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Application Note SI-02454

Fast GC analysis of natural waxes from art and museum objects

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Introduction

Beeswax was the earliest waxy material used by man and has been identified as waterproofing substance for ceramics in the Neolithic period, as embalming mixture in Egyptian mummies but also, more recently, in the manufacture of seals, for example.

Waxy substances as beeswax, Japan wax or animal fats have also been employed in modeling and sculpture manufacture, especially in the 19th century. Some additives such as pine resin could also be mixed with these waxy materials to change the physical properties of the modeling material. These natural products are molecular mixtures, which contain compounds with a wide range in polarity and molecular weight structures such as diterpenoids, n-alkanes, long-chain alcohols and fatty acids, esters and triacylglycerols.

Due to the small sample sizes that must be taken from works of art, often at micrometric scales, sensitivity of analytical procedures is one of the main challenges of organic analysis on materials from museum objects.

Gas chromatography is a useful tool to identify these waxy substances using an analytical procedure based on a short column (15 m x 0.32 mm x 0.1 μ m) fitted with on-column injection to maximize sensitivity. For additional sensitivity, some tests on natural products can be performed using a high speed, small diameter thin film Varian FactorFour™ VF-1ms column of 10 m x 0.15 mm x 0.10 μ m. This note describes the sensitivity gain achieved by transferring the analysis from a 0.32 mm ID to a 0.15 mm ID column.

Materials and Methods

Technique: GC/FID
Column A: VF-1ms, 15 m x 0.32 mm, df = 0.10 μ m (Part no. CP8918) + 1 m x 0.32 mm retention gap, methyl deactivated
Temperature: 50 °C (1 min), 10 °C/min, 350 °C (10 min)
Carrier Gas: Helium, programmed flow, 2 mL/min (17 min), 4 mL/min (5 min), 6 mL/min (15 min)

Column B: VF-1ms, 10 m x 0.15 mm, df = 0.10 μ m (Part no. CP9030) + 1 m x 0.32 mm retention gap, methyl deactivated
Temperature: 50 °C (1 min), 20 °C/min, 380 °C (4 min)
Carrier Gas: Helium, constant flow, 1 mL/min (9 min), 2 mL/min (2.5 min), 3 mL/min (7.5 min)
Inj. Volume: 1 μ L
Injector: On-column, tracking oven temperature
Detector: FID, 350 °C

Sample preparation

Fifty μ L of sample (1 mg/mL CH_2Cl_2) was evaporated at 40 °C under N_2 . Silylation was performed with 50 μ L BSTFA/TMCS for 30 minutes at 80 °C, and subsequently evaporated to dryness at 40 °C under N_2 . The sample was reconstituted in 200 μ L dichloromethane for the 0.32 mm column or 600 μ L dichloromethane for the 0.15 mm column. As a result, the sample on the 0.15 mm column had a 3x lower concentration level.

Results and Discussion

Figure 1 illustrates the elution of a mixture of test components ranging from (un)saturated fatty acids, alkanes, di- and triglycerides and sterols. Figure 1A shows the analysis as originally performed on the 10 m x 0.32 mm column. The higher speed of analysis on the 10 m x 0.15 mm column was evident from Figure 1B, resulting in analysis times that are almost half of the original analysis. The on-column injection on the 0.15 mm column was facilitated by a 1 m x 0.32 mm retention gap coupled by a glass quick-seal connector to the 0.15 mm ID column. The shorter analysis results in a higher FID signal for each eluting component due to decreased band broadening effects in the capillary column. It thus provides an improved signal-to-noise ratio and better sensitivity. Figure 1C illustrates the effect of an optimized temperature program to 380 °C for the VF-1ms column, to improve elution of the late eluting high-boiling triglycerides. As a result, peak shape and signal of the triglycerides were significantly improved. Although sample concentration levels for B and C were 3x lower than for A, the signal intensity was identical.

The maximum temperature of the VF-1ms column was normally set at 350 °C. However, the highly temperature stable VF-1ms liquid phase can be routinely pushed to 380 °C without any loss of column performance. A slightly higher column bleed level at 380 °C can be observed under these conditions (Figure 1C).

Figure 2 shows the comparative chromatograms of a beeswax sample. The analysis on the 10 m x 0.15 mm VF-1ms GC column (Figures 2B, 2C) provided a high-resolution separation in a significantly shorter analysis time and with improved sensitivity. Identified components included normal alkanes with odd carbon number (from C₂₃ to C₃₃, with C₂₇ as the main alkane), saturated free fatty acids (from C₂₂ to C₃₄), and a number of palmitate esters ranging from C₄₀ to C₄₈ with C₄₆ as the main ester. This characteristic composition of beeswax is in agreement with those already described¹⁻³. This initial composition may change with aging and depend on the environment. For example, on Egyptian objects (a quite hot and waterless conservation climate), sometimes only the heaviest compounds (esters) remain. Under high relative humidity, these esters could be hydrolyzed to palmitic acid

and corresponding long chain alcohols.

Figure 3 illustrates the analysis of Japan wax where tripalmitin was the main compound of a triacylglycerol pattern. This vegetal fat could be the extract from the coating of kernel from a Rhus species growing in Japan or China.

The fast GC analysis (Figure 4) of the slightly more polar constituents of pine resin (Figure 5) can be performed in less than 15 minutes with a good resolution for these kinds of diterpenoid isomers.

Figure 6 shows the GC/FID analysis of animal fats, revealing the presence of common triglycerides.

Conclusions

Only very small samples can be taken from museum objects and works of art. Using a small diameter, thin film Varian FactorFour™ VF-1ms column, tests on natural waxes can be performed at high speed and with high sensitivity, despite the small sample size.

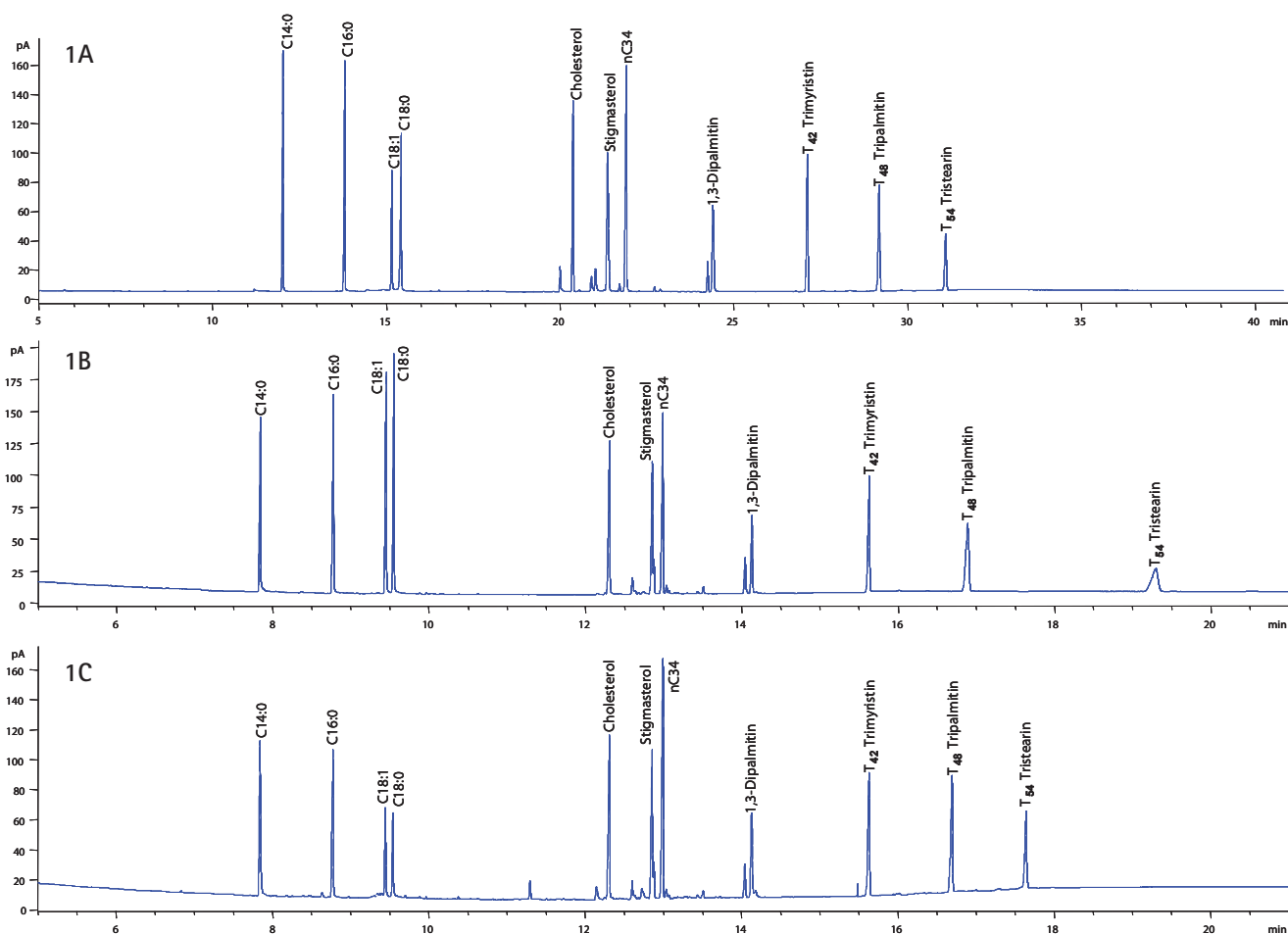


Figure 1. GC analysis of a test mixture on VF-1ms, 15 m x 0.3 2mm x 0.1 μm (1A) and 10 m x 0.15 mm x 0.1 μm (1B and 1C). 1B, maximum oven temperature 350 °C; 1C, maximum oven temperature 380 °C.

References

1 Heron, C., Nemcek, N., Bonfield, K.M., Dixon, D. and Ottaway, B.S. (1994) The Chemistry of Neolithic Beeswax. *Naturwissenschaften*, 81: 266-269.

2. Kolattukudy, P.E. (Ed.) (1976) *Chemistry and Biochemistry of Natural Waxes*. Elsevier, Amsterdam, Oxford, New York.
 3. Tulloch, A.P. and Hoffman, L.L. (1972) Canadian beeswax: analytical values and composition of hydrocarbons, free acids and long chain esters. *J. Am. Oil Chem. Soc.*, 49: 696-699.

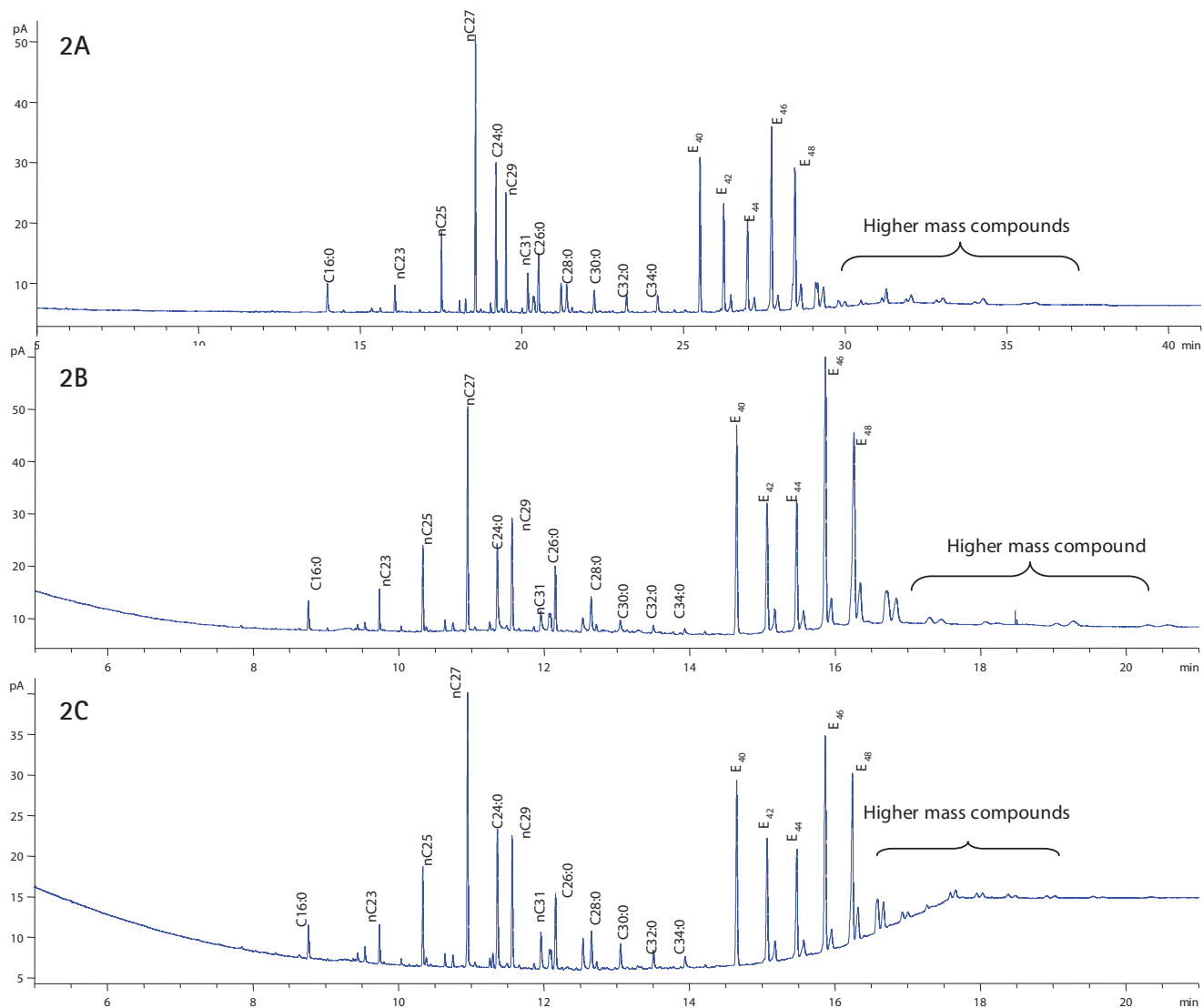


Figure 2. GC/FID analysis of beeswax on VF-1ms, 15 m x 0.32 mm x 0.1 μ m (2A) and 10 m x 0.15 mm x 0.1 μ m (2B and 2C). 2B, maximum oven temperature 350 °C; 2C, maximum oven temperature 380 °C.

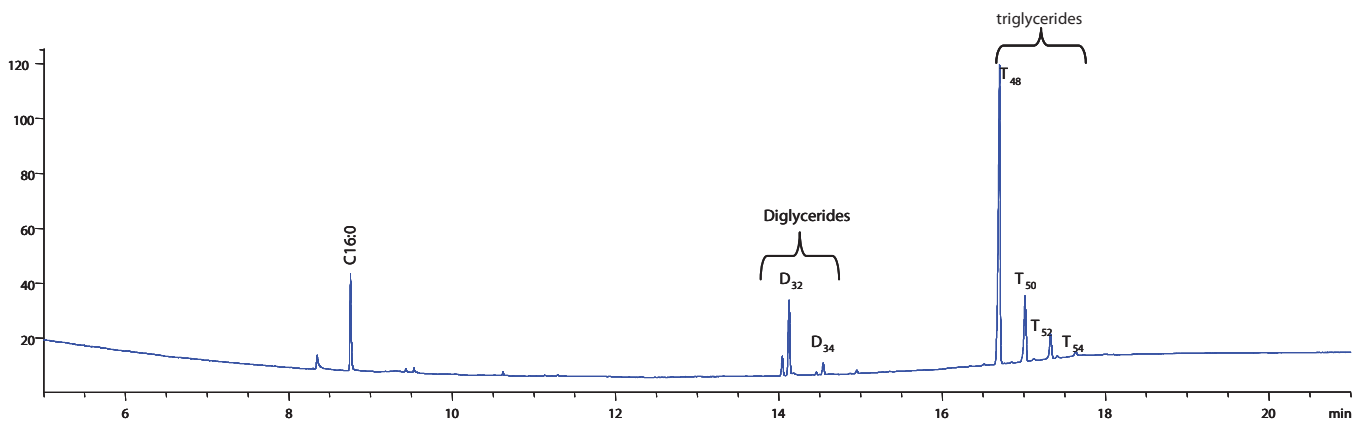


Figure 3. GC/FID analysis of Japan wax.

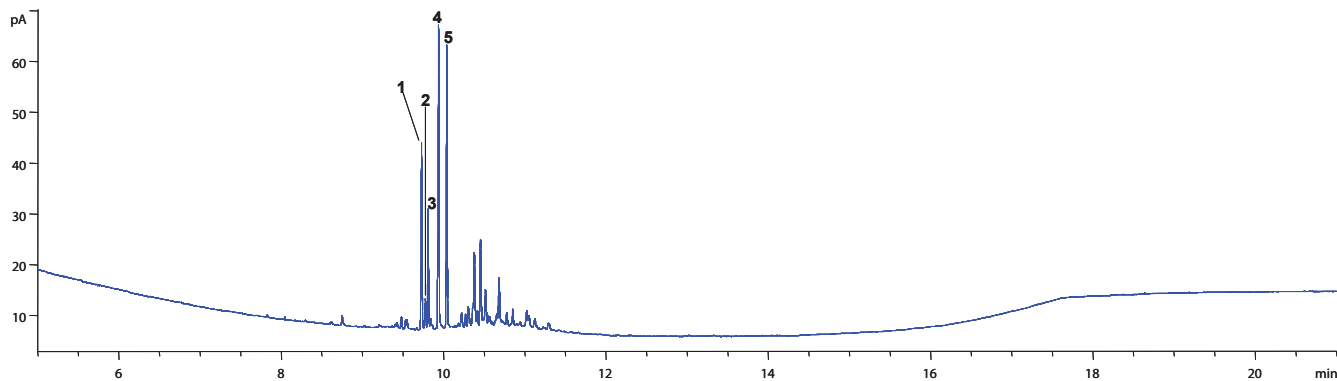


Figure 4. GC/FID analysis of pine resin.

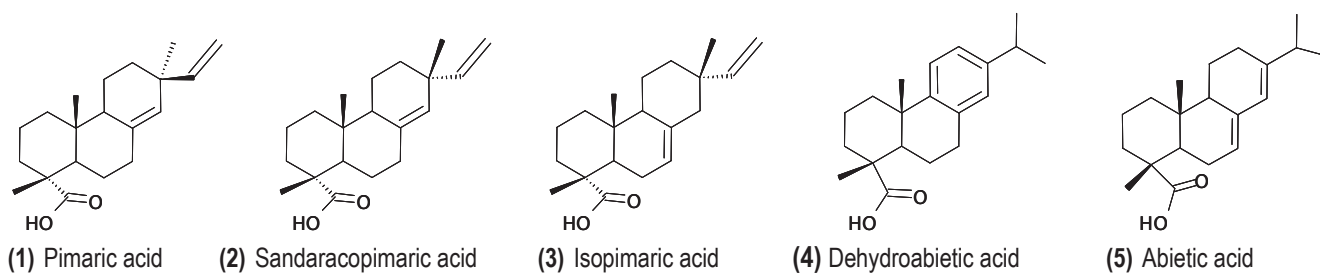


Figure 5. Structures of some pine resin constituents.

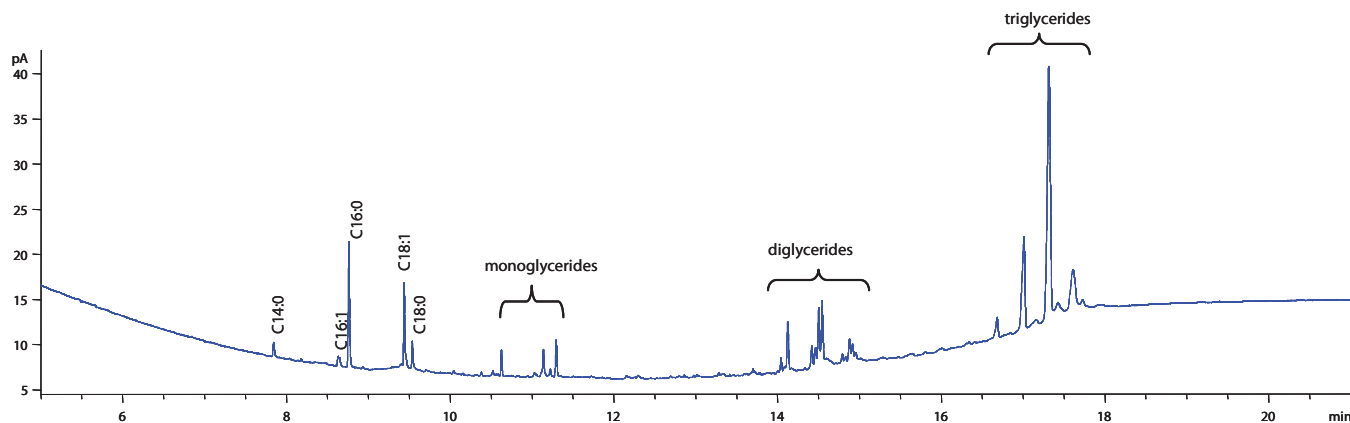


Figure 6. GC/FID analysis of animal fat.

These data represent typical results.

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