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Analysis of triglyceride degradation products in drying oils and oil paints using LC–ESI–MS

Eliane P. van Dam^{a,1}, Klaas Jan van den Berg^{a,b,*}, Art Ness Proaño Gaibor^{a,c},
Maarten van Bommel^b

^a Cultural Heritage Agency of the Netherlands, Amersfoort/Amsterdam, The Netherlands

^b University of Amsterdam, Conservation and Restoration of Cultural Heritage, Amsterdam, The Netherlands

^c Van Gogh Museum, Amsterdam, The Netherlands

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ABSTRACT

An LC–ESI–MS method is presented as a novel approach for the study of aged drying oils and oil paints in various stages of oxidation and hydrolysis. The method involves separation and detection of glycerides and fatty acids on a reversed phase column using a polar gradient ranging from methanol/water to methanol/isopropanol with post-column addition of NH_4Ac to facilitate electrospray ionisation. This setup allows for a reasonable separation of non-polar triglycerides in drying oil as well as very polar oxidised and hydrolysed tri, di and monoglycerides as well as free fatty acids. Detection is performed by using both positive and negative ionisation mode: positive ions for glycerides, negative ions for carboxylic acid containing degradation products and free fatty acids.

In this way, distinction can be made between components in oil and metal stearate mixtures by independently probing the palmitic acid/stearic acid (P/S) ratios of the free fatty acids which mostly derive from the metal stearates, and the glycerides which derive only from the drying oil components.

Analyses of 10 year-old titanium white oil paints with medium exudations and 62 year-old paints from Winsor&Newton are presented as examples to show the applicability of the method.

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1. Introduction

Vegetable drying oils such as linseed and safflower oil continue to be used in artists' paints [1], together with pigments and additives such as dryers, stabilisers and fillers. These paints will dry chemically after application in a series of autoxidation reactions with atmospheric oxygen. In these reactions, the triacylglycerides (TAGs) form crosslinks which result in a polymer network, binding the pigments together.

In order to understand the optical and mechanical changes that occur in paintings in the course of time, it is important to use micro-analytical techniques that give information about the underlying chemical changes. GCMS has long been the method of choice for analysis of oil paint binders in paintings, but the way the method has been applied traditionally, the information this method brings

forward is limited. Direct Temperature-resolved MS (DTMS) is used to some extent as a quick sensitive method to give qualitative information on paints and their ageing behaviour [2,3]. Especially the degree of hydrolysis of oil paints has been neglected to some extent in the literature, partly due to the difficulty in quantifying this with GCMS, with few exceptions [4].

Reactivity in curing and ageing oil paints comprises a complex set of reactions. Polymerisation is in competition with oxidative, chain scission degradation reactions which form small aldehydes, diacids and other products that do not participate in the polymerisation process [5,6]; in addition, hydrolysis will take place in the course of time, which results in a lower degree of polymerisation (see Fig. 1) [7]. These reactions may lead to relatively weak paint films sensitive to organic solvent swelling [8]. Soap formation of the resulting carboxylic acid groups in the presence of neutral or alkaline polyvalent metal salts such as lead may in turn lead to more stable, solvent resistant paints [7,8].

It has been shown that formation of diacid by-products takes place primarily on the surface of paints [9] and/or in the absence of inorganic materials [10]. Since diacids are soluble in water, these may therefore be responsible for sensitivity of paints to water [1,11].

* Corresponding author at: Cultural Heritage Agency of the Netherlands, PO box 1600, 3800 BP Amersfoort, The Netherlands.

E-mail address: k.van.den.berg@cultureelerfgoed.nl (K.J. van den Berg).

¹ Present address: FOM Institute AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands.

Fig. 1. Scheme indicating the different polymerisation and degradation reactions of a drying oil. Polyunsaturated triacyl glycerides (TAGs) may polymerise and/or be oxidised. The system may then hydrolyse in the course of time. Non-polymerised TAGs may form extractable diacyl glycerides (DAGs), monoacyl glycerides (MAGs) and free fatty acids (FFA's) [7,8].

It is important for an optimal conservation of paintings that original contents, the application and the state of degradation of paints is well understood in addition to historical climate conditions met by the paintings [12,13]. In recent years, many studies have been performed on degradation phenomena of paintings, focusing particularly on pigments and pigment-binding medium interactions. For the analysis of binding media, many new approaches have been developed including GCMS methodology [4,14] and direct electrospray [9,15].

As an approach towards understanding all reaction paths (polymerisation, oxidation and hydrolysis reactions) together this paper describes an LC–MS method for the identification of organic degradation products from oil in paint which was adapted specifically for oxidised and hydrolysed products of oil paint. Information is presented which aids in the interpretation of the positive and negative ion mass spectra, through NH_4^+ and Na^+ adducts and $[\text{M}-\text{H}]^-$ ions, respectively. The LC method was based directly on a procedure recently set up for the analysis of TAGs in fresh oil paints, by La Nasa et al. [16,17]. The method includes post-column addition of NH_4Ac to facilitate ionisation [18,19]. The adapted method was applied on young paint reconstructions and on naturally cured and aged artists' oil paints that were more than sixty years old.

2. Materials and methods

2.1. Chemicals

Solvents used: methanol LC–MS grade (Fluka), isopropanol LC–MS grade (Fluka) and ethanol absolute (Sigma Aldrich). The standards of mono-, di- and triglycerides that were used for method development were: trilaurin, trimyristin, tripalmitin, tristearin, mixture of monoolein, diolein and triolein obtained from Supelco (all purity 98.5%), dipalmitin ($\geq 99\%$), monostearin ($\geq 99\%$), distearin ($\geq 99\%$), monolinolein ($\geq 97\%$) and dilinolein ($\geq 97\%$), obtained from Sigma Aldrich. Phosphoric acid (reagent grade) was purchased from Sigma Aldrich. Ammonium acetate (chem. pure) was from Lamers & Pleuger. Water was prepared with a Millipore Simplicity MilliQ water system.

2.2. Paint samples

Paint reconstructions were prepared by mixing c. 1 g of pigment (titanium dioxide CR-826, Tronox) with c. 1 g of linseed oil (bleached linseed oil, van Beek). Paints were ground by mixing with an automatic paint miller. After mixing, the paint was applied on Melinex (polyester film) with a draw down bar, forming a layer of

Fig. 2. Overlaid extracted ion chromatograms of fresh linseed oil. The single ion chromatograms are based on the most prominent $[\text{M}+\text{Na}]^+$ or $[\text{M}+\text{NH}_4]^+$ ions. Compound identification is presented in Table 1.

Table 1

Most prominent compounds identified in fresh linseed oil. TAG = triglyceride, OX-TAG = oxidised triglyceride, n DB = number of double bonds in the fatty acid chains, n O = number of oxygen atoms incorporated in the fatty acid chains by oxidation. (TAG identification: P = palmitic acid, S = stearic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid. M: more than two TAG compositions possible). See Section 3.5 for the Mass spectrometric identification.

	retention time (min)	formula	structure	m/z	ion
1	5.22	C ₅₇ H ₉₄ O ₇	OX-TAG, 3C18, 8 DB, 1 O	913.9	[M+Na] ⁺
2	6.04	C ₅₇ H ₉₆ O ₇	OX-TAG, 3C18, 7 DB, 1 O	915.9	[M+Na] ⁺
3	7.00	C ₅₇ H ₉₈ O ₇	OX-TAG, 3C18, 6 DB, 1 O	912.9	[M+NH ₄] ⁺
4	8.12	C ₅₇ H ₁₀₀ O ₇	OX-TAG, 3C18, 5 DB, 1 O	919.9	[M+Na] ⁺
5	10.16	C ₅₇ H ₉₂ O ₆	TAG, 3C18, 9 DB (LnLnLn)	890.9	[M+NH ₄] ⁺
6	11.40	C ₅₇ H ₉₄ O ₆	TAG, 3C18, 8 DB (LLnLn)	892.9	[M+NH ₄] ⁺
7	12.46	C ₅₅ H ₉₄ O ₆	TAG, 2C18 + C16, 6 DB (PLnLn)	868.8	[M+NH ₄] ⁺
8	12.73	C ₅₇ H ₉₆ O ₆	TAG, 3C18, 7 DB (OLnLn/(LLnLn))	894.9	[M+NH ₄] ⁺
9	13.66	C ₅₅ H ₉₆ O ₆	TAG, 2C18 + C16, 5 DB (PLnLn)	870.8	[M+NH ₄] ⁺
10	13.97	C ₅₇ H ₉₈ O ₆	TAG, 3C18, 6 DB (M)	896.9	[M+NH ₄] ⁺
11	14.88	C ₅₅ H ₉₈ O ₆	TAG, 2C18 + C16, 4 DB (POLn/(PLL))	872.8	[M+NH ₄] ⁺
12	15.18	C ₅₇ H ₁₀₀ O ₆	TAG, 3C18, 5 DB (M)	898.9	[M+NH ₄] ⁺
13	15.97	C ₅₅ H ₁₀₀ O ₆	TAG, 2C18 + C16, 3 DB (M)	874.8	[M+NH ₄] ⁺
14	16.42	C ₅₇ H ₁₀₂ O ₆	TAG, 3C18, 4 DB (M)	900.9	[M+NH ₄] ⁺
15	17.14	C ₅₅ H ₁₀₂ O ₆	TAG, 2C18 + C16, 2 DB (POO/PSL)	876.8	[M+NH ₄] ⁺
16	17.42	C ₅₇ H ₁₀₄ O ₆	TAG, 3C18, 3 DB (M)	902.9	[M+NH ₄] ⁺
17	18.54	C ₅₇ H ₁₀₆ O ₆	TAG, 3C18, 2 DB (SSL/SOO)	904.9	[M+NH ₄] ⁺

Table 2

Comparison between method La Nasa and optimised method in this article. See Section 3.5 for the mass spectrometric identification.

Name	Short name	Method Di Nasa			Method optimised for hydrolysis and oxidation products		
		Retention time (min)	m/z	ions	Retention time (min)	m/z	ions
monolinolein	C18:2	1.86	731.58	[2M+Na] ⁺	14.53	377.28	[M+Na] ⁺
monoolein	C18:1	1.94	735.55	[2M+Na] ⁺	15.88	379.31	[M+Na] ⁺
monostearin	C18:0	2.04	739.62	[2M+Na] ⁺	16.72	381.32	[M+Na] ⁺
dilinolein	2C18:2	3.89	1256.01	[2M+Na] ⁺	23.27	639.51	[M+Na] ⁺
dipalmitin	2C16:0	4.83	1160.04	[2M+Na] ⁺	24.79	591.55	[M+Na] ⁺
diolein	2C18:1	5.11	638.55	[M+NH ₄] ⁺	25.6	643.57	[M+Na] ⁺
trilaurin	3C16:0	6.74	656.59	[M+NH ₄] ⁺	28.26	661.55	[M+Na] ⁺
distearin	2C18:0	6.96	647.59	[M+Na] ⁺	29.01	647.59	[M+Na] ⁺
trimyristin	3C14:0	11.92	740.71	[M+NH ₄] ⁺	39.5	740.71	[M+NH ₄] ⁺
tripalmitin	3C16:0	17.04	824.79	[M+NH ₄] ⁺	50.66	866.91	[M+60] ⁺
triolein	3C18:1	18.02	902.81	[M+NH ₄] ⁺	51.87	907.86	[M+Na] ⁺
tristearin	3C18:0	21.09	908.92	[M+NH ₄] ⁺			

200 µm. The paint films were left to dry under ambient conditions for one month.

Three historical, naturally aged paints were selected from Winsor & Newton artists' oil paints made and painted out in 1953. The paint swatches were obtained from Tate (London), that holds a large collection of these swatches donated by the paint company. The cobalt blue paint contained cobalt aluminate pigment bound in oil, extended with magnesium carbonate, and some zinc and calcium compounds. The cadmium yellow paint contained cadmium zinc sulphide in oil extended with barium sulphate, magnesium carbonate and aluminium stearate. Indian yellow paint contained iron oxide in linseed oil, magnesium carbonate and aluminium hydroxide [20,21].

Samples of the painting 'Z79' by Oldenhof were taken by mechanical removal of the yellow exudate, the surface layer and the bulk paint, using a scalpel.

2.3. Sample preparation

For the analysis of paint samples, approximately 0.1–0.5 mg of paint sample was extracted with 50 µl ethanol for 60 min at room temperature. After extraction, the sample was homogenised and centrifuged, using a National Labnet Co. minicentrifuge C-1200, for 1 min.

For the analysis of oil samples, 0.2 µl of oil was dissolved in 1 ml ethanol and diluted 10 times with ethanol. For HPLC–MS analysis, the extracts and solutions were mixed 1:1 v/v with ethanol.

2.4. Instrumental

HPLC was performed using a Waters HPLC system, equipped with a Waters 616 quaternary gradient pump, a Waters 717plus autosampler and a Waters temperature control module. The HPLC effluent was delivered to the MS system with a microprobe. Chromatographic separation was performed using a Phenomenex Luna C₁₈ column (100 × 2.00 mm, 3 µm particle size), equipped with a guard column, kept at 45 °C. The injection volume was 5 µl. The method developed by La Nasa and applied in this study involved a gradient of methanol and isopropanol: from 95% methanol to 5% methanol in 25 min, then held for 5 min, and re-equilibrated in 10 min. The flow was set at 0.3 ml/min. NH₄Ac was added post column to the HPLC effluent. A solution of 2 mM NH₄Ac pumped with 0.02 ml/min with a Waters 515 HPLC pump, and connected to the HPLC outlet tubing with a T-connector. Of the total HPLC effluent, 1/9th was delivered to the MS and 8/9th to waste. MS analysis was carried out with a Micromass Q-tof-2, equipped with an ESI source. Operating conditions were: desolvation gas: nitrogen, 150 °C, 2 l/min, nebuliser gas: nitrogen, 1.5 l/min, cone gas: nitrogen, 2 l/min, collision gas: Argon. The source temperature was set at 80 °C, cone voltage 30 V, capillary voltage 3.0 kV, collision energy 10 V. The mass axis was calibrated using phosphoric acid.

For the LC method optimised for the separation of polar compounds, the same setup was used, but the gradients were different: a gradient of water, methanol and isopropanol was used: starting with 30% eluent A (10% MeOH in H₂O) and 70% eluent B (MeOH), held for 5 min, in 2 min to 100% eluent B, kept at 100% eluent B for

Fig. 3. Overlaid extracted ion chromatograms, based on the most prominent $[M+Na]^+$ or $[M+NH_4]^+$ ions, of different mono-, di- and triglycerides. (a) Analysis performed with adapted method from La Nasa. (b) Analysis performed with method developed for analysis of polar compounds. Peak identification: see Table 2.

Fig. 4. BPI chromatogram of ethanol extract of cured oil paint. The paint contained titanium white and linseed oil paint without additives and had cured under ambient conditions for 199 days. Peak identification: see Table 3.

25 min, in 20 min to 60% eluent B and 40% eluent C (isopropanol), then held for 5 min and re-equilibrated in 10 min.

LC–MS data were collected and interpreted with MassLynx software. The chromatograms are displayed by the (overlaid) extracted ion chromatograms (EIC). Furthermore, the base peak

intensity chromatogram (BPI) is used, which gives more detail than the TIC. However, for quantitative information peak surface areas from extracted ion chromatograms are used. All chromatograms are smoothed for better display, either by a 2 time 10 point Savitsky Golay smoothing or 2 times 10 point FFT smoothing.

Table 3

Identified compounds in extract of cured oil paint. TAG = triglyceride, DAG = diglyceride, DiCn = diacid chain of n carbon atoms, OX-TAG = oxidised triglyceride, n DB = number of double bonds in the alkyl chain, n O = number of oxygen atoms incorporated in the alkyl chain.

	retention time (min)	structure	m/z (+)	ions (+) ^a	m/z (–)	ions (–)
1	1.77	Diacid-MAG, DiC9	285.2	[M+Na] ⁺	261.2	[M–H] [–]
2	1.90	Diacid-DAG, 2DiC8	427.3	[M+Na] ⁺		
3	1.90	Diacid-DAG, 2DiC9	455.2	[M+Na] ⁺	431.2	[M–H] [–]
4	2.01	Diacid-TAG, 3DiC8	583.3	[M+Na] ⁺		
5	2.05	Diacid-TAG, DiC9 + 2DiC8	595.3	[M+Na] ⁺		
6	2.17	Diacid-TAG, 2DiC9 + DiC8	611.3	[M+Na] ⁺		
7	2.30	Diacid-TAG, 3DiC9	625.3	[M+NH ₄] ⁺		
8	4.40	OX-MAG, C18, 2 DB, 1 O	393.3	[M+Na] ⁺		
9	4.50	OX-MAG, C18, 1 DB, 1 O	395.3	[M+Na] ⁺		
10	15.00	MAG, C16	353.3	[M+Na] ⁺		
11	15.39	MAG, C18:1	379.3	[M+Na] ⁺		
12	15.55	Diacid-OX-TAG, C18 + 2DiC9, 2 DB, 1 O	733.5	[M+Na] ⁺		
13	16.32	Free FA, C16			255.2	[M–H] [–]
14	16.51	Diacid-DAG, C16 + DiC8	509.4	[M+Na] ⁺	485.4	[M–H] [–]
15	16.69	Free FA, C18:1			281.3	[M–H] [–]
16	16.81	Diacid-DAG, C16 + DiC9	523.4	[M+Na] ⁺	499.4	[M–H] [–]
17	17.13	Diacid-DAG, C18:1 + DiC9			525.4	[M–H] [–]
18	17.2	Diacid-OX-TAG, C18:1 + C16 + DiC9 1 O	803.5	[M+Na] ⁺		
19	17.87	Diacid-TAG, C16 + 2DiC8	665.5	[M+Na] ⁺		
20	17.96	Free FA, C18			283.3	[M–H] [–]
21	18.21	Diacid-DAG, C18 + DiC9	551.4	[M+Na] ⁺	527.4	[M–H] [–]
22	18.21	Diacid-TAG, C16 + DiC9 + DiC8	679.6	[M+Na] ⁺		
23	18.54	Diacid-TAG, C16 + 2DiC9	693.5	[M+Na] ⁺	669.5	[M–H] [–]
24	19.55	Diacid-TAG, C18 + DiC9 + DiC8	707.6	[M+Na] ⁺		
25	19.72	Diacid-TAG, C18 + 2DiC9	721.6	[M+Na] ⁺	697.5	[M–H] [–]
26	20.30	OX-DAG, C18 + C16, 1 DB, 1 O	633.6	[M+Na] ⁺		
27	20.85	Diacid-Short Chain-TAG, C16 + DiC9 + C8			625.4	[M–H] [–]
28	21.88	Diacid-OX-TAG, C18:1 + C16 + DiC9 1 O			779.6	[M–H] [–]
29	22.30	OX-DAG, 2C18, 1 DB, 2 O	677.6	[M+Na] ⁺		
30	22.35	Diacid-OX-TAG, C18 + C16 + DiC9, 2DB, 1 O	801.6	[M+Na] ⁺		
31	23.83	Diacid-OX-TAG, 2C18 + DiC9, 2 DB, 1 O	829.7	[M+Na] ⁺		
32	24.63	DAG, 2C16	591.5	[M+Na] ⁺		
33	24.97	DAG, C18:1 + C16	617.6	[M+Na] ⁺		
34	25.35	DAG, 2C18:1	643.6	[M+Na] ⁺		
35	26.24	Diacid-TAG, 2C16 + DiC8	747.7	[M+Na] ⁺		
36	26.43	DAG, C18 + C16	579.6	[M+NH ₄ –NH ₃ –H ₂ O] ⁺		
37	26.67	Diacid-TAG, 2C16 + DiC9	761.6	[M+Na] ⁺	737.6	[M–H] [–]
38	26.85	DAG, C18:1 + C18	645.6	[M+Na] ⁺		
39	27.31	Diacid-TAG, C18:1 + C16 + DiC9	787.6	[M+Na] ⁺	763.6	[M–H] [–]
40	27.74	Diacid-TAG, 2C18:1 + DiC9			789.6	[M–H] [–]
41	28.15	OX-DAG, 2C18, 1 DB, 1 O	656.6	[M+NH ₄] ⁺		
42	28.39	Diacid-TAG, C18 + C16 + DiC8	775.6	[M+Na] ⁺		
43	28.75	Diacid-TAG, C18 + C16 + DiC9	789.8	[M+Na] ⁺	765.6	[M–H] [–]
44	28.78	DAG, 2C18	647.6	[M+Na] ⁺		
45	29.37	Diacid-TAG, C18 + C18:1 + DiC9			791.6	[M–H] [–]
46	31.18	Diacid-TAG, 2C18 + DiC8	803.7	[M+Na] ⁺		
47	31.90	Diacid-TAG, 2C18 + DiC9	817.7	[M+Na] ⁺	793.6	[M–H] [–]
48	44.44	TAG, 3C18, 8 DB	892.9	[M+NH ₄] ⁺		
49	45.20	OX-TAG, 3C18, 3 DB, 1 O	918.9	[M+NH ₄] ⁺		
50	47.48	TAG, 3C18, 7 DB	899.8	[M+Na] ⁺		
51	49.99	OX-TAG, 3C18, 1 DB, 1 O	927.8	[M+Na] ⁺		
52	51.04	TAG, C18:1 + 2C16	855.8	[M+Na] ⁺		
53	51.39	TAG, 2C18:1 + C16	881.8	[M+Na] ⁺		
54	51.80	TAG, 3C18:1	907.8	[M+Na] ⁺		
55	53.00	TAG, C18 + C18:1 + C16	883.8	[M+Na] ⁺		
56	53.37	TAG, C18 + 2C18:1	909.9	[M+Na] ⁺		
57	54.82	TAG, 2C18 + C18:1	911.9	[M+Na] ⁺		

^a The molecular ions listed here are the most prominent in the spectrum. Other ions are formed as well (see 3.5 Mass spectrometry).

3. Results and discussion

3.1. Analysis of linseed oil

The method as published by La Nasa was adapted and optimised. The main adjustments were (a) the use of a normal column instead of a core shell column; and (b) the application of post column mixing of NH₄Ac to facilitate ion formation.

The results from the analysis of fresh linseed oil (from unwashed, windmill pressed *Sophie Flax seeds*) are displayed in the form of overlaid extracted total ion chromatograms in Fig. 2. Assigned triglyceride (TAG) components of the peaks are presented

in Table 1. On the fatty acid level, linseed contains c. 10–11% saturated fatty acids (P; palmitic and S; stearic acid), 19–22% oleic acid (O; one double bond (DB)), 14–17% linoleic acid (L; 2 DBs) and 52–55% α-linolenic acid (Ln; 3DBs) [22]. The fatty acids have preferences for the position in the triglyceride, but this effect is not measurable with the methods commonly used in conservation science and lost in the course of ageing [23]. With the LC–MS analysis, mainly TAGs with alkyl chains lengths of 16 and 18 carbon atoms and 2–9 double bonds were identified, in relative amounts that are in accordance with literature values for fresh linseed oils. It should be noted that in case of some triglycerides several isomers are possible. This is the case, for example, for peak 10 in Fig. 2, which is a

Fig. 5. BPI chromatograms in positive mode of extracts of three Winsor & Newton paint outs created in 1953. From top to bottom: Cobalt blue, Cadmium yellow and Indian Yellow. Peak identification is listed in Table 3.

TAG with 3 fatty acids with 18 carbon atoms and 6 double bonds, which could be of the LLL, OLLn or SLnLn configuration. In addition, e.g. the SLnLn has two isomers, with the stearic acid on the side or in the middle. Although all conformations probably have a similar polarity, a peak broadening may be expected.

In addition to the original glycerides, a small fraction of oxidised TAGs was detected. The chromatogram shows that oxidation of a TAG has a great effect on its retention time. For example, if compound 1 (3C18, 8DB, 10) and compound 6 (3C18, 8DB) are compared, a retention time difference of 6 min is observed. This is due to the increased polarity of the triglyceride after oxidation.

Table 1 shows that the assignment of the oxidised TAGs is based on Na^+ adduct ions, while the original TAGs are represented by their NH_4^+ ions. This particular feature of the ESI mass spectrometry in the present system is discussed in Section 3.5.

3.2. Comparison method La Nasa with method Van Dam

The LC–MS method described by La Nasa et al. enables the analysis of relatively non-polar triglycerides in oil paint primarily. However, for the chromatographic separation of hydrolysis and oxidation products that are formed e.g. in dried oil paints, this method is not sufficient.

Fig. 3a presents the LC–MS analysis of different standards of mono-, di-, and triglycerides performed with the adjusted La Nasa method. The chromatogram shows that particularly the mono- and diglycerides are not well separated, implying that more polar oxidised and hydrolysed compounds will not be separated at all.

Therefore, the method was modified employing a more polar LC gradient as described in the method Section 2.4 and displayed in Fig. 3b. The results obtained with both methods are summarised in Table 2.

Interestingly, although the method is not quantitative and subject to variation in e.g. ionisation efficiency between runs, the new method also shows improved separation with narrower and thus more intense peaks for the more polar compounds. With the new method, the limit of detection was determined for a selection of standards. The observed limits of detection are: monoolein 0.045 µg/ml, monostearin 0.115 µg/ml, diolein 0.048 µg/ml, distearin 0.121 µg/ml, tripalmitin 0.364 µg/ml and triolein 0.024 µg/ml.

The feasibility of the new method for the analysis of degradation compounds in cured oil paints was evaluated with an extract of dried oil paint. The BPI chromatogram of this measurement is shown in Fig. 4 and peaks are assigned in Table 3.

With the new method, a fair separation of the different compounds in the dried oil paint extract was achieved. In this paint 27 compounds could be identified. Most of the identified compounds are diglycerides and triglycerides containing at least one diacid moiety, primarily azeleic acid (nonanedioic acid). Furthermore, other oxidised fatty acid containing glycerides were detected containing hydroxyl or epoxy fatty acid moieties which are commonly found in cured oil paints, in addition to some unreacted triglycerides. Especially the 9,10 epoxy- and the 9,10-dihydroxy stearic acids (corresponding to peak 14 and 25, respectively in Fig. 4 and Table 3) are commonly found using more traditional GCMS methods [3].

This analysis shows that extensive oxidation had occurred during the over six months drying time, but little hydrolysis.

In the course of time, further oxidation and hydrolysis will certainly take place. To show this, the method was tested on older artists' quality oil paint films made by the paint manufacturer Winsor & Newton in 1953. Three different colours were tested: cobalt blue, cadmium yellow and Indian yellow. Fig. 5a shows the BPI chromatograms of these three paints in positive mode.

As expected, the paints have both oxidised and hydrolysed to a further extent than the young paint presented in Fig. 4. In the paints, a total of 23 compounds could be identified. The identified compounds are summarised in Table 3. These chromatograms reveal that the degradation products of the cobalt blue paint are very different than that of the Indian yellow and cadmium yellow paints. Cobalt blue contains mostly diacids-DAG and DAG compounds, all fully saturated. This means that all the original double bonds in the extractable components in the oil paint have reacted. The Indian yellow and cadmium yellow paints however, contain unsaturated diacid-DAG, DAG and TAG compounds. This difference can be explained by the catalytic properties of Co, which acts as a surface drier. The TAG compounds are present in a higher concentration in cadmium yellow than in Indian yellow. The results are largely confirmed by analysis using direct ESI-MS [9].

3.3. W&N negative mode

Analyses were also performed in negative mode, which gives a higher sensitivity and single molecular ions for organic acids such as the diacid containing glycerides described above. The BPI chromatograms in negative mode are shown in Fig. 5b. In negative mode, 11 compounds were identified. The identified compounds are listed in Table 3.

With the measurements performed in negative mode, fewer compounds are detected than with positive mode. This is because in negative mode, only compounds containing carboxylic acid groups are detected (free fatty acids and diacid compounds).

The cobalt blue paint shows the most pronounced differences in negative mode. In cobalt blue, diacid-MAG, diacid-DAG and free fatty acids are found. In Indian yellow and cadmium yellow, also diacid-TAG compounds were found. This means that in cobalt blue paint, more hydrolysis has taken place. In Indian yellow and cad-

Fig. 6. 'Z79' by Erik Oldenhof (c.2005). (a) detail, x 6.8 × 8.4 cm, (b) micrograph showing the yellow exudation of oil on the paint surface, 1.9 × 2.5 mm. (Photographs Sarah Bayliss).

mium yellow paint, the same compounds have been detected, but in different ratios. In cadmium yellow, the relative amount of the diacid-TAG compounds is lower, meaning that Indian yellow is more hydrolysed than cadmium yellow. These results are consistent with analyses using direct ESI-MS [9].

In summary, in both positive and negative mode the observation was made that in cobalt blue the most hydrolysis has taken place, less hydrolysis occurred in Indian yellow, and the least hydrolysis has taken place in cadmium yellow. In addition, positive mode measurements show a high abundance of compounds with one double bond. This observation was not confirmed in negative mode since most compounds with double bonds do not contain carboxylic (di)acid moieties.

3.4. Oxidation profiles in oil paint: surface, bulk and medium exudation in a painting by Erik Oldenhof

The LCMS method was used for analysis of a painting 'Z79' by Dutch painter Erik Oldenhof (Fig. 6). On certain areas in this work, where paint was thickly applied, yellow exudate had formed as a consequence of the specific storage conditions of the work. After creating the painting in c. 2005, and after leaving it to dry for several months in the light, the painting was stored in the dark, wrapped in bubble wrap. In 2012, yellow exudates had formed. Bayliss et al.

Fig. 7. BPI chromatograms (a) positive and (b) negative mode) of extracts of different parts of the painting Z79 by Erik Oldenhof. From top to bottom: bulk of the paint, paint surface and the yellow exudate. The identified peaks in this chromatogram are listed in Table 3.

Fig. 8. Mass spectra (analysis with method for polar compounds) in positive mode of (A) monoolein (C18:1), (B) diolein (2C18:1) and (C) triolein (3C18:1).

Table 4

Intensities and ratios of selected peaks in LCMS spectra from 'Z79' and reconstructions (Rec.) stored in dark and light after curing. Negative mode, areas of extracted ion chromatograms.

Peak number	13	20	16	21	23	25	P/S ratio		
ID	Free FA	Free FA	Diacid-DAG	Diacid-DAG	Diacid-TAG	Diacid-TAG	Free FA (13/20)		
Sample	C16	C18	C16 + DiC9	C18 + DiC9	C16 + 2DiC9	C18 + 2DiC9	DAG (16/21)		
Oldenhof Exudate	131,86	139,92	239,48	79,30	42,16	11,31	0,94	3,02	3,73
Oldenhof Surface	18,66	29,02	34,27	11,24	11,10	1,73	0,64	3,05	6,42
Oldenhof Bulk	24,04	41,44	34,29	11,59	5,42	2,77	0,58	2,96	1,96
Rec. Dark Exudate	98,99	214,47	161,59	56,73	142,16	47,83	0,46	2,85	2,97
Rec. Dark Surface	16,25	49,10	34,15	11,11	65,27	22,61	0,33	3,07	2,89
Rec. Dark Bulk	35,24	93,89	41,00	14,15	7,85	4,33	0,38	2,90	1,81
Rec. Light Surface	21,24	65,65	32,17	11,01	29,29	9,40	0,32	2,92	3,12
Rec. Light Bulk	33,96	87,71	47,99	20,03	8,54	4,15	0,39	2,40	2,06

[11] concluded that this exudation was caused by the lack of light causing the medium in the not fully dried, thick paint to exude. This was confirmed by a series of paint reconstructions of Titanium white tube paints, which showed the same phenomenon on thickly applied paints stored in the dark, whereas reconstructions stored in ambient light showed no exudation.

The chemical composition of the exudate, paint surface and bulk paint of 'Z79', the exudate, paint surface and bulk paint of the reconstruction stored in the dark and the paint surface and bulk paint of the reconstruction stored in the light were analysed with LC-MS. The results for 'Z79' are given in Fig. 7a (positive) and b (negative). The identified peaks in this chromatogram are listed in Table 3.

In the yellow exudate of 'Z79', relatively high amounts of small diacid containing glycerides (**1** DiC9, **2** 2DiC8 and **3** 2DiC9; **23** C16 + 2DiC9, **37** 2C16 + DiC9, **39** C18:1 + C16 + DiC9) are found in the positive mode, some of which are not found in the surface and bulk. On the paint surface and in the bulk paint, relatively high amounts of unsaturated, oleic acid (C18:1) containing glycerides are detected (**11** C18:1, **33** C18:1 + C16, **34** 2C18:1), while these are hardly detected in the exudate.

Consistent with the information obtained in positive mode, small diacid compounds (**1** DiC9, **3** 2DiC9) are found relatively prominently in the negative mode.

Glycerides from different plants and animals can be distinguished based on their respective palmitic (C16)/to stearic (C18) or P/S ratios as these are generally the only acids to survive ageing in paints [24]. Metal stearates contain high relative proportions of stearic acid, with a P/S ratio of c. 0.7, whereas lipids have higher P/S ratios of e.g. 1.4 (linseed) and 2.2 (safflower) [25]. The P/S ratios in combined MS, more specifically LCMS, analysis of glycerides and free fatty acids can be thus used to distinguish triglyceride lipids from metal stearate extenders [14,26,27].

In the present results, the free fatty acid C16 (**13**) content is higher in the exudate, while the other free fatty acids (**15** C18:1, **20**

C18) are present in abundance in the bulk of the paint. The P/S ratios are as a result lower in the bulk of the paint than in the exudate whereas the P/S ratio of the measured from a set of glycerides is more or less the same in every case, which is consistent with the use of safflower oil (Table 4). This is consistent with earlier findings based on GCMS [11] and indicates that in the process of exudation the oil components have to some extent separated from the metal stearates which have mostly stayed within the bulk of the paint.

The BPI chromatograms (not shown) of the paint reconstructions stored in the dark show a similar trend. Again, the exudate shows relatively more small diacid compounds (**1** DiC9, **2** 2DiC9 etc.) compared to the surface and bulk. Furthermore, the trend in P/S ratios is the same. For the reconstructed painting stored in the light throughout the process, there is no exudate. The surface shows a much more oxidised surface; however, there is no clear trend indicating separation of oil and metal stearates. It should be noted, however, that the sampling of the surface paints is difficult since the surface layer is very thin and can never be fully separated from the bulk paint.

3.5. Mass spectrometry; positive ion formation in triacylglycerides and their hydrolysis and oxidation products

The HPLC-ESI-MS method described in this paper involves post-column addition of NH₄Ac to facilitate the formation of positive and negative ions. Negative ions ([M-H]⁻) will be formed from proton abstraction from free fatty acids and azelate or other diacid containing glyceride oxidation products.

While for negative ions, the ion formation is straightforward, the adduct ions in the positive mode show a different pattern. In addition to NH₄⁺ adducts ([M+18]⁺), also Na⁺ adducts are formed ([M+23]⁺) and occasionally even Li⁺ adducts ([M+6]⁺). The metal ions come from traces of corresponding salts in the eluent, and may vary. Within chromatograms, mass spectra resulting from stan-

dards of mono-, di- and triglycerides show different ratios of the adduct ions. Fig. 8 shows the comparison of the MS spectrum of monoolein, diolein and triolein.

Interestingly, the monoglycerides show mainly Na^+ and Li^+ adducts whereas ammonium adducts for the diglycerides, ammonium adducts become more prominent, also accompanied by expulsion of water and NH_3 : $[\text{M}+\text{NH}_4-\text{NH}_3-\text{H}_2\text{O}]^+$. The triglycerides show a yet unknown adduct, $[\text{M}+60]^+$; its even mass indicates that the ion probably contains one ammonium group. All spectra presented in this paper show that Na^+ adduct ions are formed mostly from molecules high in hydroxyl and carboxylic acid functionalities, whereas ammonium adducts form preferentially with non-polar glycerides.

4. Conclusions and outlook

This paper describes the development of an LC–MS method for the analysis of lipids and their degradation products in both fresh oils and extracts of cured oil paint. The method was successfully applied to a range of cured paints from young (200 days) to old (62 years) age. With this method, triglycerides, diglycerides, monoglycerides, free fatty acids, oxidised fatty acids and diacids were identified in paint films. The analysis in both positive and negative mode give partly complementary information from the same sample; in positive mode the triglycerides are preferentially ionized and detected, but not free fatty acids, for example; in the negative mode, only free fatty and diacids are detected together with diacid containing glycerides.

The internal ratios of peaks can be used to give more information on the type of oil used, the state of oxidation and hydrolysis, both in the paint and towards the surface. Because free fatty acids and glycerides are analysed simultaneously, the P/S ratio of both can be calculated separately giving a good indication of the type of oil as well as the presence of metal stearate additives.

It was found that Na^+ adduct ions are formed mostly from molecules high in hydroxyl and carboxylic acid functionalities, whereas ammonium adducts form preferentially with non-polar glycerides. Whether this would warrant standard addition of low amounts of sodium salts to increase the sensitivity of polar glycerides in the positive mode.

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