Identification of non-cross-linked compounds in methanolic extracts of cured and aged linseed oil-based paint films using gas chromatography–mass spectrometry

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Abstract

Methanolic extracts of paint samples of different composition and age were qualitatively investigated by GC–MS using an on-column injector after off-line methylation or trimethylsilyl derivatisation, and on-line thermally assisted (trans)methylation with tetramethylammonium hydroxide using Curie-point pyrolysis–GC–MS. The combination of these three analytical strategies led to the identification of typical oxidation products of unsaturated fatty acids by interpretation of their mass spectrum. Some of the identified compounds have not been reported before. Both the off-line and on-line GC–MS strategy show series of short-chain fatty (di)acids and C_{16} and (oxidised) C_{18} fatty acids. The major advantage of the on-line pyrolysis–GC–MS approach is that chemical work-up is minimal and very quick. With this technique both the carboxylic acid functionalities, and hydroxyl groups are methylated. Young paint films are shown to contain relatively more oxidised C_{18} fatty acids and less diacids compared to older paints, which is indicative for the on-going oxidation processes within the paint. After trimethylsilylation, monoacylglycerols are detected indicative for hydrolytic processes, which reflect the relative distribution of the most prominent silylated fatty acids present. Relatively more C_{16} and C_{18} monoacylglycerols are found in young paints, whereas older paints contain higher amounts of monoacylglycerols of diacids.   © 2002 Published by Elsevier Science B.V.

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1. Introduction

It is known since the 11th and 12th century descriptions by Theophilus that certain vegetable oils (linseed, poppy and walnut) are capable of forming an elastic and insoluble paint film [1]. These oils, which are still used today for painted works of art or in a modified form in 20th century household alkyd paints, are called drying oils because of their ability to dry chemically, i.e. to cross-link to a semi-solid.

The chemical drying of oil is a result of auto- and/or photoxidation [2,3] of the doubly and triply unsaturated fatty acid moieties (linoleic and linolenic acids, respectively) that are present in high amounts in these oils (>65%) [4]. The subsequent free radical chain reactions give rise to a variety of cross-linked

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materials [5–7] and degradation products that are partially lost by evaporation [8–10].

Furthermore, partial hydrolysis of ester bonds of the triacylglycerols (TAGs) occurs, leading to free fatty acids. Part of these free acids will react with pigments or driers present in the paint and metal salts will be formed [11]. This leads to a paint system consisting of a solvent removable fraction (the so-called "mobile" phase) and a cross-linked polymeric system (the "stationary" phase) [12,13]. The mobile phase includes oxidised- and de-esterified fatty acids, glycerol, mono-, di- and triacylglycerols and oligomeric structures. Since every oil paint has its own characteristic formulation, history of storage, restoration and cleaning and age, the ratio and chemical composition of both phases will be different.

The process of film formation has been the subject of elaborate analytical chemical investigations. Information on the fundamental mechanisms is available on cross-linking, the different types of cross-links, the types of functional groups present in the polymeric system and the size of the cross-linked material. These studies include (high-performance) size-exclusion chromatography [14,15], different types of mass spectrometry [7], (Fourier transform) infrared spectroscopy [16–18], and swollen or solid state ^{13}C NMR [19,20]. However, these methods only provide general information on the structure and size of the high-molecular-mass material and almost no detailed structural information due to the complexity of the material and insufficient resolution of the analytical techniques applied.

The analysis of the low-molecular-mass fraction of oil paint samples gives more detailed structural information. One of the common analytical techniques for the analysis of this fraction is gas chromatography, often combined with off-line chemical derivatisation [21–26].

Surprisingly, the authors of these studies on paint samples only used reagents capable of methylating carboxylic acid groups. Other polar functional groups, like hydroxy, keto or epoxy groups that are expected to be formed upon oxidation seemed to have been overlooked or at least have not been reported. Furthermore, triacylglycerols nor their hydrolysis/oxidation products seem to be present, whereas in tempera-based paints these compounds were identified [13]. Only recently, two keto-substi-

tuted oxidised fatty acids were reported to be present in aged oil paint [27] and simultaneous analysis of fatty acids, mono-, di- and triacylglycerols present in extracts with high-temperature GC–MS [28] was reported. No oxidised acylglycerols were detected despite the age of the paint sample (18th century). The authors, however, did report the presence of fully saturated triacylglycerols, which is remarkable and may be explained by interesterification or contamination since these compounds are not present in fresh linseed oil [29,30]. In another recent study, an extended range of (oxidised) mono- and diacylglycerols was identified in solvent extracts from relatively young oil paints by LC–MS [31].

In this paper, the results are described of three different analytical strategies to qualitatively characterise the extractable fraction of an oil paint sample. As extraction solvent, methanol was chosen because of its high polarity and the ability to extract oxidised fatty acids, acylglycerols and polar oligomeric material. Methylation of all free carboxylic acid groups using trimethylsilyl-diazomethane is one of the two off-line derivatisations applied. Other functional groups are not derivatised under the conditions used. Methylation of hydrolysed oil paint or oil paint extractables has been used most often in conservation science [21,23,26]. The results are compared to off-line trimethylsilyl (TMS) derivatisation of the extract using bis(trimethylsilyl)trifluoroacetamide. This derivatisation reagent transforms free carboxylic acid groups and hydroxyl groups of both acylglycerols and oxidised fatty acids into their TMS derivatives [32]. This method is occasionally used for the analysis of paint samples [33].

On-line pyrolysis (Py) and the subsequent introduction of the pyrolysate into a chromatographic system has been shown to be suitable for the analysis of the organic part of alkyd and oil paint samples [34–37] without extensive chemical work-up. Only the addition of low amounts of aqueous or methanolic solutions of quarternary ammonium salts like tetramethylammonium hydroxide (TMAH) is needed for the on-line derivatisation [35,38–40]. This reagent transmethylates all ester bonds and methylates all free acids and partially also hydroxyl groups, depending on the pK_a [41]. Although both low- and high-molecular-mass materials can be investigated using this method, in this case only the extractable

fraction was analysed in order to compare the results with the two previously mentioned methods.

In this study, the results of the analyses of extracts of a relatively young 5-year-old stand oil film without pigmentation, a 26-year-old lead white pigmented oil paint and a 373-year-old paint material of an unknown composition are reported. These three samples were selected from a much larger survey study. The main objective of this study is to obtain a better qualitative chemical picture of the composition of these paint films by identification of most of the compounds present in the extractable fraction and to relate the results to the age of the paint material. It may be clear that a more systematic study is needed to identify quantitative differences and changes in ageing oil paint systems in more detail [22,24,42].

2. Experimental

2.1. Chemicals

Tetramethylammonium hydroxide pentahydrate (minimum 97%), and (trimethylsilyl)diazomethane (TMS-diazomethane; in hexane, 2 M) were obtained from Aldrich (Zwijndrecht, Netherlands). Deuterated tetramethylammonium hydroxide pentahydrate (98%) was supplied by Cambridge Isotope Labs. (Andover, MA, USA). Bis(trimethylsilyl)trifluoro-acetamide (BSTFA; ≥99%) was purchased from Fluka (Zwijndrecht, Netherlands). Cremnitz white (basic lead carbonate or lead white) was supplied by Old-Holland Classic Oilcolours (Driebergen, Netherlands).

2.2. Paint films/samples

A stand oil (linseed oil that has been prepolymer-ised by heating to 280 °C) (Talens, Apeldoorn, Netherlands) film of about 0.5 mm thickness was made by spreading out oil on a glass slide. This test paint was stored under room conditions on the window-sill at our institute for 5 years. This typical sample was chosen since oils used to be prepolymer-ised quite often according to historical sources [43].

The lead white pigmented film was made in 1973 by H.-C. von Imhoff by grinding basic lead carbon-ate (Schmincke) with linseed oil (Mühlfellner-Rupf,

Zurich, Switzerland) in such a ratio that a workable paint was obtained. The paint was applied on primed linden wood and was hung up at the Canadian Conservation Institute (CCI), Ottawa, under room conditions. Samples were taken by carefully scraping off the paint layer and homogenised prior to use.

The old paint was sampled from a fragment of a canvas painting from 1626 made by an unknown artist, and supplied by the Rijksmuseum (Amster-dam, Netherlands). The (restoration) history of this painting is unknown. The paint system consists of six layers with a total thickness of 0.3 mm, including a red ochre ground and a varnish layer. The third and fourth layers contain azurite with smaller amounts of black and white particles. The second layer contains lead white. The paint mixture was obtained by carefully scraping off the mixture of thin paint layers. The powder was homogenised prior to analy-sis by extensive grinding.

2.3. Methylation derivatisation procedure (off-line)

An extract of the paint sample was obtained by repetitive immersion of the paint film in methanol. After evaporation of the combined extracts to dry-ness, for every milligram of sample, 40 µl benzene and 10 µl methanol are added. The dissolved sample is methylated by addition of 5 µl of a 2.0 M TMS-diazomethane solution in hexane. After 5 min, the reaction mixture was dried under a gentle stream of nitrogen and immediately dissolved in dichloro-methane (DCM), either with or without hexadecane as internal standard (50 ng/µl). It is important not to dry too long or under extreme conditions in order to avoid loss of relatively volatile components as much as possible.

2.4. Trimethylsilylation derivatisation procedure (off-line)

The extractable material was collected as previ-ously described. The solvent was dried under a gentle stream of nitrogen and dissolved in 250 µl hexane (for every milligram of extractable material). Subsequently, 13 µl/mg of BSTFA was added and the mixture was kept at 75 °C for 60 min. Every 15 min, the reaction vial was thoroughly shaken. Imme-diately after evaporation to dryness the residue was

dissolved in DCM, either with or without hexadecane as internal standard (50 ng/μl).

2.5. GC–MS with on-column injection

A 1-μl aliquot of derivatised sample was directly injected into a SGE BPX5 column (25 m×0.32 mm I.D., 0.25 μm film thickness) using an AS 800 on-column autoinjector (Fisons Instruments). The GC–MS system and conditions are the same as described in Section 2.6 unless otherwise stated. In a number of cases more than one sample of the same paint was prepared and analysed in order to test reproducibility. For the paints investigated, similar chromatograms were obtained with minor differences in the relative intensities.

2.6. TMAH derivatisation–Curie-point Py–GC–MS

About 15 μl of the extracted material was applied onto a rotating 610 °C Curie-point wire and 3–4 μl of a 2.5% aqueous solution of TMAH was added before the sample was dried in vacuo. The ferromagnetic wire was inserted in a glass liner, flushed with argon to remove air and subsequently placed into the pyrolysis unit. Curie-point pyrolysis was performed with a FOM 5-LX pyrolysis unit [44]. The ferromagnetic wire was inductively heated for 6 s in a 1 MHz radio frequency (RF) field to its Curie-point temperature (610 °C). Pyrolysis fragments were flushed (splitless) into a SGE BPX5 column (25 m×0.32 mm I.D., 0.25 μm film thickness) mounted in a Carlo-Erba gas chromatograph (series 8565 HRGC MEGA 2) which was coupled directly to the ion source of a JEOL DX-303 double focusing (E/B) mass spectrometer via a laboratory-built interface which was kept at 280 °C. Helium was used as carrier gas at a flow-rate of approximately 2 ml/min as regulated with a CP-CF 818 pressure/flow control box (Fisons Instruments). The initial temperature of the gas chromatograph was 50 °C which was maintained for 2 min. The oven temperature was programmed with a ramp of 6 °C to an end temperature of 320 °C [50(2)–6–320]. Ions were generated by electron impact ionisation (70 eV) or chemical ionisation using isobutane at a pressure of 10^{-3} Pa in the ionisation chamber (180 °C), accelerated to 3 keV, mass separated and postaccelerated to 10 keV

before detection. The mass spectrometer was scanned from m/z 40 to 700 with a cycle time of 1 s. A Jeol MP-7000 data system was used for data acquisition and processing. The compounds were identified based on their 70 eV electron impact mass spectrum [45–47].

3. Results and discussion

3.1. Off-line methylation combined with on-column injection and GC–MS

The GC–MS total ion current (TIC) trace (Fig. 1) of the methylated methanol extract of the 5-year-old stand oil film shows short-chain fatty acids (C_7–C_{10}), diacids (C_7–C_{11}), saturated long-chain fatty acids (C_{16}–C_{18}, C_{20}–C_{22}), a cyclic C_{18} fatty acid and some unsaturated and/or oxidised C_{18} fatty acids (see Table 1 for full list). These compounds could be identified by interpretation of their 70 eV mass spectrum or comparison with published spectra in literature or mass spectral databases. In case of low-intensity peaks or overlapping peaks, the mass spectra were obtained using selected ion monitoring, (background) subtraction, and comparison with spectra of neighbouring homologues, when part of a series. The identified compounds all arise from the initial triacylglycerols either via hydrolysis or oxidative degradation. Esterified carboxylic acid groups from acylglycerols or cross-linked low-molecular-mass materials are not (trans)methylated with the derivatisation technique and therefore will not pass the chromatographic system used. The ratio of palmitic to stearic acid (P/S) of 1.3, based on peak areas, is well within the range for linseed oil-based paints [21]. The doubly and triply unsaturated fatty acids, originally present in high amounts, are not detectable anymore, whereas the relative amount of monounsaturated C_{18} fatty acids is still reasonably high. Upon autoxidation, the monounsaturated fatty acids are less reactive compared to the doubly and triply unsaturated fatty acids [48]. Since this paint is only 5 years old, reasonably thick compared to traditional paint layers, and no driers or pigments are present that speed up the oxidation, they have not reacted away and are still detectable. The doubly and triply unsaturated C_{18} fatty acids, however, already
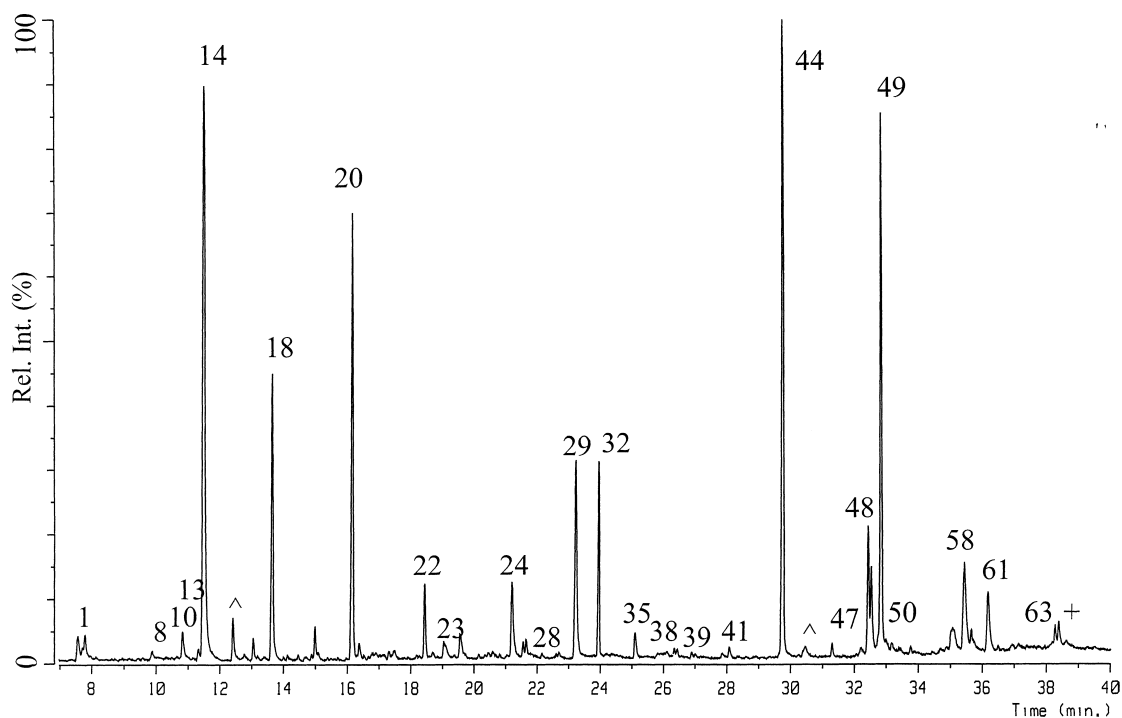
Fig. 1. Total ion chromatogram of a methanolic extract of a 5-year-old stand oil film after methylation and GC–MS analysis using on-column injection. Temperature program: 50(5)–6–320 (See Experimental section for further explanations.) Numbers correspond to Table 1. Peaks labelled × and + are identified as polysiloxanes and diisoctylphthalate, respectively.

have reacted away, partially by Diels–Alder cyclisations upon heating during the prepolymerisation, giving rise to cyclic C_{18} fatty acids and carbon–carbon linked oligomers of triacylglycerols [49–51], or are converted to oxidised products or incorporated into the cross-linked polymeric fraction. The short-chain fatty acids found are cleavage products formed upon oxidation of the initial unsaturated fatty acids [8,10]. Most of the acid groups of these fatty acids probably are formed upon oxidation of intermediate aldehydes, although some of these acids may arise from hydrolysis of degradation products formed via a different pathway [10,52]. Other degradation products that are formed upon oxidation of unsaturated fatty acids, like short-chain aldehydes, ketones, alkanes and alcohols, are volatile and are lost from the paint film by evaporation, giving the drying paint its typical smell. Furthermore, (highly) volatile compounds that will remain within the paint will be lost upon drying during sample preparation. The diacids are typical compounds from oxidised unsaturated

fatty acids. It is thought that the relative ratio of the C_8 (suberic acid; compound 24) and C_9 (azelaic acid; compound 29) diacids gives an indication whether the oil has been prepolymerised by heat treatment, although it has never been proven scientifically. Due to isomerisation of the original double bond systems relatively higher amounts of diacids other than azelaic acid are formed. In this case the oil has been heated and a ratio of suberic acid to azelaic acid of 0.4 is found. The cyclic C_{18} fatty acids are formed by cyclisation of linolenic acid upon heating [53]. These types of compounds are present in trace amounts (e.g. compound 50, M_r 290), and no absolute structures could be derived on the basis of their mass spectrum other than an indication of the presence of an aromatic ring. This is also a proof for heat-treatment of the oil. Two oxidised mono- and two doubly unsaturated C_{18} fatty acids (compounds 58 and 61, respectively) are not well resolved, which hampers structural identification. However, it is clear that these compounds contain a hydroxyl group as

Table 1
Identified methylated compounds in the methanolic extracts of oil paint samples after off-line methylation in combination with on-column
GC–MS or on-line (trans)methylation using Curie-point pyrolysis–GC–MS (on the basis of 70 eV EI spectra)

No.	Molecular mass	Compound
1	108	Methoxybenzene
2	116	Pentanoic acid, methyl ester
3	120	1,3-Dimethoxy-2-propanol
4	134	1,2,3-Trimethoxypropane
5	120	2,3-Dimethoxypropanol
6	130	Hexanoic acid, methyl ester
7	132	Propanedioic acid, dimethyl ester
8	94	Hydroxybenzene
9	142	Heptenoic acid, methyl ester
10	144	Heptanoic acid, methyl ester
11	158	2-Ethylhexanoic acid, methyl ester
12	144	Butenedioic acid, dimethyl ester
13	146	Butanedioic acid, dimethyl ester
14	166	Silane, trimethylphenoxy
15	160	α-Methylbutanedioic acid, dimethyl ester
16	136	Benzoic acid, methyl ester
17	156	Octenoic acid, methyl ester
18	158	Octanoic acid, methyl ester
19	160	Pentanedioic acid, dimethyl ester
20	172	Nonanoic acid, methyl ester
21	174	Hexanedioic acid, dimethyl ester
22	186	Decanoic acid, methyl ester
23	188	Heptanedioic acid, dimethyl ester
24	202	Octanedioic acid, dimethyl ester
25	194	1,2-Benzenedicarboxylic acid, dimethyl ester
26	216	α-Methyloctanedioic acid, dimethyl ester
27	230	α,α-Dimethyloctanedioic acid, dimethyl ester
28	214	Dodecanoic acid, methyl ester
29	216	Nonanedioic acid, dimethyl ester
30	232	α-Methoxyoctanedioic acid, dimethyl ester
31	230	α-Methylnonanedioic acid, dimethyl ester
32	226	Hexadecane (internal standard)
33	244	α,α-Dimethylnonanedioic acid, dimethyl ester
34	228	Tridecanoic acid, methyl ester
35	230	Decanedioic acid, dimethyl ester
36	246	α-Methoxynonanedioic acid, dimethyl ester
37	244	α-Methyldecanedioic acid, dimethyl ester
38	242	Tetradecanoic acid, methyl ester
39	244	Undecanedioic acid, dimethyl ester
40	260	α-Methoxydecanedioic acid, dimethyl ester
41	256	Pentadecanoic acid, methyl ester
42	258	Dodecanedioic acid, dimethyl ester
43	268	Hexadecenoic acid, methyl ester
44	270	Hexadecanoic acid, methyl ester
45	272	Tridecanedioic acid, dimethyl ester
46	256	Hexadecanoic acid
47	284	Heptadecanoic acid, methyl ester
48	296	Octadecenoic acid, methyl ester (*cis/trans*)
49	298	Octadecanoic acid, methyl ester
50	290	Octadecatetraenoic acid, methyl ester (9-(*o*-propylphenyl) nonanoic acid, methyl ester)

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Table 1. Continued

No.	Molecular mass	Compound
51	296	Octadecadienoic acid, methyl ester
52	284	Octadecanoic acid
53	326	8-Methoxy-9-octadecenoic acid, methyl ester
54	326	11-Methoxy-9-octadecenoic acid, methyl ester
55	326	9-Methoxy-10-octadecenoic acid, methyl ester, 10-methoxy-8-octadecenoic acid, methyl ester
56	312	9,10-Epoxyoctadecanoic acid, methyl ester
57	312	4-Oxooctadecanoic acid, methyl ester
58	312	9-Hydroxy-10-octadecenoic acid, methyl ester, 10-hydroxy-8-octadecenoic acid, methyl ester
59	312	9-Oxooctadecanoic acid, methyl ester, 10-oxooctadecanoic acid, methyl ester
60	326	Icosanoic acid, methyl ester
61	310	9-Hydroxy-10-octadecenoic acid + :1, methyl ester, 10-hydroxy-8-octadecenoic acid + :1, methyl ester
62	358	9,10-Dimethoxyoctadecanoic acid, methyl ester
63	354	Docosanoic acid, methyl ester
64	368	Tricosanoic acid, methyl ester
65	382	Tetracosanoic acid, methyl ester
66	396	Pentacosanoic acid, methyl ester

can be deduced from their mass spectra (not shown: molecular ions at m/z 312 and 310, respectively, and the loss of water 18, leading to fragment ions m/z 294 and 292). Based on retention time, the doubly unsaturated compounds most probably contained another hydroxyl group that is eliminated upon electron impact (EI) ionisation, after separation on the GC column. Glycerol, mono-, and diacylglycerols which can be present in the paint sample due to hydrolytic processes, are not detectable with this analytical procedure.

The chromatogram of the methylated extract of 17th century paint material (Fig. 2, Table 1) shows basically the same compounds but in different relative quantities. No attention has been paid to the varnish constituents of this paint since this falls outside the scope of the article. The oil is identified, based on a P/S ratio of 1.4, as a linseed oil. The ratio of the suberic to azelaic acid of 0.2 points to an oil, which has not or hardly been heat-treated prior to usage. No cyclic fatty acid structures were detected. Short-chain fatty acids are present in relatively low amounts, partially because they must have evaporated from the paint film, or have been removed by previous restoration treatments. Moreover, relatively increased amounts of hydrolysed fatty acids and

diacids are expected. Almost no monounsaturated and/or oxidised C_{18} fatty acids are detected since these must have reacted away to a large extent in time.

3.2. Off-line trimethylsilylation combined with on-column injection and GC–MS

The two methanol extracts described in Section 3.1 were also analysed with GC–MS in combination with on-column injection after trimethylsilylation. Now all free carboxylic and hydroxyl groups are trimethylsilylated, whereas ester bonds remain intact. The P/S ratios of 1.3 and 1.5, and C_8/C_9 diacid ratios of 0.5 and 0.3, for the 5-year-old stand oil and the 17th century paint are in good agreement with the results obtained with the methylation procedure for this extract. The TIC of the analysis of the stand oil extract (Fig. 3, Table 2) shows several new peaks that were not detected in the experiment with methylation only. First, co-eluting with the C_8 short-chain fatty acid, is trimethylsilylated glycerol (compound 5, Table 2). Secondly, new compounds are the monoacyl glycerols of suberic, azelaic, sebacic, palmitic and stearic acids (compounds 32, 34, 38, 40/42, and 44/46, respectively; see Table 2). Both
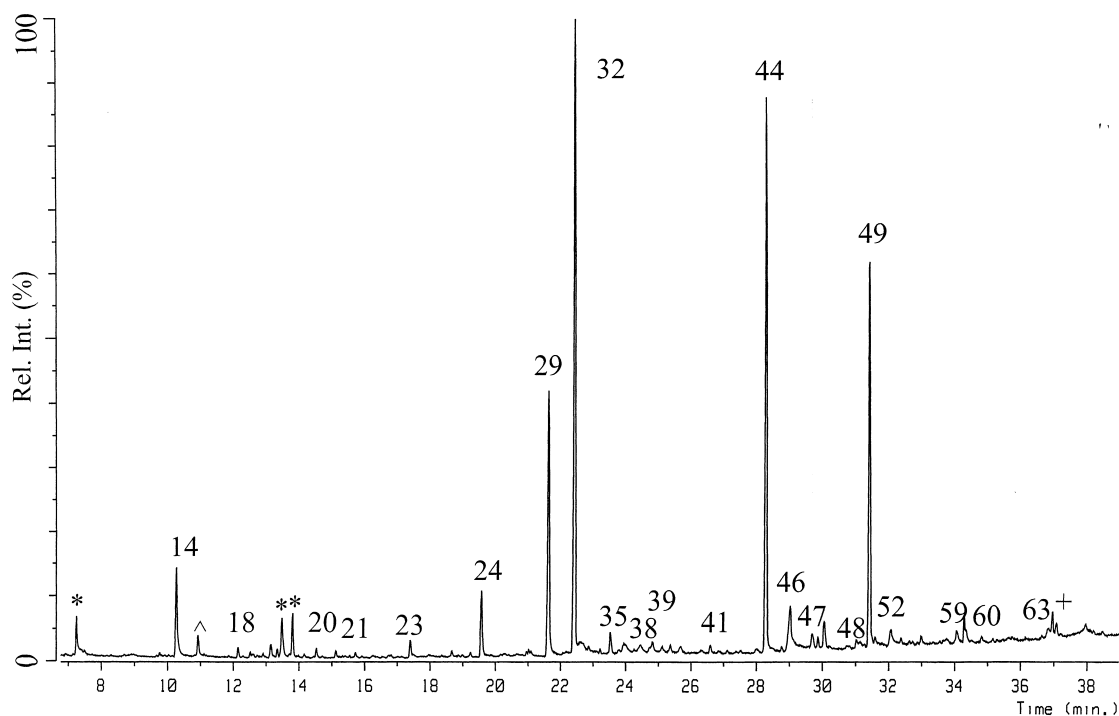
Fig. 2. Total ion chromatogram of a methanolic extract of 17th century paint after methylation and GC–MS analysis using on-column injection. Temperature program: 50(2)–6–320. Numbers correspond to Table 1. Peaks labelled * are presently unidentified, whereas peaks labelled $\times$ and + are ascribed to polysiloxanes and diisoctylphthalate, respectively.

the 1- and 2-substituted monoacyl glycerols of the C_{16} and C_{18} fatty acids are found. Two of the three original ester bonds have been hydrolysed in this case. Since the oil is reasonably young and oxidation is still proceeding, smaller amounts of monoacylglycerols of diacids, relative to those of saturated fatty acids, are detected. Di- and traces of triacylglycerols are expected to be present as well but cannot be eluted with the GC system used. The oxidised unsaturated fatty acids (compounds 35–37), which also were observed in the previous experiments (compounds 58, Table 1), and their monoacylglycerols (compound 47) could now be positively identified on the basis of the mass spectrum. As in the methylation experiment, short chain fatty acids are present in high amounts and no monoacylglycerols of these compounds are detected. This clearly indicates that their acid group has been formed to a large extent upon oxidation and was not esterified to glycerol originally. Two series of new compounds were identified based on their mass spectra: silylated

α- and β-hydroxy diacids (compounds 18, 19, 22, 23, 26, 27). The α-hydroxy diacids most probably are derived from dihydroxy fatty acids whereas the β-hydroxy diacids, only present in trace amounts, most probably originate from epidioxides, both formed upon autoxidation [54,55].

Most of the other compounds identified were also observed in the previous analyses of the methylated extracts. The main difference between the two analytical methods is the relative amount of diacids detected, which is higher in the case of the silylation experiment. Apart from hypothesising larger response factors of trimethylsilylated diacids relative to saturated fatty acids derivatives when compared with methylated compounds, there is no explicit explanation for the origin of this difference.

This last phenomenon was also observed for the 17th century paint (compare Figs. 2 and 4). Again relatively low amounts of short chain- and oxidised (unsaturated) C_{18} fatty acids are found. One oxidation product however, is present in moderate
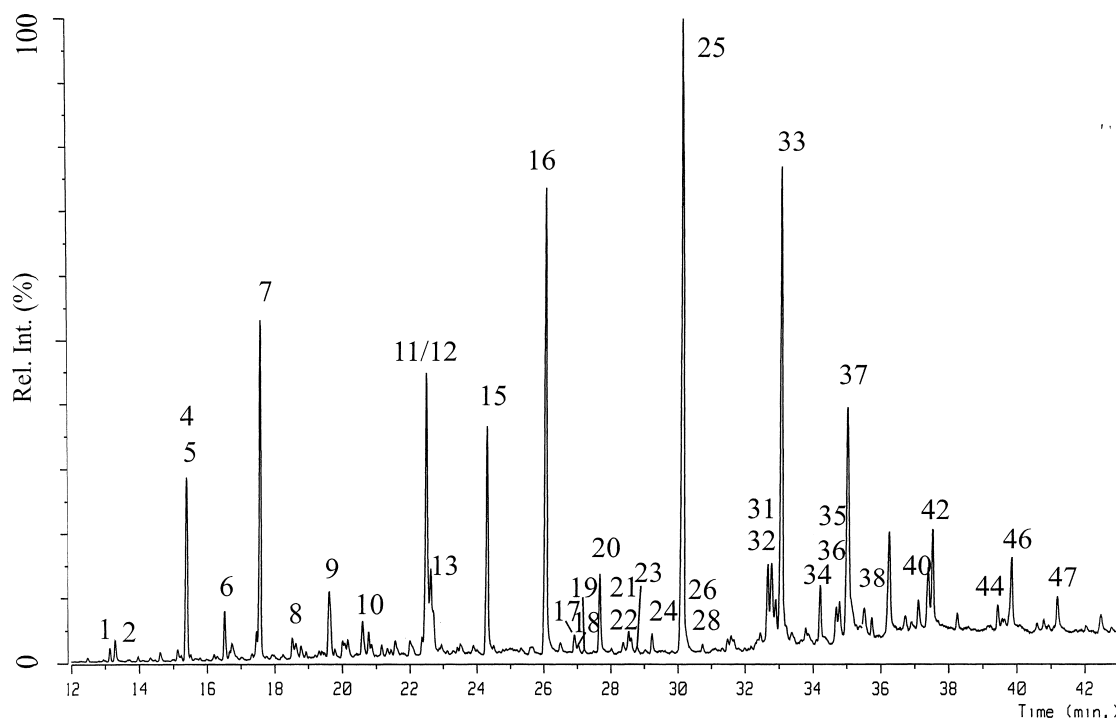
Fig. 3. Total ion chromatogram of a methanolic extract of 5-year-old stand oil film after trimethylsilylation and GC–MS analysis using on-column injection. Temperature program: 50(2)–6–320.

amounts: the completely silylated derivative of 9,10-dihydroxyoctadecanoic acid (compound 41). This compound is often detected when analysing old oil paint layers. Although this paint is 373 years old, ester bonds still intact were detected in the form of monoacylglycerols. The relative amount of mono-acylglcerols of the diacids is higher relative to those of the saturated C_{16} and C_{18} fatty acids, which were only present in low amounts (compounds 42 and 46). This can be explained by the ongoing oxidation of the unsaturated (and oxidised) fatty acids, which leads to higher amounts of diacids. In addition, an increased number of their monoacylglycerols will be detected due to ongoing hydrolysis.

3.3. On-line Curie-point pyrolysis–GC–MS

In Fig. 5, the TIC of a Curie-point Py–GC–MS analysis of a 5-year-old stand oil film is shown. The same compounds as in Sections 3.1 and 3.2 are identified (Table 1) but previously unidentified material as well. Compounds 3–5 are all methylated

derivatives of glycerol, which was liberated during the transmethylation reaction of the acylglycerols. Since this film still contains high amounts of ex-tractable tri-, di- and monoacylglycerols, next to (trans)methylated fatty acids, glycerol is detected in relatively high amounts. The relative abundance of the short C_7–C_{10} fatty acids, formed upon oxidative degradation, is relatively lowered compared to the methylation/silylation experiments because of the additional transmethylation of the esterified com-pounds present in the extract.

A series of unsaturated short-chain fatty acids is detected as well. These are not seen in the methyla-tion and silylation experiments and are supposed to be formed upon pyrolytic elimination from the extracted (cross-linked) oligomeric material in the extract [56–58]. Another series of new compounds identified are α-methylated and α,α-dimethylated diacids. These are by-products formed by reaction of the TMAH derivatisation reagent with the diacids as was proven for pure azelaic acid reference material [44]. A second series of compounds that can be

Table 2
Identified compounds in the methanolic extracts of oil paints after off-line trimethylsilylation and on-column GC–MS (on the basis of 70 eV
EI spectra)

| No.	Mol. mass	Compound
1	202	Heptanoic acid, TMS ester
2	236	Glycerol, 1,3-bis[(TMS)oxy]
3	214	Octenoic acid, TMS ester
4	216	Octanoic acid, TMS ester
5	308	Glycerol, 1,2,3-tris[(TMS)oxy]
6	262	Butanedioic acid, di-TMS ester
7	230	Nonanoic acid, TMS ester
8	276	Pentanedioic acid, di-TMS ester
9	244	Decanoic acid, TMS ester
10	290	Hexanedioic acid, di-TMS ester
11	304	Heptanedioic acid, di-TMS ester
12	226	Hexadecane (internal standard)
13	390	1,2-Benzenedicarboxylic acid, diethyl ester
14	282	4-[(TMS)oxy)]benzoic acid, TMS ester
15	318	Octanedioic acid, di-TMS ester
16	332	Nonanedioic acid, di-TMS ester
17	300	Tetradecanoic acid, TMS ester
18	406	Octanedioic acid, α-OTMS ether, di-TMS ester
19	406	Octanedioic acid, β-OTMS ether, di-TMS ester
20	346	Decanedioic acid, di-TMS ester
21	314	Pentadecanoic acid, TMS ester
22	420	Nonanedioic acid, α-OTMS ether, di-TMS ester
23	420	Nonanedioic acid, β-OTMS ether, di-TMS ester
24	360	Undecanedioic acid, di-TMS ester
25	328	Hexadecanoic acid, TMS ester
26	434	Decanedioic acid, α-OTMS ether, di-TMS ester
27	434	Decanedioic acid, β-OTMS ether, di-TMS ester
28	374	Dodecanedioic acid, di-TMS ester
29	342	Heptadecanoic acid, TMS ester
30	388	Tridecanedioic acid, di-TMS ester
31	354	Octadecenoic acid, TMS ester
32	464	Octanedioic mono-TMS ester, 2,3-bis(TMS)oxy propyl ester
33	356	Octadecanoic acid, TMS ester
34	478	Nonanedioic mono-TMS ester, 2,3-bis(TMS)oxy propyl ester
35	442	9-Octadecenoic acid, 8-TMS ether, TMS ester
36	442	9-Octadecenoic acid, 11-TMS ether, TMS ester
37	442	10-Octadecenoic acid, 9-TMS ether, TMS ester; 8-octadecenoic acid, 10-TMS ether, TMS ester
38	492	Decanedioic mono-TMS ester, 2,3-bis(TMS)oxy propyl ester
39	384	Icosanoic acid, TMS ester
40	474	Hexadecanoic acid, 1,3-bis(TMS)oxy propyl ester
41	532	Octadecanoic acid, 9,10-bis[(TMS)oxy], TMS ester
42	474	Hexadecanoic acid, 2,3-bis(TMS)oxy propyl ester
43	398	Docosanoic acid, silyl ester
44	502	Octadecanoic acid, 1,3-bis(TMS)oxy propyl ester
45	500	Octadecenoic acid, 2,3-bis(TMS)oxy propyl ester
46	502	Octadecanoic acid, 2,3-bis(TMS)oxy propyl ester
47	588	10-Octadecenoic acid, 9-TMS ether, 2,3-bis(TMS)oxy propyl ester; 8-octadecenoic acid, 10-TMS ether, 2,3-bis(TMS)oxy propyl ester
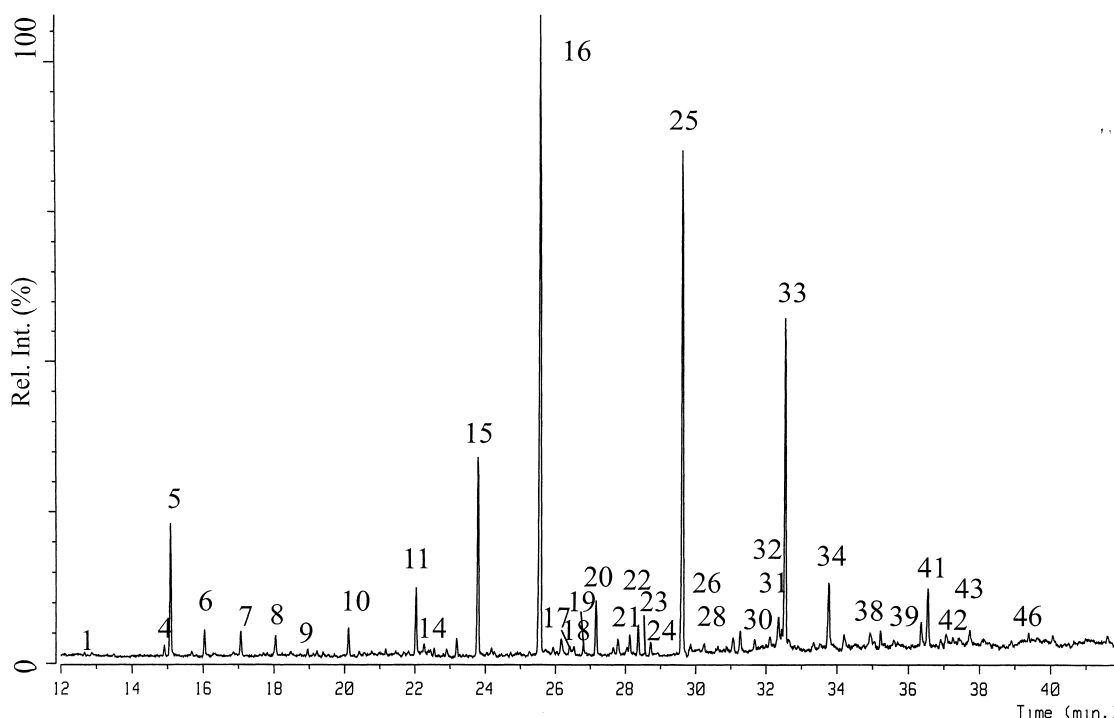
Fig. 4. Total ion chromatogram of a methanolic extract of 17th century paint after trimethylsilylation and GC–MS analysis using on-column injection. Temperature program: 50(2)–6–320. Numbers correspond to Table 2.

found in the chromatogram are the α-methoxylated C_8–C_{10} diacids (compounds 30, 36 and 40), which were also detected after trimethylsilylation of the extract. The β-methoxylated diacids were not detected.

The (trans)methylation procedure results in new mass spectra that not have been reported before to our knowledge. In Fig. 6A–C, the EI mass spectrum, the EI mass spectrum using deuterated TMAH reagent and the chemical ionization (CI) mass spectrum using isobutane are depicted for 2-methoxy-nonanedioic acid dimethyl ester (36). Here α-cleavage next to the methoxy group gives rise to the most intense fragment ions (m/z 187) followed by two times the loss of methanol (m/z 155 and 123, respectively). The molecular mass of m/z 246, found by chemical ionisation using isobutane (Fig. 6C), is in agreement with the proposed molecular structure.

Compounds 53, 54, 55a and b are identified as octadecenoic acid methyl esters substituted with a methylated hydroxy group. These types of compounds were also visible in both the methylated and

silylated extracts and the combination of these three measurements led to the positive identification. As an example, the mass spectrum of 8-methoxy-9-octadecenoic acid methyl ester (compound 53) is depicted in Fig. 7. In the 70 eV EI mass spectrum (Fig. 7A) the most intense ion m/z 183 is formed by α-cleavage next to the methoxy group. An α-cleavage next to the double bond gives rise to fragment ions of m/z 213. The result of the experiment with deuterated TMAH supports the proposed structure. An increase of 6 u was observed for the α-cleavage fragment next to the methoxy group, whereas the other fragment ion only increased by 3 u (Fig. 7B). Chemical ionisation using isobutane showed the molecular mass of the compound to be m/z 326 (Fig. 7C). Other oxygenated fatty acids identified include 9,10-epoxyoctadecanoic acid methyl esters (56) and 9,10-dimethoxyoctadecanoic acid methyl esters (62). Similar to the methylation and silylation experiment the cyclic C_{18} fatty acid was also detected with TMAH derivatisation–Py–GC–MS. It could now be identified as 9-(o-propylphenyl)nonanoic acid,
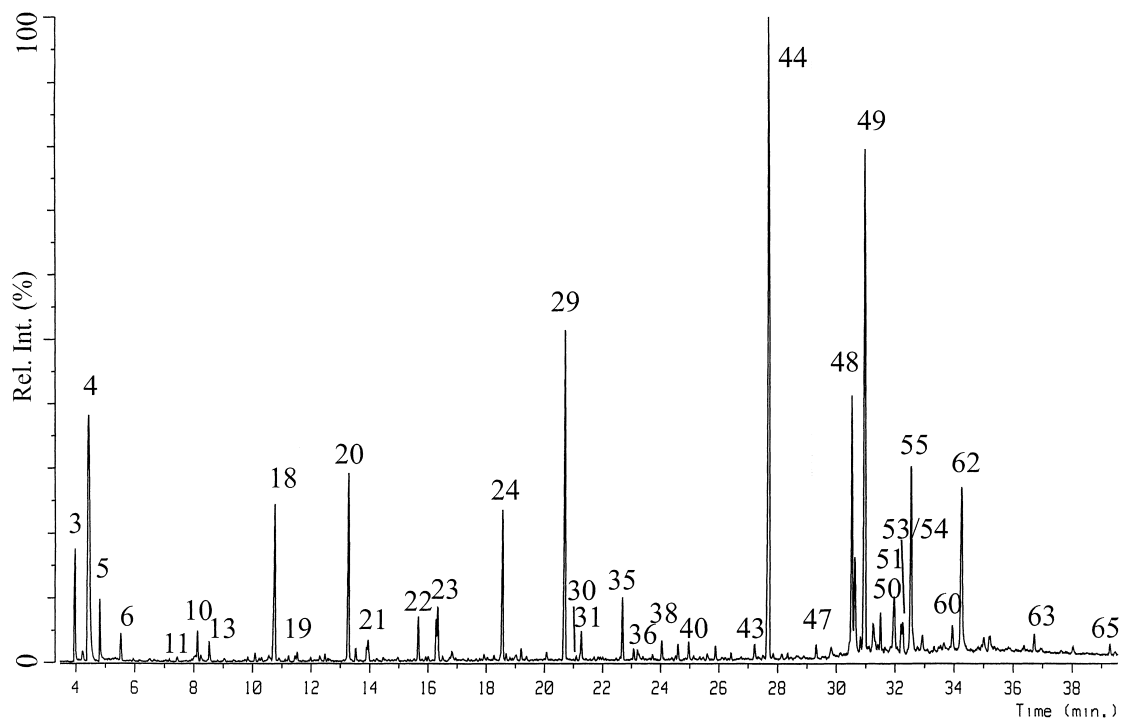
Fig. 5. Total ion chromatogram of a methanolic extract of a 5-year-old stand oil film after Curie-point pyrolysis assisted with on-line (trans)methylation using TMAH. Temperature program: 40(6)–6–320. Numbers correspond to Table 1.

methyl ester, because of the more specific fragment ions visible. In addition to this particular fatty acid, trace amounts of other isomeric aromatic C_{18} fatty acids were detected as well. With the previous two methods only free fatty acids and some monoacyl-glycerols are monitored. On-line derivatisation of the methanol extract with tetramethylammonium hydroxide, assisted by 610 °C Curie-point pyrolysis, however, transmethylates esterified fatty acids as well. Therefore, a more quantitative picture can be obtained of the composition of the extract. Furthermore, hydroxyl groups, including those of glycerol, are (partially) methylated which leads to better chromatographic separation and MS identification. The only materials that cannot be analysed straightforward this way are the cross-linked oligomeric compounds formed upon oxidative or heat-induced polymerisation.

The P/S and C_8/C_9 diacid ratios of the extract were 1.3 and 0.4, respectively. These values are identical to the results obtained with the off-line derivatisation procedures.

A methanolic extract of a 26-year-old lead white pigmented linseed oil paint sample has been analysed by TMAH derivatisation–Py–GC–MS as well in order to obtain a complementary result in between the very young and very old paint. However, it should be remembered that these three samples have a different composition and history. The same compounds are found as in the extract of the 5-year-old film, except for the cyclic fatty acids (Fig. 8, Table 1). The P/S ratio of 1.4 is within the range of linseed oil. Cyclic C_{18} fatty acids were not detected and the ratio of C_8 to C_9 diacids of 0.3 is an analytical confirmation that the oil has not been heat-treated [59]. As expected, only minor amounts of monounsaturated C_{18} fatty acids are detected in the extract. These species have reacted away upon oxidation and are partially incorporated in the oil network. The relative amount of oxidised C_{18} compounds is still high and comparable to the 5-year-old paint. The relative amount of diacids, however, is somewhat lower. Formation of lead soaps with these diacids is expected, and more favourable relative to the fatty
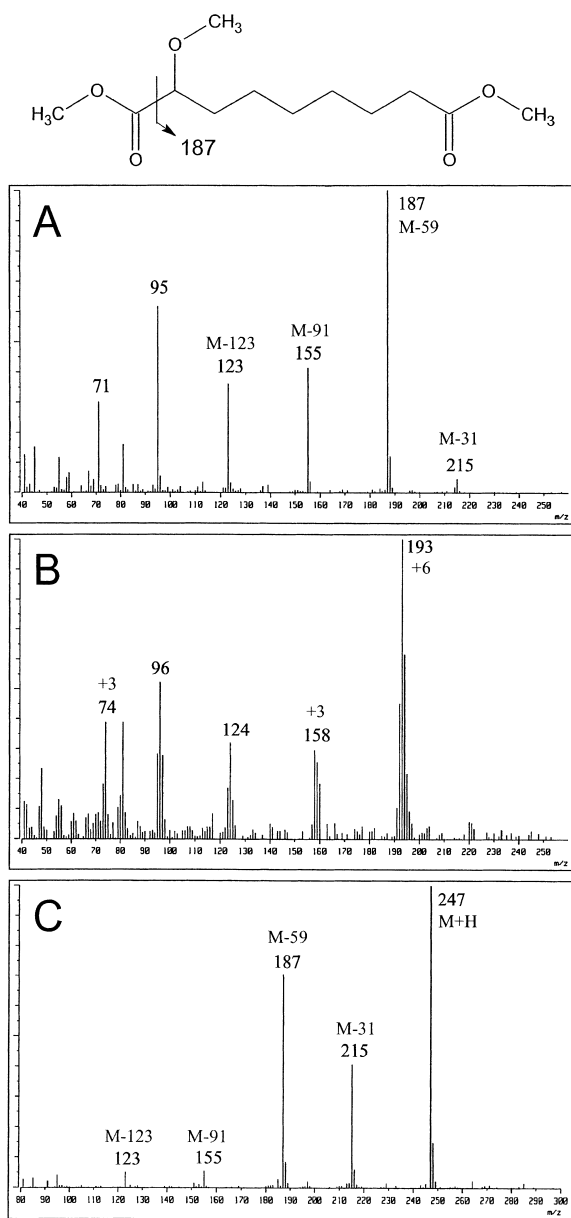
Fig. 6. Mass spectrum of nonanedioic acid, α-methoxy, dimethyl ester using (A) 70 eV EI, (B) 70 eV EI and deuterated TMAH, and (C) chemical ionisation using isobutane.
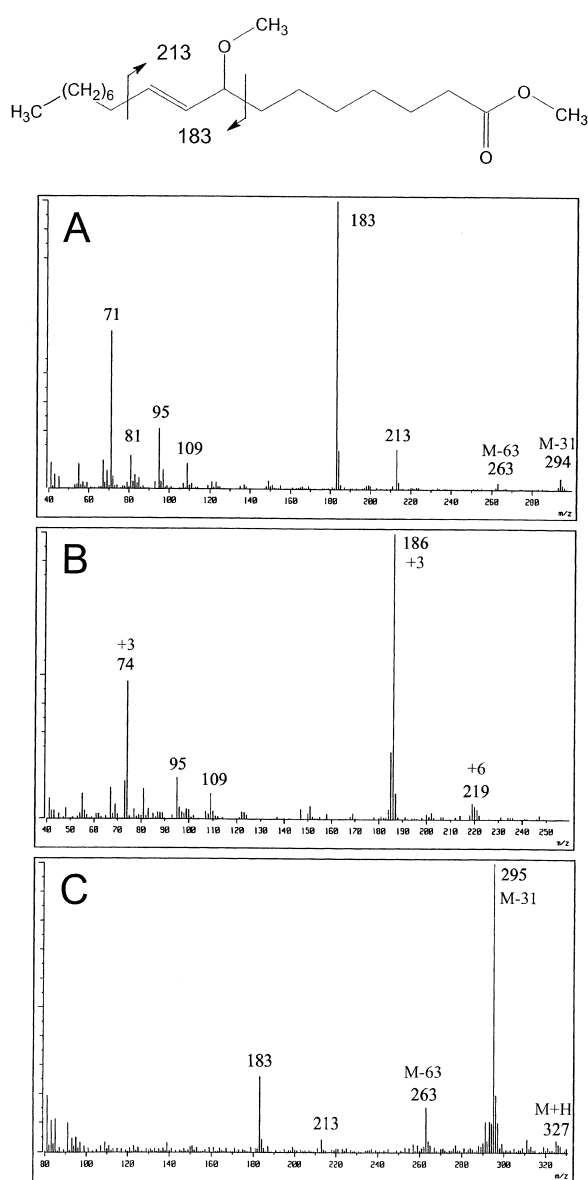

Fig. 7. Mass spectrum of 8-methoxy-9-octadecenoic acid, methyl ester using (A) 70 eV EI, (B) 70 eV EI and deuterated TMAH, and (C) chemical ionisation using isobutane.

acids containing one acid group. This leads to lower amounts of extractable fatty diacids. The relative amounts of short-chain fatty acids are lower compared to the previous analysis and low amounts of glycerol derivatives are detected. Analysis of the

paint residue by TMAH derivatisation–Py–GC–MS after extraction showed that relatively high amounts of glycerol are still present. It is therefore suggested that due to a high degree of polymerisation only low amounts of acylglycerols can be extracted. This is to be expected for a lead white pigmented oil film since
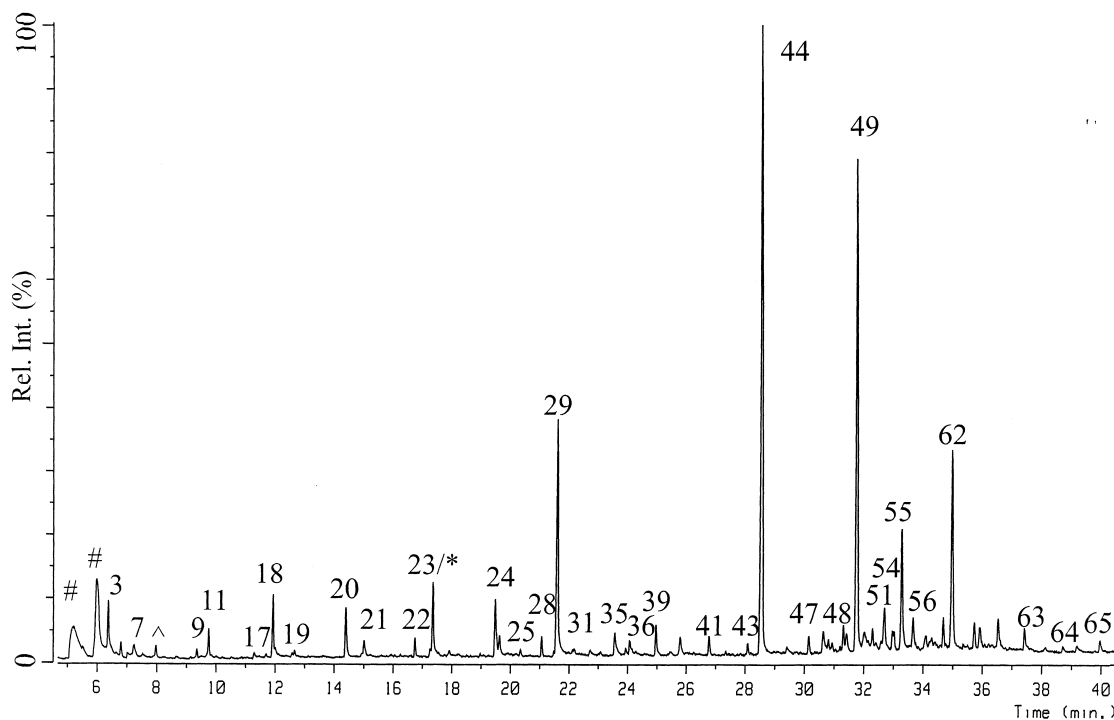
Fig. 8. Total ion chromatogram of a methanolic extract of a 26-year-old lead white pigmented linseed oil paint after Curie-point pyrolysis assisted with on-line (trans)methylation using TMAH. Temperature program: 50(2)–6–320. Peaks labelled # and × are identified as TMAH reagent-derived by-products and polysiloxanes, respectively. Numbers correspond to Table 1. The peak labelled 23/* contains an unidentified compound.

lead is known for its positive effect on the polymerisation [60,61]. Furthermore, the lead soap formation with carboxylic acid groups present in the oligomeric material is also expected to result in reduced extractability of glycerol-containing material, as long as the number of hydrolysed ester bonds is low. Most of the glycerol will be bound to the networks in this latter case.

The analysis of the methanol extract of the 17th century paint is presented in Fig. 9 (see also Table 1). The same types of compounds are found when compared to the other extracts investigated. Both the P/S and C_8/C_9 diacid ratios obtained are comparable to the numbers measured with the previously described off-line strategies. Again the main difference is the relative amount of the different ageing products detected. Glycerol cannot be detected anymore. Hydrolysis of the ester bonds, in combination with evaporation and the (unknown) restoration history has led to the loss of glycerol from the paint.

Oxidised C_{18} fatty acids are present in relatively low amounts, whereas the diacids are detected in relatively high quantities compared to the younger paint samples. This can be ascribed to the ongoing oxidative degradation of the paint material, in combination with evaporative losses.

A comparison of the results obtained with the three analytical strategies applied in this study shows that for the major components present, similar qualitative information is obtained. The palmitic-to-stearic acid and suberic-to-azelaic acid ratios of each extract were identical for all three methods. The off-line methylation procedure, despite the fact that it only takes 15 min to derivatise the sample, is the least favourable of the three methods. Only free fatty acids are monitored and polar groups on the fatty acids are not derivatised, which has a negative influence on the chromatographic separation. Trimethylsilylation, however, is capable of derivatising hydroxy groups and chromatographically resolved
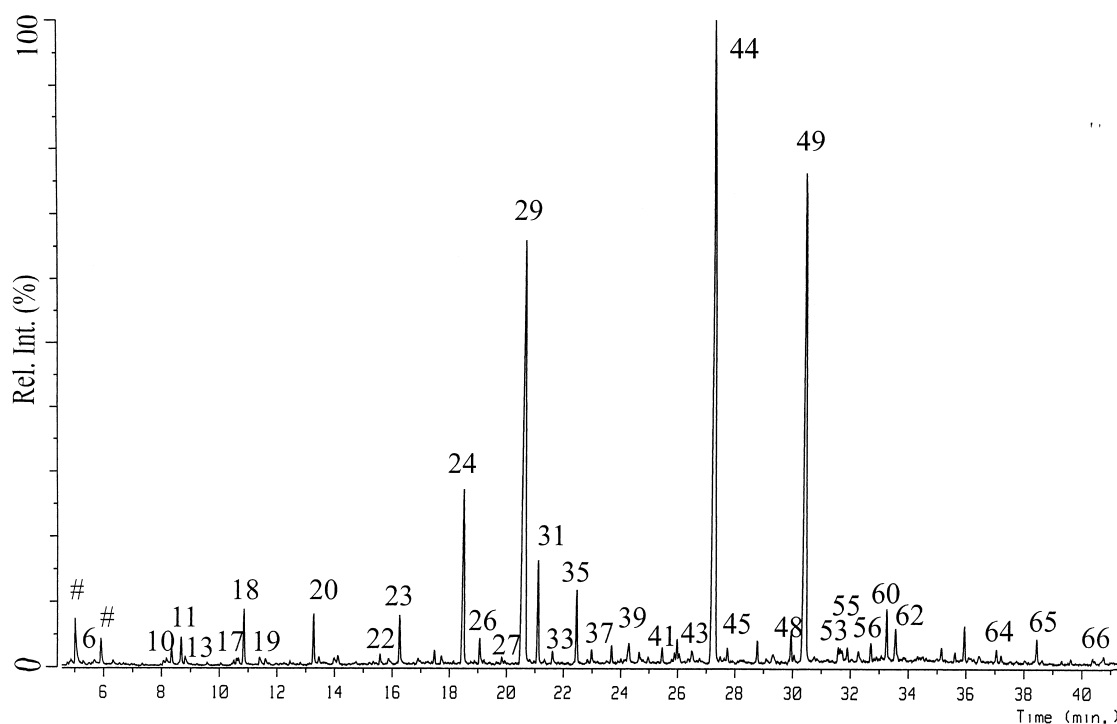
Fig. 9. Total ion chromatogram of a methanolic extract of 17th century paint after Curie-point pyrolysis assisted with on-line (trans)methylation using TMAH. Temperature program: 50(2)–6–320. Numbers correspond to Table 1. Peaks labelled # are identified as TMAH reagent-derived by-products.

oxidation products can now be identified on the basis of their mass spectrum. Furthermore, monoacylglycerols are chromatographable and their relative abundance can give information to what extent hydrolytic processes have taken place. A disadvantage is the longer reaction time needed, the drying steps that may lead to loss of volatile low-molecular-mass compounds when not carefully performed and the fact that di- and triacylglycerols and other esterified oligomeric materials are not included in the analysis. This would require high-temperature columns or an LC–MS technique [62,63]. The TMAH derivatisation–pyrolysis–GC–MS technique is of the three strategies the only one capable of analysing all the extractable non-cross-linked compounds. Both free and esterified fatty acids are methylated and can be detected whereas for the methylation and trimethylsilylation experiments only free fatty acids (or monoacylglycerols in the case of a TMS derivatisation) are monitored. In this sense the on-line (trans)methylation technique is comparable to the ''classic'' saponification with KOH, followed by extraction and methylation. A disadvantage, however, of the TMAH derivatisation–Py approach is the formation of by-products, which are not seen with the other methods mentioned. On the other hand, there are three major advantages. First, the chemical work-up is much quicker and easier, and secondly, there is no loss of material due to extraction and drying steps. Last but not least, due to the methylation capabilities of TMAH, hydroxyl groups are also derivatised, which makes separation and identification of oxidised fatty acids favourable.

4. Conclusion

There are numerous factors that influence the chemical composition of an oil paint. Not only does age play a role but also other important factors like the kind of oil and the processing methods, the type and amount of pigment, the environmental condi-

tions, and the (restoration) history of the paint. It is not known whether certain synergistic effects will play a role and how all these factors together will influence the development of the oil paint system.

However, there are many common features in the development of oil paints. In the extracts of paint films of different ages, always products can be found from de-esterification of the triacylglycerols and/or degradation of the oxidised fatty acids. In young paint samples, oxidised C_{18} fatty acids, short chain fatty acids and glycerol are found in relatively high amounts. Some of the more polar compounds identified with both trimethylsilylation using on-column injection combined with GC–MS and TMAH derivatisation–Py–GC–MS were reported for the first time. Most of these compounds are lost from old paint films due to oxidative degradation, evaporation from the surface of the paint or and/or perhaps repetitive cleaning procedures. Diacids, the relatively stable end products of the oxidation of unsaturated fatty acids, are relatively more prominent in aged paint samples.

Both the off-line trimethylsilylation and methylation strategy are suitable for the analysis of the extractable non-cross-linked fraction with GC–MS in combination with on-column injection. Trimethylsilyl derivatisation not only gives information on free (oxidised) fatty (di)acids but also makes it possible to identify monoacylglycerols. The relative amount of these species gives semi-quantitative information on the degree of hydrolysis of the paint. Other esterified fatty acids, however, cannot be monitored. TMAH derivatisation–Py–GC–MS on the other and methylates all esterified and free fatty acids present in the extract and the totality of non-cross-linked material can be detected very easily in this way.

Acknowledgements

References

[1] A.P. Laurie, The Painter's Methods, Dover Publications, Mineola, NY, 1988.

[2] H.W.-S. Chan, in: H.W.-S. Chan (Ed.), Autoxidation of Unsaturated Lipids, Academic Press, London, 1987.

[3] H.W.-S. Chan, J. Am. Oil Chem. Soc. 54 (1977) 100.

[4] C. Sultana, in: A. Karleskind, J.-P. Wolff (Eds.), Oils and Fats Manual, Vol. 1, Lavoisier, Paris, 1996, p. 159.

[5] H.W. Gardner, in: H.W.-S. Chan (Ed.), Autoxidation of Unsaturated Lipids, Academic Press, London, 1987, p. 51.

[6] W.J. Muizebelt, J.J. Donkerbroek, M.W.F. Nielen, J.B. Hussem, M.E.F. Biemond, R.P. Klaasen, K.H. Zabel, J. Coat. Technol. 70 (1998) 83.

[7] W.J. Muizebelt, M.W.F. Nielen, J. Mass Spectrom. 31 (1996) 545.

[8] W. Grosch, in: H.W.-S. Chan (Ed.), Autoxidation of Unsaturated Lipids, Academic Press, London, 1987, p. 95.

[9] V. Pinnel, J. Vandegans, J. High Resolut. Chromatogr. 19 (1996) 263.

[10] R.A. Hancock, N.J. Leeves, Prog. Org. Coatings 17 (1989) 321.

[11] L.A.O. O'Neill, R.A. Brett, J. Oil Chem. Assoc. 52 (1969) 1054.

[12] J.D.J. van den Berg, K.J. van den Berg, J.J. Boon, in: J. Bridgland (Ed.), Preprints of the 12th Triennial ICOM CC meeting, Lyon, 1999, p. 248.

[13] J.J. Boon, S.L. Peulve, O.F. Van den Brink, M.C. Duursma, D. Rainford, in: T. Bakkenist, R. Hoppenbrouwers, H. Dubois (Eds.), Early Italian Paintings Techniques and Analysis, Limburg Conservation Institute, Maastricht, 1997, p. 35.

[14] W.J. Muizebelt, J.C. Hubert, R.A.M. Venderbosch, Prog. Org. Coatings 24 (1994) 263.

[15] A. Hopia, Lebensm. Wiss. Technol. 26 (1993) 563.

[16] J.H. Hartshorn, J. Coat. Technol. 54 (1982) 53.

[17] J. Mallégol, J.-L. Gardette, J. Lemaire, J. Am. Oil Chem. Soc. 76 (1999) 967.

[18] J. Mallégol, J.-L. Gardette, J. Lemaire, J. Am. Oil Chem. Soc. 77 (2000) 249.

[19] G.L. Marshall, J.A. Lander, Eur. Polym. J. 21 (1985) 959.

[20] G.L. Marshall, M.E.A. Cudby, K. Smith, T.H. Stevenson, K.J. Packer, R.K. Harris, Polymer 28 (1987) 1093.

[21] J.S. Mills, Stud. Conserv. 11 (1966) 92.

[22] M.R. Schilling, H.P. Khanjian, in: J. Bridgland (Ed.), Preprints of the 11th Triennial ICOM CC Meeting, Edinburgh, 1996, p. 220.

[23] M.R. Schilling, H.P. Khanjian, D.M. Carson, Techne 5 (1997) 71.

[24] N. Stolow, G.W. Rogers, in: W.J. Young (Ed.), Preprints of the Conference Application of Science in Examination of Works of Art, Boston, MA, 1970, p. 213.

[25] D. Erhardt, J.-S. Tsang, in: J.S. Mills, P. Smith (Eds.), Preprints of the Conference Cleaning, Retouching and Coatings: Technology and Practice for Easel Paintings and Polychrome Sculpture, Brussels, 1990, p. 93.

[26] J. Koller, U. Baumer, Z. Kunsttechnol. Kons. 9 (1995) 286.

[27] R. White, A. Roy, Stud. Conserv. 43 (1998) 159.

[28] C.S. Tumosa, J. Millard, D. Erhardt, M.F. Mecklenburg, in: J. Bridgland (Ed.), Preprints of the 12th Triennial ICOM CC Meeting, Lyon, 1999, p. 347.

[29] R.A. Hites, Anal. Chem. 42 (1970) 1736.

[30] T. Rezanka, P. Mares, J. Chromatogr. 542 (1991) 145.

[31] K. Sutherland, N. Shibayama, in: J. Bridgland (Ed.), Preprints of the 12th Triennial ICOM CC Meeting, Lyon, 1999, p. 341.

[32] R.P. Evershed, in: K. Blau, J. Halket (Eds.), Handbook of Derivatives for Chromatography, Wiley, Chichester, UK, 1993, p. 92.

[33] M.P. Colombini, F. Modugno, M. Giacomelli, S. Francesconi, J. Chromatogr. A 846 (1999) 113.

[34] G. Chiavari, G.C. Galetti, G. Lanterna, R. Mazzeo, J. Anal. Appl. Pyrol. 24 (1993) 227.

[35] J.M. Challinor, J. Anal. Appl. Pyrol. 18 (1990) 233.

[36] J.D.J. van den Berg, J.J. Boon, K.J. van den Berg, I. Fiedler, M.A. Miller, Anal. Chem. 70 (1998) 1823.

[37] J.M. Challinor, J. Anal. Appl. Pyrol. 37 (1996) 185.

[38] E.W. Robb, J.J. Westbrook, Anal. Chem. 35 (1963) 1644.

[39] J.M. Challinor, J. Anal. Appl. Pyrol. 16 (1989) 323.

[40] J.W. de Leeuw, M. Baas, J. Anal. Appl. Pyrol. 26 (1993) 175.

[41] W.C. Kossa, J. MacGee, J. Chromatogr. Sci. 17 (1979) 177.

[42] J.V. Gimeno-Adelanto, R. Mateo-Castro, M.T. Doménech-Carbó, F. Bosch-reig, A. Doménech-Carbó, M.J. Casas-Catalán, L. Osete-Cortina, J. Chromatogr. A 922 (2001) 385.

[43] L.A. Carlyle, A critical analysis of artists' handbooks, manuals and treatises on oil painting published in Britain between 1800–1900: with references to selected eighteenth century sources. Courtauld Institute of Art, London, 1991, p. 390.

[44] J.D.J. van den Berg, J.J. Boon, J. Anal. Appl. Pyrol. 61 (2001) 45.

[45] R. Ryhage, E. Stenhagen, Ark. Kemi 14 (1959) 497.

[46] R. Ryhage, E. Stenhagen, Ark. Kemi 15 (1960) 545.

[47] W.W. Christie, Lipids 33 (1998) 343.

[48] E. Ucciani, A. Debal, in: A. Karleskind, J.-P. Wolff (Eds.), Oils and Fats Manual, Vol. 1, Intercept, Andover, 1996, p. 325.

[49] A. Crossley, T.D. Heyes, B.J.F. Hudson, J. Am. Oil Chem. Soc. 39 (1962) 9.

[50] C. Boelhouwer, J.T. Knegtel, M. Tels, Fette Seifen Anstrichm. 69 (1967) 432.

[51] J.C. Martin, M.C. Dobarganes, M. Nour, G. Marquez-Ruiz, W.W. Christie, F. Lavillonnière, J.L. Sébédio, J. Am. Oil Chem. Soc. 75 (1998) 1065.

[52] R.J. Horvat, W.H. McFadden, N.G. Hawkins, D.R. Black, W.G. Lane, R.M. Teeter, J. Am. Oil Chem. Soc. 42 (1965) 1112.

[53] E.G. Perkins, W.T. Iwaoka, J. Am. Oil Chem. Soc. 50 (1973) 44.

[54] E.N. Frankel, W.E. Neff, K. Miyashita, Lipids 25 (1990) 33.

[55] E.N. Frankel, W.E. Neff, K. Miyashita, Lipids 25 (1990) 40.

[56] F. Gelin, J.-P.L.A. Gatellier, J.S. Sinninghe Damsté, P. Metzger, S. Derenne, C.C. Largeau, J.W. de Leeuw, J. Anal. Appl. Pyrol. 27 (1993) 155.

[57] S.C. Moldoveanu, Analytical Pyrolysis of Natural Organic Polymers, Elsevier, Amsterdam, 1998.

[58] W.A. Hartgers, J.S. Sinninghe Damsté, J.W. de Leeuw, J. Anal. Appl. Pyrol. 20 (1991) 141.

[59] H.-C. Imhoff, A. Voûte, Research project on pigment-identification and controlled natural alteration through age. Canadian Conservation Institute, Ottawa, 1972.

[60] Z.W.J. Wicks, F.N. Jones, S.P. Pappas, Organic Coatings: Science and Technology, Vol. 1: Film Formation, Components, and Appearance, Wiley, New York, 1992.

[61] S.M.P. Meneghetti, R.F. de Souza, A.L. Monteiro, M.O. de Souza, Prog. Org. Coatings 33 (1998) 219.

[62] L. Steenhorst-Slikkerveer, A. Louter, H.G. Janssen, C. Bauer-Plank, J. Am. Oil Chem. Soc. 77 (2000) 837.

[63] W.C. Byrdwell, W.E. Neff, J. Chromatogr. A 905 (2001) 85.