl ester calibration standards

The trimethylsilyl esters (TMSE) of palmitic, stearic and oleic acid were synthesised as described by Noda and Bode [6]. After heating a Schlenk flask at 130 °C overnight, the flask was kept airtight and cooled in Ar flow. First, approximately 0.25 g of palmitic, stearic or oleic acid was added. Then, 0.7 ml of anhydrous MeCN and 0.7 ml of BSTFA were added to the

flask and the mixture was stirred in Ar flow for 3 h at 60 °C. Following, the volatiles were removed under vacuum at 60 °C during 3 h. The purities of synthesised palmitic acid TMSE (synthesis yield 79%, purity 99.1%), stearic acid TMSE (yield 47%, purity 95.4%) and oleic acid TMSE (yield 37%, purity 99.2%) were determined with the quantitative NMR (qNMR) method.

Quantitative NMR analysis of synthesised fatty acid trimethylsilyl ester

In the qNMR method, the purity of a substance (x) is calculated (equation 1) using an internal standard (std) and measuring the 1D ^{1}H spectrum of the mixture [7].

$$m_{(x)} = P_{(std)} \cdot \frac{MW_{(x)}}{MW_{(std)}} \cdot \frac{nH_{(std)}}{nH_{(x)}} \cdot \frac{m_{(std)}}{P_{(x)}} \cdot \frac{A_{(x)}}{A_{(std)}} \qquad (1)$$

In the equation, the *m* stands for the mass in g, *MW* is the molecular weight in g/mol, *P* is the purity, *nH* corresponds to the number of protons contributing to the signal for integration, *A* is the area for the selected peak. The peaks of the internal standard may not overlap with the peaks of the TMSE-s.1,4-Dimethoxybenzene fits this criterion, therefore, a solution of $CDCl_3$, 1,4-Dimethoxybenzene and one of the synthesised TMSE with known concentrations was made.

References

[1] B. Witkowski, A. Duchnowicz, M. Ganeczko, A. Laudy, T. Gierczak, M. Biesaga, Identification of proteins, drying oils, waxes and resins in the works of art micro-samples by chromatographic and mass spectrometric techniques, J. Sep. Sci. 41 (2018) 630–638.

[2] J.D.J. van den Berg, K.J. van den Berg, J.J. Boon, Determination of the degree of hydrolysis of oil paint samples using a two-step derivatisation method and on-column GC/MS, Prog. Org. Coat. 41 (2001) 143–155.

[3] J.D.J. van den Berg, Analytical chemical studies on traditional linseed oil paints, FOM-Institute for Atomic and Molecular Physics (AMOLF), Amsterdam, The Netherlands, 2002.

[4] F.C. Izzo, 20th century artists′ oil paints: a chemical-physical survey, Ca' Foscari University of Venice, Venice, Italy, 2010.

[5] A. Thomas, B. Matthäus, H.-J. Fiebig, Fats and Fatty Oils, in: Ullmanns Encycl. Ind. Chem., Wiley-VCH Verlag GmbH & Co. KGaA, 2000.

[6] H. Noda, J.W. Bode, Synthesis and chemoselective ligations of MIDA acylboronates with O-Me hydroxylamines, Chem. Sci. 5 (2014) 4328–4332.

[7] T. Rundlöf, M. Mathiasson, S. Bekiroglu, B. Hakkarainen, T. Bowden, T. Arvidsson, Survey and qualification of internal standards for quantification by 1H NMR spectroscopy, J. Pharm. Biomed. Anal. 52 (2010) 645–651.