SUPPLEMENTARY MATERIAL

Natural products such as adhesives in oil paintings

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The study and the analysis of the materials employed in artistic paintings provides deeper knowledge about the history of the work of art, including restoration efforts made in the past, and the development of painting techniques through the centuries. Gas chromatography coupled to mass spectrometry is the main analytical technique employed, as it proved to be the most suitable technique for the analysis of complex mixtures, thanks to its combination of sensitivity, wide range of applicability and versatility. Further, μ FT-IR technique has also been employed to get a preliminary screening of the samples taken from paintings. **In this paper, the analytical protocol based on these two techniques has been applied for analyzing natural terpenic resins; its performance has been tested on microsamples collected from paintings of valuable artistic interest.**

Keywords: Natural resins; Diterpenoids; Triterpenoids; Colophony; Canada Balsam; Mastic; Mass spectra; GC-MS; Oil painting.

Experimental Part

Materials

The reagent used where: n-hexane, methyl alcohol, methylene chloride (Carlo Erba, Milano, Italy) diethyl ether, KOH, HCl, (Carlo Erba, RPE).

Abietic acid, dammar resin, mastic resin and elemi gum were supplied by Sigma (Germany), Venice turpentine from Zecchi (Italy) whereas Sandarac resin by Morrone (Italy) and Canada balsam by Carlo Erba (Italy). Manila Copal was obtained from Morrone (Italy). Abietic acid, dammar, mastic, elemi and Manila Copal were used also for specimens on glass, and these were

aged by the action of UV radiation for 22 days and then left to ultimate the ageing process for 18 years in the dark (kindly by the Istituto Centrale per il Restauro, Rome).

Apparatus and chromatographic conditions

GC-MS analysis was performed on a HP-5890 Series II (HP, Rome, Italy) gas chromatograph coupled to a HP-5972 mass selective detector (HP). Chromatographic separations were achieved on a fused-silica capillary column (HP-5MS), stationary phase SE54 (5% phenyl-95% methylpolysiloxane), 30 m $\times$ 0.25 mm I.D. and 0.25 μ m d_f (Russo et al. 2012; Cinelli et al 2014).

The chromatographic conditions for GC-MS analysis of terpenic resins compounds were: injector temperature 300 °C; the injections were made in splitless mode (30 s delay before opening the splitter); transfer line temperature 200 °C; initial oven temperature 120 °C, isothermal for 1 min; 30 °C min⁻¹ up to 200 °C; 5 °C min⁻¹ up 230 °C; then 30 °C min⁻¹ up to the final temperature of 290 °C. The carrier gas was helium, constant inlet gas pressure of 5 psi. The mass spectrometer was scanned from m/z 45 to 700 at 70 eV.

The chromatographic conditions for GC analysis of methyl esters of fatty acids present in drying oils were: oven temperature 180 °C (isothermal); injector temperature 280 °C; detector temperature 280 °C; carrier gas H₂ (1.2 atm).

The chromatographic conditions for GC-MS analysis were: injector temperature 300 °C; transfer line temperature 280 °C; initial oven temperature 120 °C, isothermal for 1 min; 30 °C min⁻¹ up to 200 °C and isothermal for 2 min; then 5 °C min⁻¹ up to the final temperature of 230 °C. The carrier gas was helium at a flow-rate of 1.3 mL min⁻¹. Analysis of methylesters was performed in the scan mode (mass range 50-550 amu at 70 eV).

Analytical procedures for terpenic resins analysis

In this paper three analytical methods for the analysis of terpenic resins by GC-MS are tested and compared:

1. direct methylation with diazomethane;
2. alkaline hydrolysis and subsequent acidic trans-esterification, as previously proposed elsewhere (Cartoni, et al., 2001), to determine fatty acids in drying oils;
3. two steps derivatisation procedure involving trans-esterification and subsequent methylation with diazomethane.

Method 1): a weighted amount of resin (1-2 mg) was dissolved in 0.5 mL of methylene chloride. Diazomethane in diethyl ether (Ma and Lades 1976) was added to the solution, in order to obtain methyl esters of the acidic components of resins. The solution was evaporated, to 250 μ L,

under a mild flow of N₂, to eliminate the diazomethane in excess. A known volume of internal standard solution was added. Then the analysis was performed by GC-MS.

Method 2): this method has already been reported for preparation of samples containing drying oils (Cartoni et al., 2001). It involves the alkaline hydrolysis of the tryglicerides and the subsequent derivatisation of the fatty acids obtained; these were then turned into methyl esters and analysed by GC-MS. A weighted amount of resin (1-2 mg) was dissolved in 100 µL of methyl alcohol for diterpenic resins, while triterpenic resins were dissolved in 50 µL of methanol and 50 µL of n-hexane; 100 µL of methanolic 2 M KOH were added. The solutions were stirred for about 2 minutes and left to rest for 15 minutes at 60 °C. Then 0.5 mL of a solution of concentrated HCl and CH₃OH (1+1 v/v) were added. The methyl esters were extracted using n-hexane (0.5 mL×3 times). The collected extracts were evaporated to 150 µL, under a mild flow of N₂, and after the addition of a known volume of internal standard solution, were analysed by GC-MS.

Method 3): this method is the same as method 2) with two differences: methylene chloride was used to extract methyl esters instead of n-hexane; a further step is involved: diazomethane in diethyl ether was added to the collected extracts to improve the derivatisation of carboxylic functions of resin components. The solution thus obtained was evaporated to 150 µL, under a mild flow of N₂, and, after the addition of a known volume of internal standard solution, was analysed by GC-MS.

The samples taken from the paintings, containing oils and resins were analysed using the above described methods: 2 and 3, even if the amount of sample (≈ 0.1 mg) made us reduce, in proportional way, the reagent quantities, always keeping the same ratios.

Direct diazomethane methylation (method 1) turns out to be a very efficient and direct method to analyse resins as acidic compounds are easily derivatisated. Nevertheless when a sample contains both drying oils and resins it is necessary to follow a method that involves alkaline hydrolysis and subsequent trans-esterification, in order to analyse fatty acids. Method 2 was found unsuitable to get methyl esters of acidic components of resins; moreover, solvent extraction from aliphatic phase reduces the efficiency of resin analysis. Method 3, instead, gives more efficient results than method 2 to determine, at the same time, the presence of oils and resins in microsamples collected from paintings.

These analytical procedures, developed and tested on standards, were applied to analyse real samples from old masters. Samples containing mainly drying oils were analysed following the trans-esterification analytical procedure. When the presence of both drying oils and resins in the same microsample was suspected, analytical methods 2 and 3 were followed.

References

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Table S1. List of characteristic absorption bands of terpenic resins.

Wavenumber (cm ⁻¹)	Functional group
3400	OH
2960-2930	CH ₃ , CH ₂
2875-2865	
1715-1695	C=O
1467-1448	C-H, C-O, C-C
1387-1382

Figure S1. μ FT-IR spectra of fresh (red line) and artificially aged (blue line) samples of colophony (a) and mastic resin (b).

Figure S2. Partial TICs of fresh (a) and aged (b) colophony. For peak number, see Table S1.

Figure S3. Partial TICs of pinus resin just collected from the tree (a) and fresh Canada balsam. For peak number, see Table 1.

Figure S4. Partial TICs of fresh (a) and aged (b) mastic resin. For peak number, see Table S1.

Figure S5. TIC of *Madonna con Bambino* by Rondinelli (a) and μFT-IR spectrum (b) and TIC (c) of *Madonna con Bambino e Santi* by Maratta. (TIC peaks: A: azelaic acid; B: palmitic acid; C: stearic acid; D: dehydroabiatic acid; E: 7-oxo-dehydroabiatic acid; F: methyl 15-hydroxy-7-oxo-dehydroabietate; G: 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-9-oxo-methyl ester; H: methyl moronate; I: methyl oleanonate) (μFT-IR spectrum: Maratta's painting, blue curve; Japanese paper clean fibre, red curve).