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Identification of drying oils used in pictorial works of art by liquid chromatography of the 2-nitrophenylhydrazides derivatives of fatty acids

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Abstract

A new HPLC-UV-Vis method for identification of drying oils from binding media or protective film used in pictorial works of art prior to conservation or restoration is proposed. Chromophore derivatization of fatty acids released by hydrolysis of structural drying oils is studied. The derivatization reagent selected was 2-nitrophenylhydrazine with 1-ethyl-3-(3-dimethyl animopropyl)carbodiimide hydrochloride/pyridine as catalyst. This reaction was carried out using microwave heating. Mobile phase was methanol/water/n-propanol/acetic acid (80:14:5:1) running in isocratic mode. Absorbance was measured at 400 nm. In these conditions, hydrazides of myristic, palmitic, oleic, and stearic acids were satisfactorily resolved. Method shows good sensitivity, with a detection limit of 15 μmol l^{-1}, and good linearity between 0.03 and 3 mmol l^{-1}. Peak area ratios among fatty acids derivatives allows identification of the drying oils. The stearic/palmitic ratio is the most important, because it allows to differentiate among the different drying oils. The proposed method has been successfully applied to real samples from items of the cultural heritage of Valencia (Spain).

Keywords: HPLC; Drying oils; Fatty acids; Derivatization; Art analysis

1. Introduction

To preserve and restore pictorial works of art successfully, information on the materials used by the original artist is of the utmost importance. However, these materials undergo degradation processes difficult to model, so it is difficult to know which substances were present in the original material from an ancient pictoric work of art. The difficulty is further increased by complexity of the sample as well as the small size usually available [1].

Drying oils, which operate as protective film and binding consolidants, are among the often used materials in pictorial works of art. Although the most usual oil is linseed (depending on period and pictorial technique), poppy seed, nut, and sunflower oils have also been used. These oils are formed by triglycerides, triple glycerol ester with several fatty acids (mainly myristic, palmitic, oleic, and stearic). Each drying oil is identified by relative amounts of its fatty acids. When oil deteriorates due to, for example, passage of time and environmental action, the fatty acid ratio also changes [2].

Drying oils have been widely studied by analysis of their fatty acids. Spectrophotometric techniques such as FTIR [3] or Raman [4] and electrochemical methods [5] discover the presence of lipidic substances in the ancient pictoric work of art but they usually do not make it possible to identify each drying oil. For oil identification separation methods such as GC-FID [6], GC-MS [7], and pyrolysis [8] must be used. Triglyceride hydrolysis is carried out in order to release the fatty acids and measure the relative amounts. However, until now HPLC has not been used to analyze this type of samples.

Free carboxylic acids do not contain chromophore groups, therefore, a derivatization reaction in order to created an UV-Vis absorbing agent is needed. Derivatization reagents such as *p*-phenazophenacyl [9], 1-chlormethylsatin [10],

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phenacyl [11], naphtacyl [12], *p*-methoxyanilines [13], 2-(phthalimino)ethyl [14], and nitrobenzyl [15] were used. These methods, however, have not always been satisfactory with regard to sensitivity, separation, and analysis time. The derivatization reagent tested here is 2-nitrophenylhydrazine, employed in fatty acids analysis [16], which provides good sensitivity [17].

Chromatographic separations of fatty acid derivatives are usually performed on reversed-phase columns with isocratic or gradient elution systems using methanol and water in various proportions [18–21]. The elution volumes of the fatty acid derivatives are affected principally by the number of carbon atoms and the number of unsaturated bonds in the aliphatic acids chain [18–21].

This paper presents an analytical study of drying oils by analysis of the fatty acids, obtained by acid hydrolysis of the oils, using HPLC by UV-Vis detection, with the purpose of obtaining best resolution of peaks and detector selectivity (FID) than with gas chromatography methods.

Drying oils employed in painted works of art were identified by calculation of the relative concentration of fatty acids in the sample and comparison with the with relative amounts found in natural 3-years-aged standard drying oils and in standard drying oils aged by thermic or UV irradiation attack [6]. Real samples were taken from the surface of several pictorial works of art from the cultural heritage of Valencia (Spain).

2. Experimental

2.1. Reagent solutions

A 0.02 mol l^{-1} solution of 2-nitrophenylhydrazine (2-NPH) 97% purity (Aldrich, Steinheim, Germany) was prepared by dissolving the reagent in acetonitrile/0.1 mol l^{-1} HCl methanolic (50:50 v/v). A 0.25 mol l^{-1} solution 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (1-EDC·HCl) synthesis grade (Merck, Darmstadt, Germany) was prepared by dissolving the reagent in a 3% v/v solution of pyridine in ethanol. These reagent solutions were kept below 5 °C. A 15% w/v potassium hydroxide solution (analytical grade, Merck) in methanol/water (80:20, v/v) and a 3 mol l^{-1} HCl solution (analysis grade, Carlo Erba, Val de Reuil, France) in water were prepared.

Standards of oleic acid (C18:1) (95% purity) was from Fluka (Switzerland). Standards of myristic (C14:0), palmitic (C16:0), stearic (C18:0), and arachidic (C20:0) acids (all 99% purity) were obtained from Sigma (Steindheim, Germany).

Methanol used in chromatographic separation is HPLC grade (Carlo Erba). Propanol is also HPLC grade (Scharlau, Barcelona, Spain). Acetic acid used to acidify the mobile phase is 99.99% pure (Aldrich). Hexane is analysis grade (Fisher Chemicals, Loughborough, UK). Ethanol used is HPLC grade (Scharlau). Water (nanopure II grade) was generated in laboratory by nanopure water production device (Sybrom, Barnstead).

2.2. Standards and solutions

Myristic acid (M), palmitic acid (P), oleic acid (O), stearic acid (S), and arachidic acid (Ara) were dissolved in ethanol in order to make a 250 mmol l^{-1} solution of each carboxylic acid.

Standards of the most usual drying oils (linseed oil, poppy seed oil, and nut oil from Kremer Pigments Germany) have been analyzed. To assess how each fatty acid can be affected by time ageing, standards were aged by two kinds of accelerated artificial ageing: thermic treatment and UV irradiation treatment.

Thermal ageing was carried out by putting the drying oils on slides into a Dycometal DI-100 climatic chamber at 50 °C with 40% relative humidity for 14 days [22].

For ultraviolet irradiation ageing, the slides with the drying oils were irradiated with OSRAM L36/37 fluorescent lamp at 350–400 nm and 36 W, for 4 weeks at a distance of 12 cm [23].

Natural ageing was made keeping the drying oils on slides for 3 years without any treatment.

All used glassware were washed with sulfochromic mixture, distilled water, acetone, and dried at 110 °C to avoid possible contamination.

2.3. Artistic samples

Real samples were taken from the surface of pictorial works of art with a scalpel. The following pieces from artistic heritage of Valencia (Spain) were analyzed.

Sample 1: Crucifixion, 17th century, anonymous, but assigned to Tintoretto.

Sample 2: Saint Therese, 18th century, anonymous.

Sample 3: Saint Martha image, 16th century, anonymous.

Sample 4: Unclassified and strongly damaged work, Yañez de la Almedina, 16th century (Valencia Cathedral).

Sample 5: Unclassified and strongly damaged work, Yañez de la Almedina, 16th century (Valencia Cathedral).

2.4. Apparatus

Chromatographic analysis was carried out using a Model 1100 liquid chromatograph (Agilent Technologies, Waldbrom, Germany) equipped with isocratic pump and UV-Vis variable detector. Signals were processed by the Agilent Chemstation for LC, Rev. A 08.03-847.

The separation of fatty acids was achieved in a Zorbax XDB-C8 C_8 main column (15 cm × 4.6 cm i.d., particle size 5 μm). The mobile phase used was the mixture

MeOH/H_2O/*n*-propanol/acetic acid 80:14:5:1. The pump worked in isocratic mode at 1.2 ml min^{-1}. The UV-Vis detector was monitored at 400 nm.

2.5. Hydrolysis of drying oils

Drying oils standards (0.5–1 mg) were placed in a 0.3 ml microvessel (Supelco, Bellefonte, USA) and treated with 100 μl of 6 mol l^{-1} HCl for 24 h at 110 °C, avoiding HCl vaporization [6]. After cooling, 250 μl of hexane was added and the mixture was shaken in order to favor extraction. Organic phase was separated and other two extractions were done again by adding 250 μl of hexane to aqueous phase and shaking. Finally, the three hexane phases were mixed in a Pyrex test tube (Bibbi Sterilin, Staff, UK) and dried at 70 °C. The residue obtained was dissolved in 0.1 ml of ethanol, heated in microwave oven (Firstline, 2450 MHz, 1300 W) at 20% power for 1 min. After cooling the mixture was ready to be derivatized.

For real sample analysis, solid parts scalpered from the pictoric work of art (0.5–1 mg) were derivatized in the same way as drying oils standard.

2.6. Derivatization procedure

To 100 μl of standard fatty acid solution (prepared in Section 2.2) [24] or hydrolyzed drying oils standard ethanolic solution or real sample ethanolic solution (prepared in Section 2.5), 100 μl of 2-NPH solution and 200 μl of 1-EDC·HCl solution were added [16,17]. The mixture was heated in a microwave oven (Firstline, 2450 MHz, 1300 W), introducing a vessel filled with 250 ml of water, at 20% power (260 W) for 6 min. After the addition of 50 μl of 15% KOH solution, the mixture was further heated under the same conditions for 3 min. A further 50 μl of a 3 mol l^{-1} HCl solution was added in order to neutralize the solution. Finally, the solution was centrifuged at 5000 rpm for 2 min and cooled in running tap water. An aliquot of 20 μl of the resulting hydrazyde mixture was injected directly into the chromatograph.

3. Results and discussion

3.1. Optimization of the derivatization conditions

Derivatization conditions we describe achieve sensitivity, specificity, and security adequate to carry out the analysis of sample.

The medium used for the derivatization reaction is a significant parameter. Ethanolic solutions of carboxylic acids react readily with 2-NPH·HCl dissolved in water using 1-EDC·HCl as a coupling agent and pyridine as a catalyst to give non-volatile acid hydrazides [24] in an alcoholic media. Reagent 2-NPH used in this work is not soluble in ethanol, thus, these derivatization conditions cannot be used.

A study of this reaction was carried out in some solvents, 2-NPH was found to be soluble in acetonitrile/methanol (50:50, v/v) mixture, so the reaction can be studied in alcoholic medium. Derivatization of 0.25 mmol l^{-1} ethanolic solution of myristic acid gives a peak area of 38 ± 7 in chloroform medium, and a peak area of 185 ± 3 was found in acetonitrile/methanol medium. No reaction is evident in acetone medium.

To provide good sensitivity low pH in reaction medium is needed. Thus, 2-NPH was finally dissolved in acetonitrile/0.1 mol l^{-1} methanolic HCl (50:50, v/v). At pH 7, derivatization of 0.25 mmol l^{-1} ethanolic solution of myristic acid provides a peak area of 63 ± 8, and at pH 2 a peak area of 185 ± 3 was found.

Microwave is used instead of water bath [24] for the reason that it is more repeatable and speed reaction is improved. Peaks obtained are higher and more precise. Twenty percent power was used, because it is a low power and is easily reached by the microwave oven. Heating time is also an important parameter. Peak areas for fatty acids became constant at heating times higher than 4 min, which suggest that maximum derivatization is accomplished at this time (see Fig. 1). Using a safe reaction time of 6 min, the carboxylic acids were converted into hydrazides without any deterioration. Derivatization of 0.25 mmol l^{-1} ethanolic solution of myristic acid by heating in water bath for 20 min at 60 °C

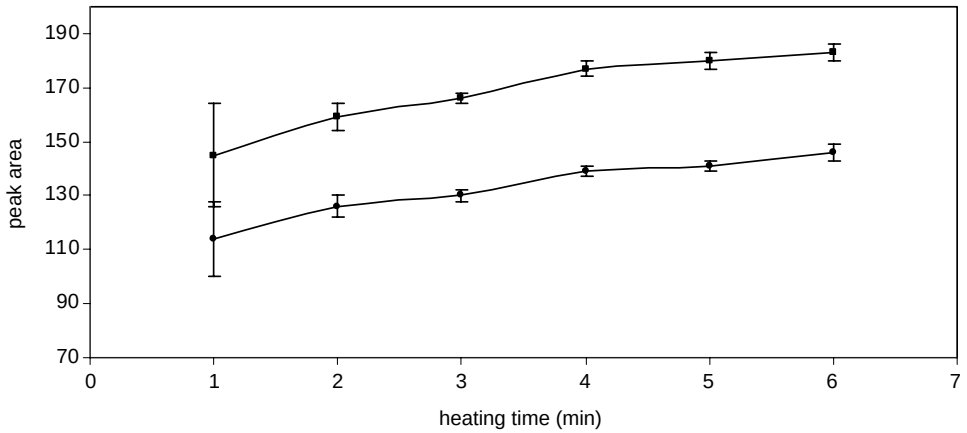

Fig. 1. Peak area values of the 2-NPH derivatives of palmitic (■) and stearic (●) acids at each heating time using microwave oven.

[24], provided a peak area of 170 ± 15. A peak area of 185 ± 3 was obtained by microwave heating under the above explained conditions.

After derivatization excess reagent and reaction by-products are destroyed by the addition of 2.7 mol l^{-1} KOH solution to the mixture [24]. If they are not destroyed, they provoke an elevation of the baseline at roughly 6 min, which interferes with the measurement of some analyte peaks. Elevation of baseline shows a stabilization around 3–4 min of heating, and sensitivity diminishes when heating time raises (see Fig. 2). At 3 min the baseline obtained is adequate, so it has been taken as the optimum time. The addition of the same quantity of 3 mol l^{-1} HCl to neutralize the mixture and avoid column deterioration is strongly recommended.

After derivatization a white precipitate due to the formation of KCl, which is insoluble in the reaction mixture, appears. To prevent this suspension from being injected into the chromatographic system, it is sufficient to centrifuge the mixture and separate the two phases.

3.2. Chromatographic separation

Retention times for these analyte derivatives decrease when the methanol level in the mobile phase increases [17]. However, trials using methanol/water mixtures showed high retention time, which is useless in chromatographic analysis, even at high amounts of methanol. To shorten retention time the polarity of the mobile phase is decreased by adding an alcohol with a longer aliphatic chain. With 5% 1-propanol in the mobile phase, peak retention times are considerably shortened, with good separation among them.

Absorption spectra of hydrazides depends on pH of media [24]. At pH > 12 hydrazides ionize which produces a deep violet color. At pH < 8.5 the absorption of hydrazides shifts to the blue region. When using chromatographic columns, pH must be maintained between 3 and 8 to avoid column damage. In this work, the mobile phase was acidified to pH 3.8 by adding acetic acid.

The choice of wavelength detection is essential for fatty acid determination. All of the carboxylic acid hydrazides show absorption maxima at 400 nm in acid medium [16], and were detectable spectrophotometrically by monitoring at this wavelength. These derivatives also showed strong absorption around 230 nm, and were monitored with a UV detector, with a sensitivity four times higher than at 400 nm. However, at 230 nm, the reaction by-products and reagent in excess interfere with the derivatived analyte [25], producing a significant elevation of the baseline after approximately 6 min, impairing the measurement of area peaks corresponding to some fatty acids [24]. Moreover, at 230 nm both noise and signal increase, therefore, the signal-to-noise ratio is not improved.

3.3. Assay with fatty acids

Fatty acids mainly found in drying oil used in pictorial works of art are myristic, palmitic, oleic, and stearic acids. Analytical parameters were determined using 250 mmol l^{-1} arachidic acid (Ara) as internal standard. Method was tested

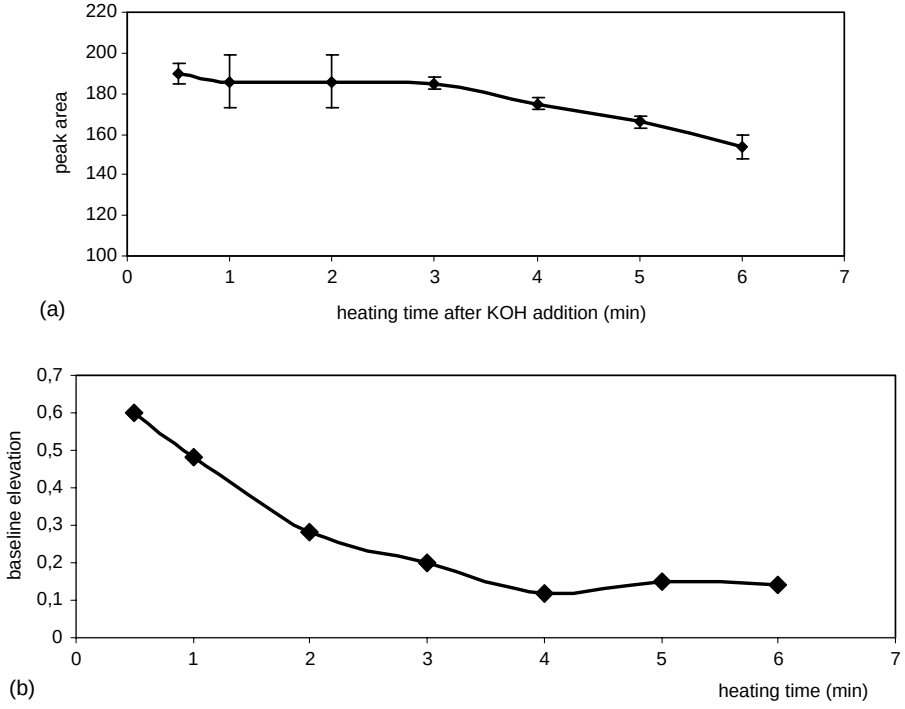

Fig. 2. (a) Peak area values of the 2-NPH derivatives of myristic acid at each heating time after KOH addition and (b) baseline elevation at each heating time after KOH addition, using microwave oven.

for myristic, palmitic, oleic, and stearic acid and were found linear between 0.03 and 3 mmol l^{-1}, following the equations:

$$\frac{A(\mathrm{M})}{A(\mathrm{Ara})} = (3.20 \pm 0.03)[\mathrm{M}] + (-0.01 \pm 0.04) \text{ with } R^2 = 0.9993,$$

$$\frac{A(\mathrm{P})}{A(\mathrm{Ara})} = (3.19 \pm 0.04)[\mathrm{P}] + (-0.02 \pm 0.05) \text{ with } R^2 = 0.9991,$$

$$\frac{A(\mathrm{O})}{A(\mathrm{Ara})} = (2.69 \pm 0.01)[\mathrm{O}] + (0.03 \pm 0.05) \text{ with } R^2 = 0.9990,$$

$$\frac{A(\mathrm{S})}{A(\mathrm{Ara})} = (2.54 \pm 0.02)[\mathrm{S}] + (0.01 \pm 0.04) \text{ with } R^2 = 0.9994,$$

for $n = 6$ and a detection limit of 15 mmol l^{-1} (measured as quotient between three times of deviation standard of the blank and sensitivity) in all cases. Derivatization yields were measured for the four fatty acids, it was found to be (65 ± 5)% for myristic acid and palmitic acid, and (53 ± 5)% for oleic acid and stearic acid. Derivatization yields were calculated comparing obtained area peak and theoretical area peak considering derivatizing yield as 100% [17].

The 250 mmol l^{-1} solution of each fatty acid standard in ethanol was analyzed. A chromatogram where the peaks of each fatty acid derivative are clearly resolved can be seen in Fig. 3. Peaks were approximately Gaussian with different areas for each fatty acid, which may be due to different derivatization yield for each analyte and different photometric absorption coefficients for each derivative. However, the M/P, O/P, and S/P peak area ratios remained roughly constant.

3.4. Assay with standard drying oils

Fresh and aged standard oils were analyzed by HPLC using the proposed method. A characteristic chromatogram of each kind of oil and ageing were obtained. The chromatograms for linseed oil can be seen in Fig. 4. For each analyzed oil, the quotients between the peak area of fatty acid derivatives and peak area of palmitic derivative were calculated, the found results for five different replicates of each oil was shown in Table 1.

Recovery was calculated spiking with 0.025 ml of a 1 mmol l^{-1} fatty acid ethanolic solution to a 100 μl hydrolyzed poppy seed oil standard, aged under UV treatment (before extraction with hexane). Myristic and oleic acid already present in this poppy seed oil sample is neglected (see Table 1). Recovery was found to be (70 ± 10)% for myristic acid and (60 ± 10)% for oleic acid.

These chromatograms were more complex than those obtained with fatty acid standard mixture, because in drying oils there were more substances, some of which appear in the chromatogram. Nevertheless, there was no peak interfering with the fatty acids studied, although peaks that appear at low retention times impair determination of fatty acids with less than 14 carbon atoms. The obtained chromatograms permit the ratio of each fatty acid to be determined.

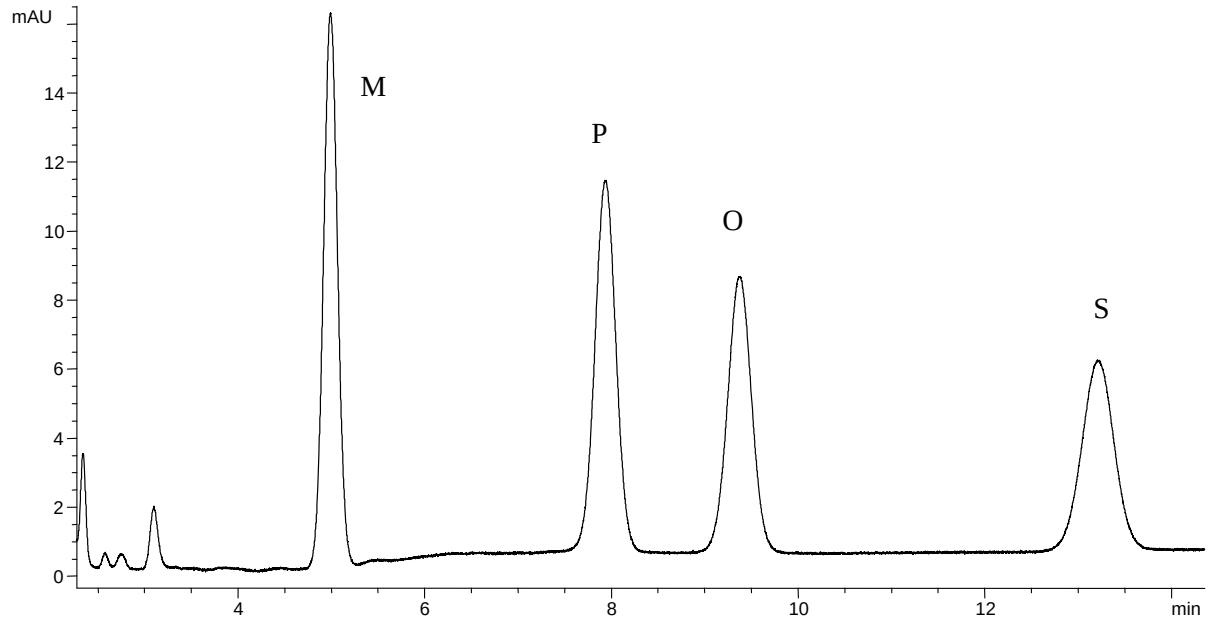

Fig. 3. Liquid chromatogram obtained by optimized derivatization with 2-NPH/EDC·HCl for a 0.25 mmol of each myristic (M), palmitic (P), oleic (O), and stearic (S) acids solutions in ethanol solution. Conditions: mobile phase: MeOH/H_2O/*n*-propanol/acid acetic (80:14:5:1) at 1.2 ml min^{-1} in isocratic mode, monitored at 400 nm, volume injected 20 μl.

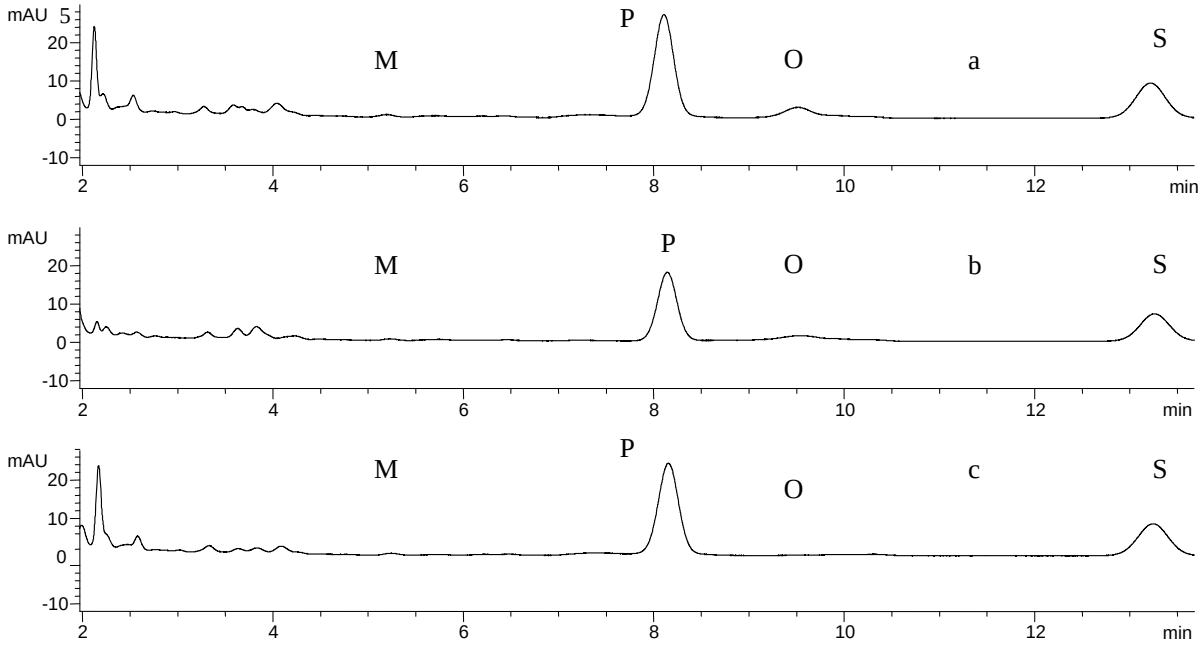

Fig. 4. Liquid chromatograms obtained by optimized derivatization with 2-NPH/EDC·HCl for linseed oil standard: (a) aged for 3 years without treatment, (b) aged by thermic treatment, and (c) aged by UV-irradiation treatment. Conditions as in Fig. 3.

As shown in Table 1, the S/P ratio is the parameter which allows identification of the used drying oil used [6]. The M/P ratio provides no information, because the amount of myristic is very small, whereas, the O/P ratio provides information related to ageing, because oleic acid disappears with UV irradiation ageing.

3.5. Analysis of drying oils from painted works of art

Samples of pictoric works of art were taken in order to identify which drying oil was used by the artist. Samples were analyzed as the standards according to the proposed method. The chromatograms were more complex than in the cases of drying oil standards and even other peaks were observed. However, resolution of the fatty acids is not impaired, so fatty acid rates can be calculated without any difficulty, as can be seen in Fig. 5. As in standard oil, M/P, O/P, and S/P ratios and their standard deviation were calculated for three replicates (three different samples taken from the pictorial work of art). The results obtained for these ratios are shown in Table 2.

In all cases, ratio standard deviations were found in samples to be higher than in aged standard oils. This is due to the considerable heterogeneity of pictorial works of art. In some samples, ratios do not totally coincide with those obtained from the standard solution. Ideal ratios were calculated by means of fresh standard drying oils artificially aged. But real ageings are neither caused by thermic attack, be-

Table 1
Values for myristic/palmitic acid (M/P), oleic acid/palmitic acid (O/P), and stearic acid/palmitic acid (S/P)

Drying oil	Type of ageing	M/P	O/P	S/P
Linseed	Fresh	0.03 ± 0.02	2.6 ± 0.3	0.54 ± 0.01
	Aged for 3 years	0.035 ± 0.005	0.16 ± 0.03	0.55 ± 0.05
	Thermic	0.029 ± 0.008	0.19 ± 0.03	0.61 ± 0.03
	UV	0.03 ± 0.02	0.02 ± 0.03	0.54 ± 0.02
Poppy seed	Fresh	0.06 ± 0.03	1.36 ± 0.01	0.18 ± 0.01
	Aged for 3 years	0.017 ± 0.008	0.035±0.004	0.21 ± 0.01
	Thermic	0.0094 ± 0.0003	0.14 ± 0.02	0.21 ± 0.02
	UV	0.009 ± 0.002	0.013 ± 0.006	0.190 ± 0.003
Nut	Fresh	0.030 ± 0.003	2.03 ± 0.01	0.30 ± 0.01
	Aged for 3 years	0.019 ± 0.003	0.18 ± 0.04	0.32 ± 0.01
	Thermic	0.030 ± 0.012	0.36 ± 0.04	0.34 ± 0.05
	UV	0.020 ± 0.017	0.010± 0.008	0.31 ± 0.04

Peak area ratios in 3 years aged without treatment and artificially aged drying oil standards. Values are average ± S.D. for $n = 5$ (different hydrolyzed standard drying oils).

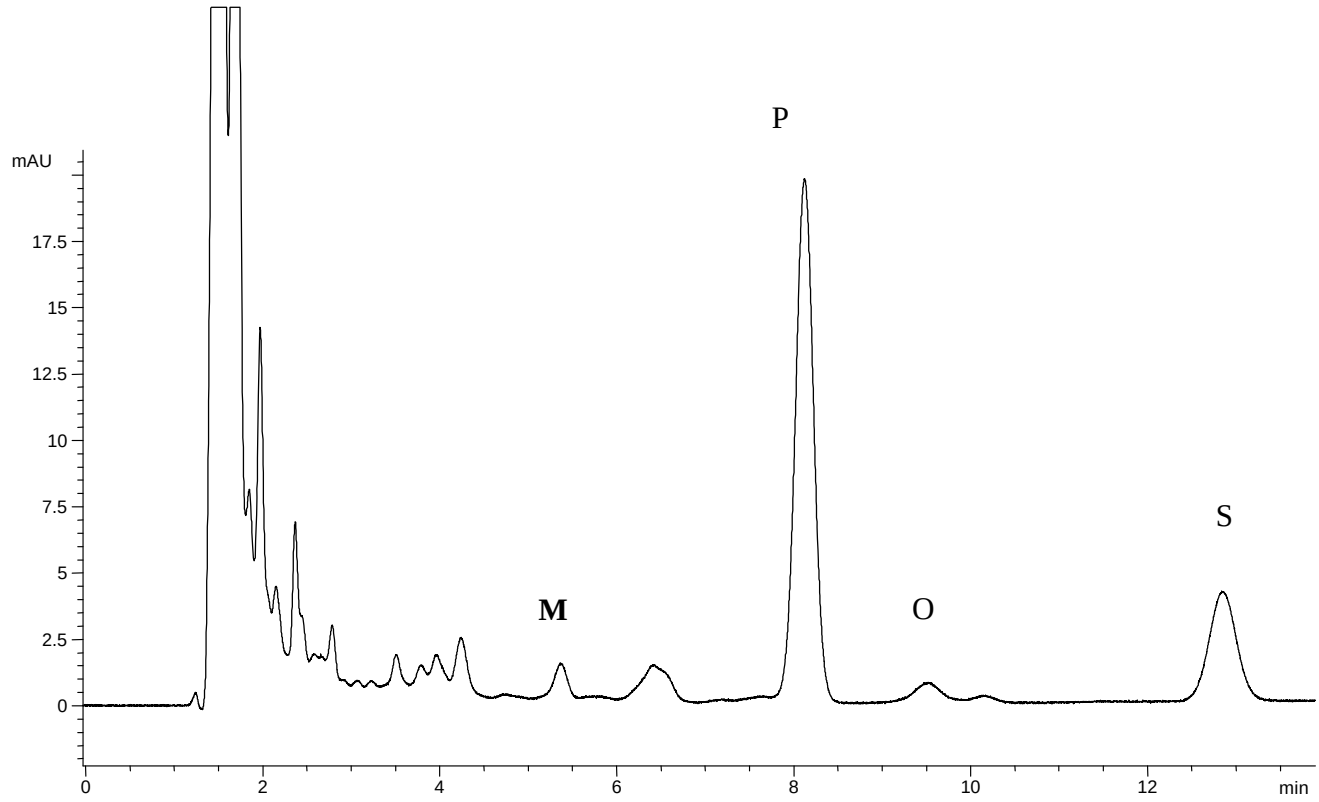

Fig. 5. Liquid chromatogram obtained by optimized derivatization with 2-NPH/EDC·HCl for sample from the painting: Crucifixion, 17th century, anonymous, but assigned to Tintoretto. Conditions as in Fig. 3.

cause pictures are not usually exposed to high temperatures (except in an occasional fire) nor by UV irradiation, because pictures are kept inside buildings (churches, museums, castles, and houses) where there are relatively protected from sun radiation. Real ageing is usually a slow attack by environmental substances (dioxygen, moisture, microorganisms, and pollution agents) and it is not feasible to model. As a result, artificial ageing processes are quite different from real ageing processes and only provide a little information about changes in fatty acid relative amounts. Drying oils aged without treatment are neither representative because this ageing process is for 3 years and real pictoric works of art are aged at least for three centuries. Ageing process also depends on the presence of pigments, which can inhibit or retard oxidation of some fatty acids. Although stearic and palmitic acids peak areas are affected, the S/P ratio can be considered reliable [26].

Table 2
Values for myristic acid/palmitic acid, oleic acid/palmitic acid, and stearic acid/palmitic acid

Sample	M/P	O/P	S/P	Suggested drying oil
1	0.08 ± 0.04	0.08 ± 0.07	0.33 ± 0.02	Nut oil
2	0.10 ± 0.01	0.10 ± 0.06	0.7 ± 0.2	Linseed oil
3	0.21 ± 0.07	0.26 ± 0.10	0.53 ± 0.07	Linseed oil
4.1	0.05 ± 0.03	0.10 ± 0.02	0.34 ± 0.03	Nut oil
4.2	0.08 ± 0.02	1.1 ± 0.2	0.55 ± 0.1	Linseed oil
5	0.07 ± 0.03	0.08 ± 0.02	0.66 ± 0.16	Linseed oil

Peak area ratios in pictorial works of art samples and identification of used drying oil. Values are average ± S.D. for $n = 3$ (different samples taken from the pictorial work of art).

All samples present M/P ratios higher than the ideal ratio, suggesting that this is caused by a process in real ageing that does not take place in thermic or irradiation ageing process.

In sample 1, the S/P quotient clearly points to nut oil as the drying oil used. The oleic/palmitic ratio suggests the ageing process is similar to an irradiation attack (see Fig. 5).

Sample 2 shows a high S/P ratio. The drying oil used was probably linseed oil but the ageing process has strongly affected the amount of palmitic acid, this is probably due to pigment effects [26]. O/P ratio was found to be between values corresponding to thermic and irradiation attack.

For sample 3, the S/P ratio indicates that linseed oil was used as drying oil. The oleic/palmitic ratio is high which suggests that the ageing process has significantly reduced the amount of palmitic and stearic acids.

In sample 4, analysis shows great heterogeneity. Two kinds of samples were found, one which may contain nut oil and another which includes linseed oil. In this pictorial work of art, the artist used one oil in the external coat and another oil in the inner layer. In this case we found linseed oil with a very high amount of oleic acid, which

may be due to the chemical environment (pigments) of linseed oil, which provides an unusual protection for oleic acid [26].

For sample 5, the S/P ratio indicates that linseed oil was used as drying oil, even if this ratio is high than expected for linseed oil. The O/P ratio is placed between those obtained for UV and thermic treatment. Results was found similar to sample 2.

4. Conclusions

Analysis of drying oils in painted works of art is feasible by HPLC-UV-Vis. The methodology proposed for oil binding media and protective film in pictorial works of art needs to separate fatty acids released in oil hydrolysis by extraction with hexane, which was robust and fast. Fatty acid derivatization by 2-NPH/EDC·HCl was sensitive enough. Stearic and oleic acids were clearly resolved, despite their similar formula. The chromatograms for different standard oils (linseed, nut poppy seed) provide data for differentiating among them by means of the fatty acid area peak ratio. This procedure has permitted the study of ageing drying oils subjected to thermic or UV irradiation treatment. The suggested HPLC method allowed the easy identification of drying oils used in painting oils in pictorial works of art, which is essential to conservation or restoration treatments. Although artificial ageing treatment of drying oils seem very different to real ageing of these materials in pictorial works of art, the S/P parameter allows to identify the drying oil.

Acknowledgements

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References

[1] G. Collalucci, Scienza e Tecnica, Mondatori, Milan, 1988.
[2] R. Pancella, R. Bart, Kunssttechnol. Konservierung 3 (1989) 101.
[3] M.C.M. Moya-Moreno, D. Mendoza-Olivares, F.J. Amezquita-López, J.V. Gimeno-Adelantado, F. Bosch-Reig, Talanta 50 (1999) 269.
[4] P. Vandenabeele, B. Wehling, L. Monees, H. Edwards, M. de Reu, G. van Hooydonk, Anal. Chim. Acta 407 (2000) 261.
[5] A. Doménech-Carbó, M.T. Doménech-Carbó, J.V. Gimeno-Adelantado, V. Peris-Martínez, F. Bosch-Reig, Recent Res. Dev. Pure Appl. Anal. Chem. 1 (1998) 207.
[6] R. Mateo-Castro, M.T. Doménech-Carbó, V. Peris-Martínez, J.V. Gimeno-Adelantado, F. Bosch-Reig, J. Chromatogr. A 778 (1997) 373.
[7] R. Mateo-Castro, J.V. Gimeno-Adelantado, F. Bosch-Reig, A. Doménech-Carbó, M.J. Casas-Catalan, L. Osete-Cortina, J. de-la-Cruz-Cañizares, M.T. Doménech-Carbó, Fresenius J. Anal. Chem. 369 (2001) 642.
[8] G. Chiavari, G.C. Galletti, G. Linterna, R. Mazzeo, J. Anal. Appl. Pyrolysis 24 (1993) 227.
[9] E. Vioque, M.P. Maza, F. Millán, J. Chromatogr. A 331 (1985) 187.
[10] G. Gübitz, Anal. Chem. 46 (1974) 1849.
[11] E. Mentasti, M.C. Gennaro, C. Sarzanini, C. Baiocchi, M. Savigliano, J. Chromatogr. 322 (1985) 177.
[12] M.J. Cooper, M.W. Anders, Anal. Chem. 46 (1974) 1849.
[13] M. Hoffman, J.C. Liao, Anal. Chem. 48 (1976) 1104.
[14] K. Funazo, M. Tanaka, Y. Yasaka, H. Takigawa, T. Shono, J. Chromatogr. 481 (1989) 211.
[15] R. Baboud, G. Pratz, J. Chromatogr. A 360 (1986) 119.
[16] H. Miwa, M. Yamamoto, T. Momose, Chem. Pharm. Bull. 28 (1980) 599.
[17] H. Miwa, M. Yamamoto, C. Hiyama, J. Chromatogr. 321 (1985) 165.
[18] H. Miwa, M. Yamamoto, J. Chromatogr. A 351 (1986) 275.
[19] H. Miwa, M. Yamamoto, J. Chromatogr. B 421 (1987) 33.
[20] H. Miwa, M. Yamamoto, J. Chromatogr. A 523 (1990) 235.
[21] H. Miwa, M. Yamamoto, J. Asano, J. Chromatogr. B 568 (1991) 25.
[22] M. Odlyha, N.S. Cohen, G.M. Foster, Thermochim. Acta 365 (2000) 34.
[23] F. Modugno, E. Menicagli, R. Fuoco, A. Giacomelli, Microchem. J. 67 (2000) 291.
[24] H. Miwa, J. Chromatogr. A 881 (2000) 365.
[25] H. Miwa, M. Yamamoto, T. Nishid, K. Nunoi, M. Kikuchi, J. Chromatogr. B 416 (1987) 237.
[26] J.V. Gimeno-Adelantado, 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.