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Trimethylsulfonium hydroxide as derivatization reagent for the chemical investigation of drying oils in works of art by gas chromatography

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Abstract

A procedure for the determination of fatty acids (FA) and glycerol in oils has been developed. The method includes a derivatization step of the FAs into their methyl esters or a transesterification of the triacylglycerols with trimethylsulfonium hydroxide (TMSH), respectively. The analysis is carried out by gas chromatography with parallel flame ionization and mass spectrometric detection. The parameters involved in the transesterification reaction were optimized. Only the stoechiometric ratio of TMSH:total FA amount showed a significant influence on the reaction yield. Relative standard deviations for 10 replicates were below 3% for all FAs studied and their linearity range was 0.5–50 mmol/L, when using heptadecanoic acid as an internal standard. The final procedure was rapid and required little sample handling. It was then tested on fresh oil samples and presented satisfying results, in agreement with previous works.

Keywords: Derivatization, GC; Art analysis; Oils; Trimethylsulfonium; Triglycerides; Fatty acid methyl esters

1. Introduction

Scientific investigations of materials used in works of art provide important information in order to gain a better knowledge of the technologies used by the artists but can also be useful in studies of provenance or authentication. Additionally, chemical analyses support conservators and conservation scientists with valuable information for preserving the objects.

Similar to carbohydrates, proteins, gums, resins and waxes, drying oils represent an important group of natural binders used in works of art. Natural drying oils such as linseed, poppyseed and walnut oil are composed of triglycerides, consisting of one molecule of glycerol linked to three fatty acids (FA). The identification of the oil is carried out by determination of the FA composition [1]. Among others [2], methods based on chemical analysis of FAs by gas chromatography (GC) have proven to be a powerful tool for the characterization of the composition of oils, typically after derivatization.

A number of sample preparation procedures have been suggested in the literature for converting the free FAs into their esters [3,4], such as silylation [5–9], or reaction with alkyl chloroformates [10–13] and for transesterification of the triglycerides [14–23], typically leading to the fatty acid methyl esters (FAMEs). Transesterification reactions are mainly one-step techniques including no or little sample preparation compared to other techniques [24,25].

Trimethylsulfonium hydroxide (TMSH) was introduced in 1979 as a methylating agent for carboxylic acid compounds [18]. Since that time, it has proven to be a powerful derivatization/transesterification reagent for the GC analysis of FAs in fats and oils [19,20,26,27]. Applied to thermally assisted hydrolysis and methylation gas chromatography (THM-GC), TMSH has also become a powerful reagent for pyrolysis–GC due to its good reaction yields and the fact that no time-consuming sample pre-treatment is required [21,22,28].

The present study concerns the establishment of a simultaneous derivatization technique for FAs and glycerol. One of the main advantages of this technique is the fast reaction and analysis time combined with only little sample manipulation. The relevant parameters have been investigated in order to obtain optimized response and reproducibility.

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2. Materials and methods

2.1. Chemicals

Ten of the most frequently encountered FAs in fresh and aged oils were considered, i.e. pelargonic acid (1C9:0)[1], suberic acid (2C8:0), myristic acid (1C14:0), azelaic acid (2C9:0), sebacic acid (2C10:0), palmitic acid (1C16:0), stearic acid (1C18:0), oleic acid (1C18:1), linoleic acid (1C18:2) and α-linolenic acid (1C18:3, in the following denoted only as linolenic acid). These compounds, as well as anhydrous glycerol and solvents, were all purchased from Fluka (Steinheim, Germany) with a purity of at least 97%, except linolenic acid which was a natural product (∼70% purity). Heptadecanoic acid (1C17:0) was used as an internal standard (I.S.) and was also obtained from Fluka, with a purity of 99%. Linseed oil, linseed stand oil, poppyseed oil and walnut oil were purchased from Kremer (Aichstetten, Germany). TMSH was obtained from Macherey–Nagel (Düren, Germany) as a 0.2 mol/L solution in methanol (denoted in the following as "TMSH reagent"). All chemicals and reagents were stored at 4 °C.

A stock solution containing a mixture of glycerol and the 10 studied FAs was prepared in chloroform, at a concentration of 50 mmol/L for each FA and 250 mmol/L for glycerol. For the calibration tests, standard solutions at concentrations ranging from 0.5 to 20 mmol/L for the FAs and from 10 to 100 mmol/L for glycerol were prepared from the stock solution. The concentration of the I.S. was fixed at 10 mmol/L.

2.2. Sample preparation

The procedure for sample preparation consisted in an in-vial derivatization/extraction technique at room temperature. Ninety microliters of chloroform were introduced into a vial and 10 μL of sample were added. After the introduction of 50 μL of TMSH reagent, the vial was slightly hand-shaken until a homogeneous solution was obtained. Chloroform was chosen as a solvent due to its superior solvency, especially for dried oils. It should be noted, however, that chloroform is a suspected carcinogen. Finally, 1 μL of the reaction mixture was injected into the GC. The FAs are methylated by TMSH reagent in basic medium, following the reaction described by Yamauchi et al. [17]. The products of the reaction are water, dimethyl sulfide [$(CH_3)_2S$] and the FAMEs. The final solvent composition of the reaction medium was chloroform–methanol (2:1, v/v), which is very suitable for the extraction of lipid compounds, such as triglycerides [22].

[1] The shorthand writing denotes the number of carboxylic acid groups (first digit), the carbon chain length (the one to two digits following "C") and the number of double bonds in the molecule (last digit).

2.3. Analytical equipment and conditions

The GC analysis of FAs and glycerol was carried out using a Hewlett-Packard HP-5890 Series II gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a split/splitless injector and an Agilent 6890 Series auto-sampler. Detection was performed by flame ionization detection (FID) operated parallel with mass spectrometry (MS) (MS Engine, HP-5989 A). Separation of FAMEs and glycerol was achieved on two DB-225 fused silica capillary columns (Agilent Technologies) 30 m × 0.253 mm i.d., 0.25 μm film thickness with a pre-column split. Helium (purity > 99.9996%) purchased from Messer-Griesheim (Gumpoldskirchen, Austria) was used as carrier gas, at a flow rate of 1.5 mL/min. In order to obtain a good separation for the FAMEs, chromatographic parameters were optimized resulting in the following: the temperature of the injector used in splitless mode was set at 200 °C, and the GC oven temperature was programmed as follows, 45 °C for 2 min, then to 180 °C at a rate of 15 °C/min, and finally to 210 °C at a rate of 3 °C/min. This program enabled a separation of the corresponding methyl esters of the 10 studied FAs and glycerol within 21 min. The mass spectrometer was used in electronic impact ionization mode (70 eV). Analyzer, source and interface temperatures were set at 100, 200 and 220 °C. The FID temperature was kept at 220 °C.

3. Results and discussion

3.1. Optimization of experimental conditions

To improve the reaction yield, experiments were carried out in order to determine the optimal quantity of TMSH reagent and study the influence of temperature and time of reaction.

3.1.1. Quantity of TMSH reagent

The quantity of TMSH necessary to obtain the maximum derivatization yield was investigated. The experiments were performed at six levels of TMSH reagent volumes (0, 5, 10, 25, 50, 100 and 150 μL), with three replicate experiments. TMSH reagent was added to 10 μL of a 50 mmol/L FA standard solution and an adequate volume of chloroform in order to keep the chloroform–methanol solvent ratio (2:1, v/v) unchanged. As shown in Fig. 1, the derivatization yield of all compounds increased rapidly until a volume of 50 μL of TMSH reagent was added, which corresponds to a TMSH:FA stoechiometric ratio of 2:1. When larger volumes of TMSH reagent were added, the derivatization yield did not increase significantly. Therefore, the optimal stoechiometric ratio of TMSH reagent to the total FA amount was fixed at 2:1.

3.1.2. Reaction time

The reaction time was optimized in order to obtain an optimal reaction yield. Additionally, an ascertainment

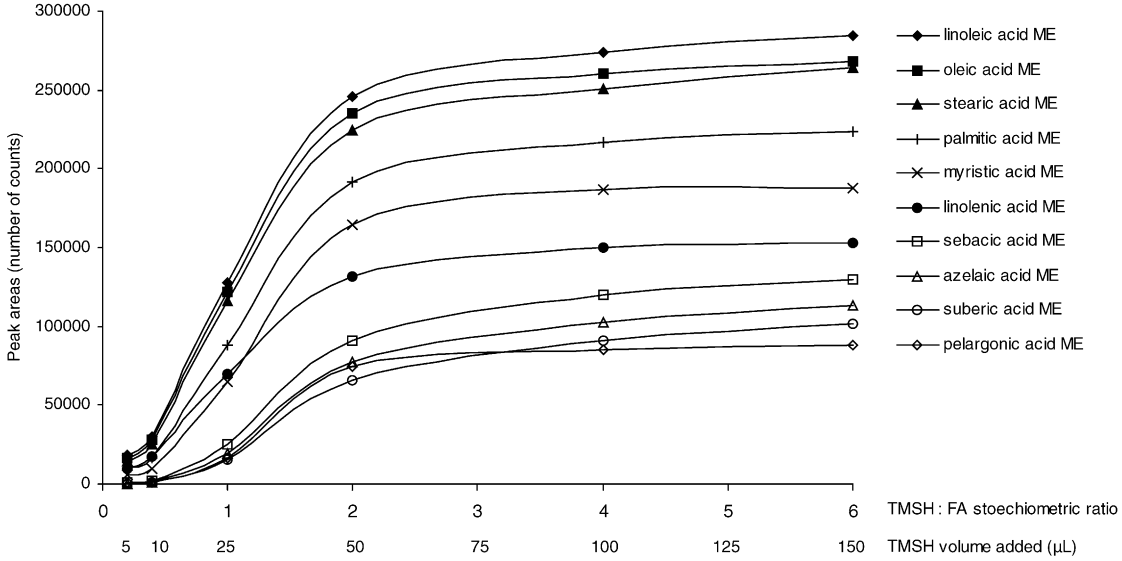

Fig. 1. GC–FID response as function of the TMSH:FA stoechiometric ratio. Up to 50 μL TMSH reagent, which corresponds to a stoechiometric ratio of TMSH:FA = 2:1, the derivatization yield shows a significant increase. All values are means of triplicate experiments.

of the stability of the FAMEs is required, as it would be impossible to inject all samples immediately after the reaction.

The samples were prepared as described previously and were then stored at room temperature for increasing times of 0, 10, 30, 50, 105, 160 and 210 min, and 24 and 48 h. This investigation was performed with a FA standard solution at an intermediate concentration level of 10 mmol/L, and with triplicate experiments.

The results of the experiments were evaluated by single factor analysis of variance (ANOVA) for all compounds of interest. In the range of 0–210 min reaction time, the ANOVA statistical test indicated that reaction time had no significant effect on the yield of derivatization, within a level of confidence $\alpha = 0.05$. After reaction times of 24 and 48 h, ANOVA evaluation showed that the reaction yield was slightly increasing for all compounds. The greatest increase, of 13% in reaction yield, was observed for linolenic acid over a period of 48 h. It was also noticed that the results for 24 and 48 h of reaction were not significantly different, according to ANOVA statistical test ($\alpha = 0.05$). Thus, no relevant improvement in the reaction yield justified a longer reaction time than 24 h.

Additional experiments were performed in order to assess the stability of the compounds obtained after derivatization. Analyses were carried out after mixing 90 μL chloroform, 10 μL of a FA standard solution at a concentration of 10 mmol/L and 50 μL TMSH reagent. The sample was injected immediately into the GC system. After 25 min storage at room temperature the sample was re-injected. The injection of the sample was repeated five times with constant intervals and a last injection was performed after 24 h storage. No significant variations in the GC responses were observed between the five first injections, performed after reaction times included in a range of 0–135 min. An increase of the response was observed for all compounds after a 24 h storage time. Depending on the compound, this increase was in the range of 18–23%. This confirmed the results obtained previously. Additionally, it was confirmed that no hydrolysis of FAMEs into FAs was observed, and therefore the stability of FAMEs was proven.

Although the derivatives were proven to be stable up to 1 day at room temperature, heptadecanoic acid was used as I.S. to improve quantitation. Consequently, variations in the reaction yield were minimized. Therefore, it is feasible to prepare a high number of samples within a short time, which is a prerequisite for high sample throughput analysis.

3.1.3. Temperature

The influence of an increase of the reaction temperature, from room temperature to 40 and 55 °C was investigated. The boiling points of methanol and chloroform are 64 and 61 °C, respectively and therefore, no higher temperatures were investigated. The experiments were performed by introducing the chemicals as described previously with a concentration level of the standard solution at 10 mmol/L for each FAs. After mixing by hand, a 20 min heating step was added, at the temperatures mentioned previously, and the samples were finally injected in the GC. The experiments were repeated three times at each temperature.

As for the study of the influence of reaction time, the results of the experiments were evaluated by ANOVA statistical test for all the FAs in the range of studied temperatures. With a level of confidence $\alpha = 0.05$, no significant variation on the yield of reaction was observed while increasing the temperature. Hence, the temperature of

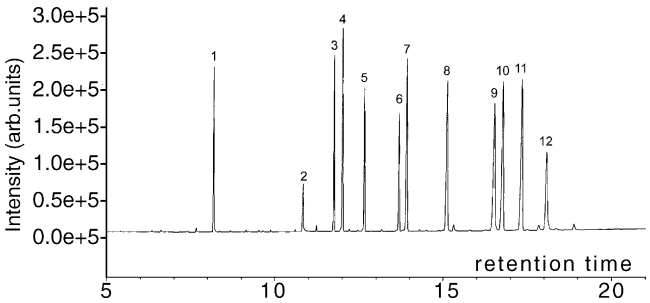

Fig. 2. GC–FID chromatogram of the analysis of FAs and glycerol in a standard solution. All FAs are at a concentration level of 10 mmol/L and glycerol is at a concentration level of 50 mmol/L. (1) Pelargonic acid ME; (2) glycerol; (3) suberic acid di-ME; (4) myristic acid ME; (5) azelaic acid di-ME; (6) sebacic acid di-ME; (7) palmitic acid ME; (8) I.S.; (9) stearic acid ME; (10) oleic acid ME; (11) linoleic acid ME; (12) linolenic acid ME. Time scale in minutes.

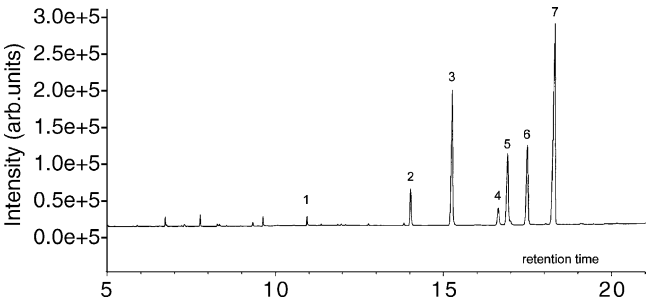

Fig. 3. GC–FID chromatogram obtained for the analysis of fresh linseed oil. (1) Glycerol; (2) palmitic acid ME; (3) I.S.; (4) stearic acid ME; (5) oleic acid ME; (6) linoleic acid ME; (7) linolenic acid ME. Time scale in minutes.

reaction did not have a significant effect on the yield of FA derivatization.

3.2. Analytical figures of merit

A typical chromatogram obtained for the analysis of FAs and glycerol in a standard solution is shown in Fig. 2. The separation of the 10 FAMEs and the glycerol was achieved in less than 19 min. A minimal resolution of 3.5 was obtained between sebacic and palmitic acid methyl esters.

In order to test the performance of the developed method, the linearity and the repeatability were estimated by applying the conditions established previously. The linearity of the method was evaluated at seven concentration levels, ranging from 0.5 mmol/L to 50 mmol/L for each FA standard. The repeatability was determined at a concentration level of 10 mmol/L with 10 replicate experiments for GC–FID and GC–MS analyses.

As the repeatability and sensitivity of FID is superior to MS detection, data are presented only for the former. In the case of FID, the relative standard deviation (R.S.D.) values were below 3% for all FAs proving that the repeatability of the method was very satisfying. Moreover, all compounds showed a good linearity in the range of 0.5–50 mmol/L with correlation coefficients greater than 0.999. For glycerol, the standard deviation equal to 9.3% was slightly higher. The calibration curve for this latter compound is fitted with a polynomial equation with a correlation coefficient of 0.9996. In conclusion, the results of these experiments were judged satisfying enough to enable the further application of the method to the determination of FAs and glycerol in fresh oils.

3.3. Analysis of fresh oils

An evaluation of FA concentrations was carried out in four fresh oils typically employed as binding media in paintings. Triplicate analyses were performed in fresh linseed oil, linseed stand oil, poppyseed oil and walnut oil samples. Prior to the derivatization step, the samples were diluted in chloroform (oil–chloroform, 1:50, v/v). Then, 80 μL of chloroform, 10 μL of the I.S. solution and a 10 μL aliquot of a diluted oil were introduced into the reaction vial. Afterwards, 50 μL of TMSH reagent were added, and immediately after mixing by hand, the solutions were injected into the GC system. The chromatogram corresponding to the analysis of the fresh linseed oil is shown in Fig. 3. The FAME concentrations measured in each fresh oil are presented in Table 1. Standard deviations did not exceed 5% for each compound, except glycerol in linseed stand oil (6.3%).

It was tested whether a larger amount of TMSH would be required in this case compared to the derivatization of free FAs. Therefore, three samples of analyzed fresh oils were selected and an additional volume of 50 μL TMSH reagent was added to each vial. They were then injected a second time into the GC. As the R.S.D. values of the pooled data sets did not increase significantly over the R.S.D. values of the two individual data sets, this was taken as a proof of the two derivatization yields being statistically equivalent. The results of these latter analyses showed that the yield of derivatization of FAs in fresh oils was not affected by the addition of a large excess of TMSH reagent.

In the field of art conservation and restoration, the identification of the nature of the oil used as binding media in paint samples is useful. For that purpose, different indicators were investigated, e.g. the palmitic acid to glycerol molar ratio (P/G ratio) and also the palmitic acid to stearic acid molar ratio (P/S ratio). To date, this latter ratio is still the most employed one and was proved to be a good indicator of the nature of the oil present as binding medium [1,16,29]. The P/S ratios were evaluated to be 1.49, 4.67 and 2.95, and the P/G ratios were estimated as 0.10, 0.20 and 0.14, respectively in linseed, poppyseed and walnut fresh oils. These values were in good agreement with the ratios published by other research groups [1,16,29]. This comparison indicated that the developed method allowed measuring the FA composition of fresh oils with a reliability equivalent to that of other methods currently applied to the determination of FAs in art samples.

Table 1
FAs and glycerol concentrations measured in linseed oil, linseed stand oil, poppy seed oil and walnut oil

FAMEs	Linseed oil			Linseed stand oil			Poppyseed oil			Walnut oil		
	Concentration (mmol/L)	R.S.D. ($n = 3$) (%)	Composition[a] (%)	Concentration (mmol/L)	R.S.D. ($n = 3$) (%)	Composition[a] (%)	Concentration (mmol/L)	R.S.D. ($n = 3$) (%)	Composition[a] (%)	Concentration (mmol/L)	R.S.D. ($n = 4$) (%)	Composition[a] (%)
Palmitic acid ME	3.1	1.5	4.5	4.0	0.8	17.8	6.3	1.8	12.5	5.3	2.5	7.9
Stearic acid ME	2.1	1.1	3.1	2.5	0.8	11.2	1.4	0.7	2.7	1.8	4.4	2.7
Oleic acid ME	8.5	1.9	12.3	13.0	1.1	57.6	8.8	1.4	17.4	10.1	2.3	15.1
Linoleic acid ME	7.8	2.1	11.3	1.5	1.1	6.8	32.4	1.8	64.1	35.5	2.0	53.2
Linolenic acid ME	46.6	2.2	67.7	1.5	3.5	6.6	1.7	1.5	3.4	14.1	1.2	21.1
Glycerol	30.4	3.3	–	26.6	6.3	–	32.2	3.3	–	38.3	3.7	–

[a] Oil composition given as molar ratio.

4. Conclusion

In this study, a method for the simultaneous analysis of FAs and glycerol by GC was developed and optimized for its application to routine FA analysis. The sample preparation is rapid, little manipulation is involved and no special equipment required. It is based on a one step hydrolysis and derivatization reaction performed by using TMSH as methylation reagent at room temperature. The linear range of the method was between 0.5 and 50 mmol/L for the 10 FAs studied in this survey and the standard deviations were below 3%. The good correlation between the concentrations of FAs determined in four fresh oils by this study and by other research groups [1,16,29] demonstrated that the developed method could be successfully applied to oil samples. Due to its rapidity and its low requirement in sample handling, this method appears to be the method of choice for high sample throughput analysis of FAs and glycerol in oils. Hence, it will be applied in forthcoming studies in order to estimate the content of FAs and the evolution of their distribution in artificially aged paint samples.

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