Preprint

Towards Robust Identification of Pleistocene Adhesives: A Critical Review of Current Analytical Approaches

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Abstract

Chemical identification of adhesive remains on prehistoric stone tools is of great interest for archaeologists, as the residues contain interesting information on tool use and the exploitation of natural resources by hominins. Adhesives were used to form a wrapping around the stone tool to protect the hand from the sharp edges and improve grip, or to secure a handle out of organic material to the stone tool. This invention, of adding a handle to a stone tool, marks a fundamental change in prehistoric technology. Adhesives can be manufactured from readily available exudates, like pine resin, but could also be man-made, in the case of birch tar that is obtain by dry distillation of birch bark. The glueing properties of the adhesives could be enhanced with the addition of an additive (e.g., charcoal, ochre, beeswax). Given that adhesive manufacture is considered to indicate planning abilities and complex thought, their identification in archaeological assemblages is important for understanding the evolution of human cognition. However, given long-term burial, organic residues on stone tools are generally significantly degraded, which raises numerous chemical challenges and interpretative difficulties that need to be tackled through close collaboration between archaeologist and chemists. Without this interaction between two vastly different research field studies can suffer from an overinterpretation of analytical data or a lack of understanding of the archaeological context. This review discusses the main pitfalls encountered in the chemical analysis of prehistoric adhesives and offers analytical recommendations to avoid them. Applying the analytical practices as proposed here will increase the reliability and credibility of the analytical results and to allow a strong chemical foundation for the archaeological interpretations. The main focus is on the use of gas chromatography-mass spectrometry for the chemical identification of prehistoric adhesives, however, other commonly used analytical techniques are also briefly discussed.

Keywords

Prehistoric adhesives, hafting, stone tools, analytical chemistry, gas chromatography, QA/QC

Abbreviations

BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide

CI	chemical ionisation
EDX	energy dispersive X-ray spectroscopy
EI	electron ionisation
eV	electron volt
FA	fatty acid
FID	flame ionisation detector
FTIR	Fourier transform-infrared spectroscopy
GC	gas chromatography
ISTD	internal standard
ka	kiloannum
LRI	linear retention index
MIS	Marine Isotope Stage
MS	mass spectrometry
MSA	Middle Stone Age
m/z	mass-to-charge ratio
OM	optical microscopy
SEM	scanning electron microscope
S/SL	split/splitless
PCA	principal component analysis
PI	photo ionisation
PTV	programmed temperature vaporization
py-	pyrolysis-
QA/QC	quality assurance / quality control

39

40 ## 1. Introduction

41 Adhesives have been part of the human toolkit since the Middle Palaeolithic. The earliest known
42 evidence may be a black sticky paste wrapped around a stone tool found in Campitello, Italy,
43 dated to at least Marine Isotope Stage (MIS) 5—and probably even earlier [1]. From around 80 ka
44 onwards, adhesives are more regularly identified on stone tools and serve to create a durable,
45 resilient bond between the stone tool and its haft [2].

46 The practice of hafting predates the use of adhesives, by c. 250 ka wear traces related to friction
47 with a haft are identified on stone tools from Europe [3] and Northeast Africa [4]. These hafting
48 systems probably involved the use of bindings, either of animal or vegetal nature. For South
49 Africa, hafting has been argued to exist as early as c. 500 ka [5], but this evidence is unreliable
50 and has been strongly contested [6]. More reliable evidence of hafting in the form of wear traces
51 and residues has been identified from about 80 ka onwards [7]. The invention of hafting
52 revolutionised prehistoric technology and fundamentally changed what tools could be made
53 (e.g., multiple stone insets) and how tools could be used. Because addition of a handle provides
54 biomechanical and physiological advantages compared to hand-held use [8–10]. Moreover, the
55 manufacturing of composite tools required greater planning and imagination, with implications
56 for human cognition [8, 11, 12].

57 A reliable determination of whether stone tools were used hafted or hand-held is important for
58 the understanding of how prehistoric technology and human behaviour evolved and varied over
59 time and space. Also, the identification of the hafting system is an important inference. Yet,
60 direct evidence of handles is rare: they were typically made from perishable organic materials
61 such as wood or antler, which only preserve in exceptional cases and only a few rare possible
62 examples dated to the Middle Palaeolithic [13, 14]. From the Upper Palaeolithic and Neolithic,
63 more direct evidence of handles with preserved stone insets has been discovered [15–17]. As a
64 result, early hafting practices usually have to be identified indirectly—either through functional
65 analysis, which studies wear patterns on the stone tool, such as those from friction with the
66 handle, or through the chemical identification of adhesive remains that are preserved on the
67 stone tools.

68 Chemical identification of residues may provide insights into tool use and hafting but also in how
69 hominins exploited natural resources. Different types of hafting adhesives have been identified:
70 natural exudates from plants, such as conifer resins [18] or mineral sources, such as bitumen
71 [19, 20], as well as synthetic adhesives like birch tar [1, 21, 22] and possibly *Podocarpus* tar
72 (specific to South Africa) [23]. Tar production requires the controlled pyrolysis of bark (birch tar)
73 or leaves (*Podocarpus* tar) [24–26], a process that implies a level of planning and understanding
74 of material transformation [27, 28]. Furthermore, the adhesive could be mixed with an additive
75 like ochre or beeswax to enhance its performance for a specific role or circumstance, which
76 requires planning and imagination of the end result. Given the more complex nature of the
77 production process of synthetic and compound adhesives, both types of glues have been used
78 to argue for complex cognition.

79 Adhesive remains generally preserve only fragmentary and often in a highly degraded form, and
80 combined with their natural origin, this complicates their chemical analysis [2]. Multiple
81 methodologies, both non-destructive and destructive as well as non-invasive or invasive to the
82 adhesive, have been developed to obtain the most accurate identification possible. An ideal
83 analytical technique is sensitive, provides a high level of information, and preserves the artefact
84 for follow-up analysis or future generations. To date, such a technique does not yet exist.
85 Spectroscopy instrumentation like scanning electron microscope coupled with energy
86 dispersive X-ray spectroscopy (SEM-EDX) or Fourier-transform infrared spectroscopy (FTIR) are
87 non-destructive, but they lack sensitivity to precisely identify weathered adhesive remains [29].
88 On the other hand, gas chromatography – mass spectrometry (GC-MS) is very sensitive and
89 provides detailed information about the adhesive, but the analysis is destructive towards the
90 residue. Its strength lies in the ability to separate and identify individual molecules from the

sample, and, thus, to distinguish between original compounds, degradation products and contamination [18]. However, a laborious sample preparation, which involves extraction of the residue and (trimethylsilyl) derivatisation, is required before GC-MS analysis is possible.

Interest in identifying residues, or more specifically adhesives on stone tools has fostered collaboration between archaeologists and analytical chemists, which requires mutual understanding of the specificities of each field [30]. Archaeologists seeking detailed insights into residues need to grasp the potential and the limitations of the analytical techniques used. As well as the factors causing possible contaminations so excavation and storage protocols can be adapted. Likewise, chemists need to appreciate the archaeological context, the complexities of taphonomy and the implications of their findings to assure that meaningful archaeological questions are addressed.

A review of the literature on the identification of Palaeolithic hafting adhesives shows that this reciprocal understanding is not always fully achieved. On the chemical side, published results sometimes lack transparency and details on quality assurance and quality control (QA/QC), making it difficult to fully assess their reliability. On the archaeological side, results are sometimes overinterpreted or interpretations lack support because essential chemical analyses were not conducted. Enhancing collaboration and communication between both disciplines would help achieving more confident conclusions that are both scientifically robust and archaeologically meaningful. In this review, we address the potential and the pitfalls of existing approaches and discuss ways in which more robust analytical practices could be developed in the future. The main topics addressed are:

1) Strengths and limitations of commonly used instrumentation in (hafting) adhesive research.
2) Current shortcomings with regard to how chemical analysis is used.
3) Methodologies considerations to improve the reliability of the chemical results, in particular in the case of GC-MS.

2. State-of-the-art of adhesive analysis

Table 1 summarises the artefacts that have been analysed for (hafting) adhesives from the Palaeolithic period in Europe and the Middle Stone Age (MSA) in Africa for a time frame dating from MIS 5 until MIS 2. Non-destructive analysis like optical microscopy (OM), scanning electron microscope coupled with energy dispersive X-ray spectroscopy (SEM-EDX) and Fourier-transform infrared spectroscopy (FTIR) are the most commonly used techniques, representing

123 almost two-thirds of the studies. About half of the studies include OM only, without chemical
124 analysis of the residues (Table 1). The other half employs an array of different analytical
125 instruments to investigate the residues. Most studies are centred on Europe and South Africa;
126 however, sites in Australia and China have also been reported (Table 1).

127

Table 1. Overview of the sites with published evidence for hafting adhesives, including the approach used, the number of tools with (possible) evidence of adhesives, and interpretation provided.

#	Site	MIS	Analytical methods	Interpretation Adhesive	# artifacts	Publication
1	Inden altdorf, Germany	5	OM, SEM-EDX	Birch tar	6	[31, 32]
2a	Campitello, Italy	5	MS, GC-MS	Birch tar	1	[33]
2b			OM, SEM-EDX, FTIR, GC-MS	Birch tar	2	[1]
3	Sodmein Cave, Egypt	5	OM	Adhesive mixed with ochre	1	[34]
4	Sibhudu, South Africa	5	OM, SEM-EDX	Resin, possibly mixed with ochre	9	[7]
5	Umm el Tell, Syria	4	GC-MS	Bitumen	3	[19]
6	Rose Cottage Cave, South Africa	4	OM	Resin mixed with ochre	48	[35]
7a	Madjebebe, Australia	4	OM	Resin mixed with ochre	1	[36]
7b			OM	Possibly resin mixed with ochre	1	[37]
8	Sibhudu, South Africa	4	OM, FTIR, GC-MS	*Podocarpus* tar	6	[26]
9	Sibhudu, South Africa	4	OM	Resin, possibly mixed with ochre	29	[38]
10	Sibhudu, South Africa	4	OM	Resinous (+ochre not all)	5	[39]
11	Sibhudu, South Africa	3	OM	Resin mixed with ochre	49	[40]
12	Sibhudu, South Africa	3	OM	Resin mixed with ochre	2	[41]
13	Umhlatuzana Rock Shelter, South Africa	3	OM	Resin mixed with ochre	2	[41]
14	Le Moustier, France*	3	FTIR, SEM-EDX	Bitumen + Ochre	5	[42]
15	Border Cave, South Africa	3	(py-)GC-MS	*Podocarpus* tar	3	[43]
16a	Königsaue, Germany	3	OM, SEM-EDX, FTIR	-	2	[44]
16b			GC-MS	Birch tar	2	[22]
16c			FTIR, GC-MS	Birch tar	2	[45]
17	Enkapune Ya Muto, Kenya	3	Macro observation	Ochre stains	?	[46]
18	Warrati rock shelter, Australia	3	OM	Resin & ochre	2 resin, 2 ochre	[47]
19	Ngarrabullgan Cave, Australia	3	OM	Resin	1	[48]

20	Gura Cheii-Râsnov, Romania	3	FTIR, EDXRF, ICP-AES, XRD, GC-MS	Bitumen	2	[20]
21a	Hummal, Syria	3	GC-MS	Bitumen	2	[49]
21b			OM, SEM, CRM, FTIR, Raman, XRD	Bitumen	2	[50]
22	Diepkloof Rock Shelter, South Africa	3	GC-MS	*Podocarpus* elongatus tar	1	[23]
23a	Border Cave, South Africa	3	OM, SEM-EDX, GC-MS	*Euphorbia* T. + Ochre + beeswax	1	[51]
23b			FIA-MS	*Euphorbia* T. + Ochre + beeswax	1	[52]
24	Grotta di Sant'Agostino, Italy	3	GC-MS	*Pinaceae* resin	6	[18]
25	Grotta del Fossellone, Italy	3	GC-MS	*Pinaceae* resin & plant lipids, 1 beeswax mix	4	[18]
26	Zandmotor, the Netherlands	3	py-GC-MS	Birch tar	1	[21]
27	Sibhudu, South Africa	3	OM, GC-MS	Resin (possible *Podocarpus*) + ochre	3	[53]
28	Cueva Morin, Spain	2	SEM, FTIR	Resin	2	[54]
29	Ohala II, Israel	2	SEM, FTIR	Carbonaceous adhesive	5	[55]
30	El Buxu, Spain	2	Raman spectroscopy, μ-FTIR	Resin + wax + (ochre)	1	[56]
31	Shanghu, China	2	OM, Raman, FTIR, py-GC-MS	Plant origin, too degraded	1	[57]

*The Le Moustier results are questioned in [58]

1 ## 2.1. Microscopy

2 ### 2.1.1. Optical microscopy (OM)

3 OM is an instrument that is commonly used for the use-wear analysis of stone tools [59, 60]. OM
4 permits to identify wear traces such as polish, edge damage, striations and rounding on the stone
5 tool surfaces and edges. These traces result from friction with the materials worked (e.g., bone,
6 hide, wood) during tool use. Methodological work has shown that traces are specific for a
7 particular contact material and use motion and can be identified based on an experimental
8 reference (Fig. 1A) [59, 60]. It could also be demonstrated that the contact with the hand and the
9 friction within a handle result in identifiable microscopic traces [61, 62], which has permitted to
10 identify that hafting appeared at least about 250 ka in Europe [3] and 200 ka in Northeast Africa
11 [4]. Similarly, residues can be deposited on stone artefacts following use (e.g.,[48]) and hafting
12 (e.g.,[34, 40, 63], though attention has to be paid to possible sources of contamination [64] and
13 the impact of taphonomic processes [65, 66]. Residues are most identified purely based on
14 visual criteria (e.g., colour, appearance) (see for instance [38]), even though it has been shown
15 that this is not sufficiently reliable in itself [67–69].

16

17 Fig. 1. Functional results, modified from Rots et al. 2017: Fig. 17 and Fig. 26 A) Example of an integrated
18 OM analysis of a stone tool with use-wear and residues. B) EDX analysis of a stone tool, suggestive for the
19 presence of ochre and a possible resinous organic residue. Rots V, Lentfer C, Schmid VC, Porraz G, Conard
20 NJ. Pressure flaking to serrate bifacial points for the hunt during the MIS5 at Sibudu Cave (South Africa).
21 PLoS ONE 2017;12:e0175151. https://doi.org/10.1371/journal.pone.0175151
22

2.2. Spectroscopy

2.2.1. Scanning electron microscope – energy-dispersive X-ray spectroscopy (SEM-EDX)

Scanning electron microscope (SEM) creates a high magnification image of the stone tool by scanning the surface with an electron beam that interacts with the atoms on the surface. In back scattering mode, the energy differences between the incoming and reflected electron beam is used to create a black-and-white image, depending on the atomic weight. In a SEM image, the heavier atoms are a lighter shade than lighter atoms, thus an organic residue shows as a light spot against the darker stone surface [70]. The size of the stone tools that can be examined depend on the size of the chamber. SEM has been used in wear studies of stone tools since the 1980's [71] and its potential for also examining residues on stone tool surfaces was quickly recognised (Fig. 1B). The first SEM instruments required that the samples were coated (usually with gold or platinum) to increase their electrical conductivity in order for optimal imaging and analysis. This coating could not be removed from the sample, destroying the adhesive for other analysis and preservation. The development of environmental SEM that no longer require artefact coating drastically increased the utility and frequency of use of SEM in functional studies of stone tools [70]. Thanks to the environmental conditions, present-day SEM can be considered as non-destructive and non-invasive [31, 70]. Environmental SEM coupled with an energy-dispersive X-ray spectroscopy (EDX) provides the possibility to analyse the atomic composition of the reside [70]. The EDX detects the characteristic X-rays which are released by the atom upon interaction with the electron beam. This is useful for an initial screening to investigate whether the residue is of organic nature (by the presence of carbon and oxygen atoms), which might be a strong indication for a possible adhesive (Fig. 1B) [7]. The combination of SEM-EDX produces an image and atomic composition at the same time. However, even though SEM-EDX analysis is highly useful, it does not provide a unique atomic pattern for each residue or adhesive, nor does it elucidate in and by itself the origin of the residue (tool use or other). Consequently, SEM-EDX analysis cannot be used on its own to identify an adhesive, but it is a crucial step within a sequential protocol that also involves OM and follow-up chemical analysis [7].

2.2.2. Fourier transformation infrared spectroscopy

Over the last decades, FTIR has significantly gained in interest and popularity as an analytical technique for the analysis of residues [72–74], including adhesives [1, 20, 50]. Advantages are the ease of operation, the fast analysis, the semi- to non-destructiveness towards the residue and the chemical information it provides. While several types of FTIR instruments are employed for studying adhesives, their basic principle is similar. In short, each molecular bond of a molecule (e.g., C-H, C-O, C=O) has a specific vibration which absorbs IR light at a different

57 wavelength. In FTIR, the sample is irradiated with all IR wavelengths at the same time and to
58 deduce which wavelengths were absorbed a Fourier-transform (FT) is performed. This
59 considerably reduces the analysis time, and it is nowadays the standard type of IR spectroscopy.
60 Every IR active organic material results in a unique spectrum, therefor, the unknown material
61 (e.g., residue) may be identified through comparison with spectra of reference materials (e.g.,
62 modern adhesives) [29, 42, 50, 54].

63 Sample preparation can be performed in two ways, both require the removal of a tiny sample (<
64 1 mg) and are thus (minimally) invasive. In the first method, the sample is finely ground before
65 being mixed with an IR inactive material, like potassium bromide (KBr) and pressed into a thin,
66 transparent pellet. This is needed to reduce scattering and reflection effects and to increase the
67 accuracy of the results. Afterwards, the sample can be recovered from the pellet and used for
68 other investigations. However, KBr is hydroscopic and needs to be prepared in a dry environment,
69 if not, water absorption might interfere in the FTIR spectrum. If water absorption (in a broad OH-
70 peak) is seen in the IR spectrum, the second derivative of the spectrum can be calculated [75,
71 76] or the sample can be re-analysed with a newly pressed KBr pellet.

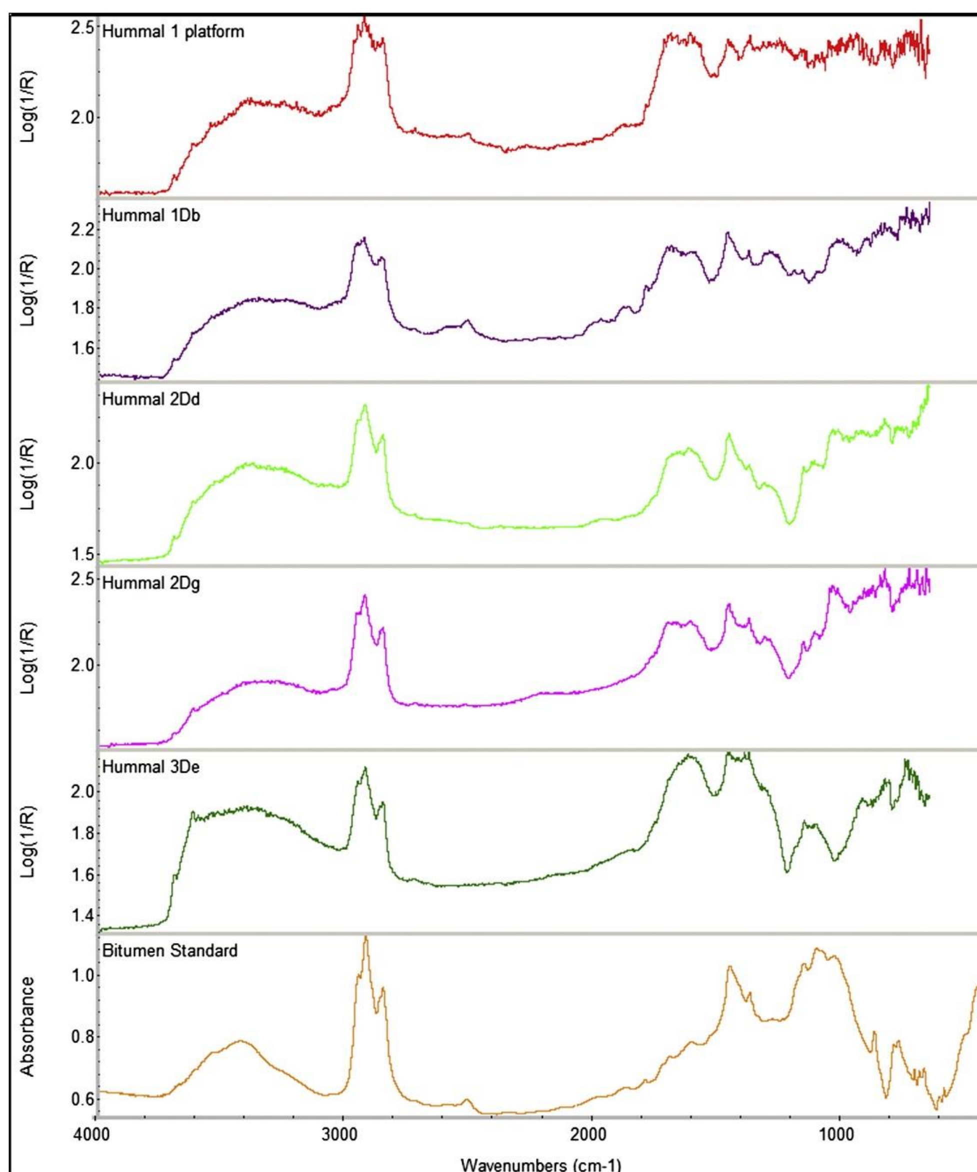
72 The second sample preparation method requires an FTIR instrument with a crystal with a high
73 refractive index, this type of instrument is also called attenuated total reflection (ATR) – FTIR.
74 Here, the finely ground sample is directly placed on top of the crystal, and the IR beam travels
75 through the crystal under total reflectance. The total reflectance angle of the IR beam causes the
76 light to only interacts with the surface of the sample, with less risk of intensity loss due to
77 scattering or reflection effects of the sample. The ATR crystal eliminates the need for a KBr pellet
78 preparation while achieving similar results. However, the KBr pellet method is slightly more
79 sensitive and is therefore still popular for Middle Palaeolithic adhesive research [20, 42, 45, 54].
80 For artefacts from the Upper Palaeolithic and younger, ATR-FTIR is more frequently used [56, 77,
81 78].

82 A non-invasive FTIR technique is the FTIR microscope (μ-FTIR), because here residue is analysed
83 without removal from the stone tool surface. This instrument works like a microscope, but it
84 comes with an extra IR light source and a tip where an ATR crystal is incorporated. The tip is
85 slightly pressed against the top of the sample, and IR light is radiated through the tip at an
86 attenuated angle. The main downside of analysing the residue directly on the stone tool with μ-
87 FTIR is that the residue is often dispersed in a very thin layer, so thin that the stone surface might
88 interfere with the results. Moreover, the surface of the samples has to be perfectly horizontal; if
89 not, some of the IR rays might scatter away (Lien et al., in press). The spectral disruption of the
90 stone surface results in a lower resolution, higher noise and negative peaks. In this case, a

91 Kramers-Kronig transformation, which mathematically reduce the spectral distortions, can be
92 performed. However, this has proven difficult for archaeological artefacts when the signals of
93 interest are hardly above the noise [29]. Nonetheless, positive results were obtained for stone
94 tools dating to MIS 3 [50] and MIS 2 [56, 57].

95 Although FTIR techniques differ in how the sample is introduced, the measurements parameters
96 are generally standardised; a spectral range of 4000-400 cm^{-1} with a resolution of 4 or 2 cm^{-1}. The
97 number of scans is depended on the type of sample, a general rule is that the higher the number
98 of scans, the higher the signal-to-noise ratio is (e.g., 32 scans [50]). Additionally, for μ-FTIR the
99 spot size needs to be selected, this depends on the size of sample and desired sensitivity as a
100 smaller aperture also means that more IR light is blocked from the detector [50].

101 The above parameters results in a detailed spectrum which includes the less specific methyl and
102 ethyl groups and the specific carboxylic groups and other functional groups. Of particular
103 importance is the region between 1500 cm^{-1} and 400 cm^{-1}, which contains peaks unique to each
104 sample, and is commonly called the fingerprint region. Spectra are usually presented as
105 absorbance (or reflectance in μ-FTIR) plotted against the wavenumber. Peak annotation can be
106 performed, and general FTIR absorbance tables exist to assist. Moreover, library comparison of
107 the obtain (μ-)FTIR spectrum is also possible, in which the full FTIR spectrum of the sample is
108 compared with a reference library [50] (Fig. 2). Although an extensive database for plant and
109 anime residues exist [73, 74], a comprehensive database for hafting adhesives—including
110 experimentally weathered samples—has yet to be developed. As a result, most studies use their
111 own small-scale reference database, which is generally composed of modern adhesive samples
112 [20, 42, 56]. This complicates comparisons, because taphonomic modifications can severely
113 alter the chemical composition of the residue, causing them to differ substantially from the
114 pristine adhesives [29]. A good example is giving in [50], see also Fig. 2, the FTIR spectrum
115 archaeological bitumen revealed a broad peak between 1700 and 1600 cm^{-1}, which is not present
116 in the FTIR spectrum of the database bitumen. The authors suggest that this broad peak might be
117 the result of oxidative degradation reactions that lead to the formation of ketones and carboxylic
118 acids, which results in absorbance peaks in that region [50]. In such cases, the sample needs to
119 be analysed with GC-MS to obtain full characterisation of the chemical composition and to
120 identify modifications induced by degradation reactions. In this case the GC-MS analysis was
121 performed before the μ-FTIR analysis. And, unfortunately, the fractionation step during the
122 sample preparation did not retain polar compounds, therefor the presumed ketones and
123 carboxylic acids by μ-FTIR were not detected with GC-MS [49].



124

125 Fig. 2. μ-FTIR spectrum of residues on archaeological artefacts and comparison with a library bitumen
126 spectrum. Reproduced with permission from Monnier GF, Hauck TC, Feinberg JM, Luo B, Tensorer JML,
127 Sakhel H al. A multi-analytical methodology of lithic residue analysis applied to Paleolithic tools from
128 Hummal, Syria. J Archaeol Sci 2013;40:3722–39. https://doi.org/10.1016/j.jas.2013.03.018 (Fig. 9).

129

130 ### 2.2.3. Raman spectroscopy

131 Raman spectroscopy is a more complex technique and is not often used in residue analysis, but
132 it is non-destructive and non-invasive. Raman spectroscopy works with a laser beam that is
133 directed towards the sample, and it measures how the light scatters to detect the vibrations of
134 the molecular bonds present in the sample. Raman spectroscopy uses a different light source
135 and interacts differently with the sample than FTIR spectroscopy. Whereas FTIR is able to identify
136 the functional groups present in the molecular composition of the residues (e.g., C=O of
137 ketones), Raman spectroscopy is most sensitive for non-polar, symmetrical molecular bonds.
138 Consequently, Raman spectroscopy is well suited for identifying carbonaceous materials (e.g.,

139 charcoal and soot), pigments (e.g., ochre) and bitumen. The main drawbacks of Raman are the
140 high fluorescence from the stone itself, which may cover the region of interest [54], and possible
141 microscopic damage of the residue due to the laser beam. Therefore, only a few studies
142 successfully used Raman spectroscopy alongside other instrumentation such as FTIR or GC-MS
143 [50, 54, 56].

144 2.2.4. Other spectroscopy instrumentation

145 In addition to the spectroscopy techniques mentioned above, X-ray diffraction (XRD) and
146 inductively coupled plasma atomic emission spectroscopy (ICP-AES) have been used. A XRD
147 identifies crystalline mineral structures found in, for example, bitumen. It provides insights into
148 possible contamination and the geological source of the bitumen. An ICP-AES quantifies
149 elemental composition, enabling the detection of trace elements that also inform about
150 degradation processes and geological provenance [20, 50]. As these techniques are used quite
151 rarely in Palaeolithic adhesive studies, they are not discussed in more detail here.

152 2.3. Gas chromatography – mass spectrometry (GC-MS)

153 GC is one of the most widely used analytical techniques for separating and identifying complex
154 mixtures. Its power lies in its ability to resolve individual molecules, providing a detailed chemical
155 profile of a sample. A basic GC consists of three parts: an injector, an oven and a detector.
156 Different injectors and detectors are present, each with their own advantages and disadvantages,
157 however the basic principle of all GC separations is the same and will be briefly explained.

158 In principle, a (liquid) sample is introduced in the inlet, where it is evaporated and directed into
159 the column by the carrier gas (mobile phase). Separation of the compounds occurs by different
160 interactions with the column (stationary phase), causing some compounds to travel faster
161 through the column than others. At the end a detector is placed, which detects the compounds
162 and produces a chromatogram. The oven, which houses the column, can be kept at an
163 isothermal temperature, or a temperature ramp can be programmed. The latter is more common
164 because it results in better separation of complex mixtures as it reduces band broadening and
165 enlarge the volatility range of separable chemicals. Because separation occurs in the gas phase,
166 only compounds that can be volatised without degradation are suitable for GC analysis. Hence,
167 both the sample preparation and the injection are optimised to ensure that all the analytes
168 present are volatised without degradation, this is further discussed in section 2.3.1 and 2.3.2.

169 The most common GC columns are narrow, open-tube fused-silica capillary columns with a
170 stationary phase made of a thin layer of polymers coated on the inner side of the capillary tube.
171 This coating can be selected depending on the chemical properties of the sample and a wide

172 variety of different stationary phases are commercially available. The ideal column coating will
173 have moderate interactions with all the analytes of the sample so that good separation is
174 obtained. If the interaction is too strong, some analytes might not elute from the column, while
175 too weak interaction results in poor separation. The chemical inertness of the fused-silica
176 ensures that the column interactions are only due to the polymer coating. A common choice is a
177 non-polar 5% diphenyl/95% dimethyl polysiloxane phase, which separates non-polar, (semi-)
178 volatile compounds primarily according to their boiling point. In addition, the fused-silica
179 columns are strong and flexible, making it possible to produce very long columns, typically 30 to
180 60 m, tightly coiled in a small diameter. The total number of distinct analytes that can be
181 separated in a single GC run is called the peak capacity. This value can be increased by
182 optimisation of the carrier gas flow rate, column length, column diameter, stationary phase, and
183 temperature program. Nevertheless, for very complex samples, such as weathered prehistoric
184 adhesives, the peak capacity is limited in practice and co-elution of two or several analytes is a
185 common phenomenon.

186 After the separation of the compounds, that exist the column, have to be detected and then
187 reported based on a so-called retention time value. One of the standard detectors is a flame
188 ionisation detector (FID), where organic eluents are burned quantitatively in an hydrogen flame
189 producing ions that are further sensed to calculate the concentration of the compounds. An FID
190 exhibits a larger linear dynamic range and is ideal for targeted quantitative analysis of analytes
191 with known retention times but is less suited for non-targeted measurements in complex
192 mixtures with unknown compounds as it lacks the ability to identify unknown molecules. For
193 those samples the hyphenation with an MS detector is a solution because it is able to provide
194 structural information. An MS apparatus has three different parts: an ion source, a mass analyser
195 and a detector, the entire set-up is being operated under vacuum. The ion source is interfaced to
196 the GC column by a heated transfer line, GC eluents are ionised, and ions are transferred into the
197 mass analyser for separation according to their mass-to-charge ratio (m/z). Several ionisation
198 techniques can be used depending on the sample's properties, but the most common is electron
199 ionisation (EI). In EI, molecules are bombarded with high-energy electrons, typically at 70
200 electron volt (eV). This collision transmits enough energy to cause fragmentation of the molecule,
201 producing a mixture of positively charged radical ions. Separated ions are used to construct the
202 fragmentation patterns of a compound and displayed in a mass spectrum. The high-energetic
203 electrons (in many cases) completely scatter the molecule, hence, an EI is a hard ionisation
204 technique. This is also the main advantage of EI, because the use of the standardised ionisation
205 energy of 70 eV results in highly reproducible mass spectra for a given compound across different

206 instruments. This reproducibility permits the use of commercial spectral libraries, such as those
207 developed by the National Institute of Standards and Technology (NIST) or Wiley, to identify
208 unknown compounds by matching their spectra. Most chromatographic software integrates
209 these databases, allowing automatic spectral searching and compound identification directly
210 from chromatographic peaks. Sometimes a softer ionisation technique is preferred, but then the
211 use of commercial libraries for compound identification is complicated because the analyte is
212 less fragmentated, see section 3.2.1.

213 ### 2.3.1. Liquid injection GC-MS

214 Liquid injection is the most widely used sample introduction method for hafting adhesive
215 analysis in GC-MS, because of its robustness and ease of operation.

216 Unfortunately, solid samples that contain a lot of polar compounds, like hafting adhesives,
217 cannot directly be injected into the GC. Most commonly 1 to 3 mg of residue from the stone tool
218 is dissolved in a semi-polar solvent followed by trimethylsilyl derivatisation, typically using N-
219 Trimethylsilyl-N-methyl trifluoroacetamide (MSTFA) or N,O-Bis(trimethylsilyl)trifluoroacetamide
220 (BSTFA). This derivatisation is required to transform the non-volatile polar compounds into semi-
221 volatile non-polar compounds, by substituting the hydrogen atoms in -OH and -NH functional
222 groups with a trimethylsilyl group. While silylation works well for most compounds classes, it
223 does not consistently derivatise fatty acids (FAs) resulting in a mix of derivatised and
224 underivatized FAs. This might cause a problem as FAs are present in almost every adhesive and
225 sometimes even used as a marker for a specific adhesive (see [79] for an example). Conversely,
226 lipidomics often employs methylation (e.g., formation of fatty acids methyl esters) for the
227 analysis of FAs with GC-MS [80]. Sometimes the solvent extract is subjected to alkaline
228 hydrolysis (e.g., saponification) [18, 33]. This step will cleave any bonds made by a base (e.g.,
229 cleavage of ester bonds and the formation of free (fatty) acids). Hydrolysis is a good sample
230 preparation to breakdown large molecules into smaller molecules that fall in the volatility range
231 of GC-MS. Afterwards, the free -OH and -NH groups are derivatised before GC-MS analysis.

232 Most liquid injections use a split/splitless (S/SL) injector. In general, 1 to 2 μL of sample is
233 injected into the heated injector port, where it is rapidly vaporised. Highly concentrated samples
234 are usually injected with the split mode. Only a small portion is injected into the column, the rest
235 is directed to the waste by the carrier gas through the split vent. The split ratio determines what
236 fraction of the sample enters the column and it is used to prevent column overload and improves
237 peak shapes. Trace-level analyses are usually conducted in splitless mode: the split vent is
238 closed, and the entire sample is transferred onto the column. This procedure maximises

239 sensitivity but also results in broader peaks and more solvent effects. Nevertheless, for the
240 analysis of highly degraded prehistoric adhesives splitless mode is often preferred. Another
241 liquid injector is a programmed temperature vaporization (PTV). However, a PTV has less inlet
242 discrimination and is thus able to better transfer also high-boiling compounds in the GC column.

243 ### 2.3.2. Pyrolysis GC-MS

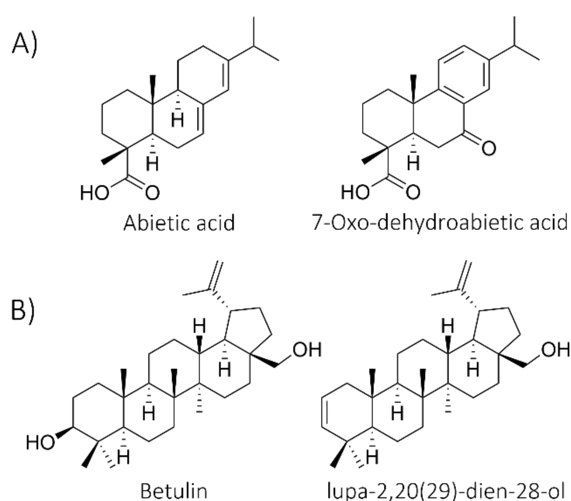
244 Pyrolysis (py) is another sample introduction method for GC-MS that is commonly used in
245 adhesive analysis. The main advantage is that it requires no sample preparation and smaller
246 sample size (≤1 mg). Moreover, adhesives that contain a large portion of insoluble compounds,
247 such as the long carbon chains alcohols, esters and FAs in beeswax or the higher molecular
248 weight hydrocarbons present in bitumen, can be easily analysed with py-GC-MS. However, py-
249 GC-MS is invasive and destructive towards the residue as it has to be removed from the artefact
250 and is consumed during the analysis.

251 In py-GC-MS, the sample is pyrolysed in the pyrolyser connected to the inlet of the GC, and the
252 smaller, volatile pyrolysis adducts are injected onto the capillary column and analysed. The
253 direct pyrolysis makes it possible to analyse polymeric structures which are normally difficult to
254 dissolve in a solvent. Furthermore, in-situ derivatisation with tetramethylammonium hydroxide
255 can be performed. In this way, the polar groups are protected, and complete pyrolysis of the
256 compounds is avoided. Despite these advantages, py-GC-MS is not often conducted on
257 Palaeolithic adhesives (but see [21, 43, 57]). In cultural heritage applications [81] or for more
258 recent time periods [82, 83] py-GC-MS is more commonly used.

259 ### 2.3.3 Targeted data analysis

260 The data analysis after the GC-MS run is a targeted search for marker compounds, called
261 “biomarkers”, which are indicatory for a specific adhesive. The development of this targeted
262 method started in the early 1990s with the groundbreaking work of Evershed and colleague [84].
263 A good marker is unique for the adhesive, chemically and thermally stable and fairly resistant to
264 degradation [84, 85]. Most di- and triterpenoids meet these requirements; plants which produce
265 diterpenoids do not produce triterpenoids and vice versa [86]. For example, a pine tree
266 exclusively makes diterpenoids and pine resin is identified with the detection of the diterpenoid
267 abietic acid and its related compounds (Fig. 3A) [18, 87, 88]. A pine tar is distinct from a resin
268 when heating markers are found, such as retene and 18-nor-abietatriene [89]. By contrast, birch
269 trees produce triterpenoid compounds. Birch tar is identified based on the detection of
270 triterpenoids betulin, lupeol, which are also naturally present in birch bark, and heating markers
271 like lupa-2,20(29)-dien-28-ol (Fig. 3B) [79, 90, 91].

272 The selection of good biomarkers is not so straightforward for archaeological residues as these
273 artefacts underwent extensive degradation, and the chemical fingerprints have been modified.
274 The degree of modification is also depending on the burial environment and taphonomic
275 processes active at the site [92, 93]. Consequently, each archaeological artefact is unique, and
276 it rarely happens that all the possible biomarkers of an adhesive are detected in an artefact (see
277 section 3.1). Good data treatment of the GC-MS analysis is, therefore, of utmost importance to
278 correctly understand the chemical composition of the sample, which is discussed in more detail
279 in section 3.2 and 3.3.



280

281 Fig. 3. A selection of important biomarkers, A) pine resin related markers and B) birch tar related markers.

282 # 3. Challenges and limitations in adhesive identification studies

283 ## 3.1. Strong focus on specific adhesives

284 Currently, attention has primarily centred on a few adhesives only (Table 1), most notably birch
285 tar in the European context [1, 21, 22, 32] and *Podocarpus* tar in the South African context [23,
286 53, 94]. The focus on tar-based adhesives probably stems from the broader debate surrounding
287 cognitive developments in Neanderthals and early modern humans [26, 95]. Tar is not readily
288 found in nature and has to be man-made by pyrolysis of bark (birch tar) or leaves (*Podocarpus*
289 tar). Some argue that tar production reflects cognitive development because it involves complex
290 transformation processes [21, 27]. Others contend, however, that it does not necessary indicate
291 advanced cognition, as a tarry substance can be obtained relatively easy [25, 96].

292 Recently, the focus on *Podocarpus* tar in the context of the MSA in South Africa has been
293 addressed. Research by Chasan et al. (2024) [93] comparing the GC-MS fingerprints of the
294 adhesives; tars and extrudates of the tree and fruits, from the genera *Podocarpus, Afrocarpus*
295 and *Widdringtonia* found minimal differences only. More problematic is that the GC-MS analysis

296 revealed that the main difference between those three genera are compounds from the pimarane
297 diterpenoid class [93]. These molecules are very susceptible to degradation due to the
298 presences of conjugated double bonds and are thus not good markers [93, 97]. In fact, all of the
299 studies published until now, in which samples were identified as *Podocarpus* tar report markers
300 which are present in all three genera. This makes it impossible to confidently state whether the
301 adhesive was indeed a tar based on *Podocarpus* [93]. Furthermore, degradation processes, such
302 as oxidation, may not only result in the removal of pimaranes but also in the formation of more
303 totarane ketones, which are only present in low quantities in modern samples [93]. Before these
304 totarane ketones can be used for diagnostic purposes, more research in their formation is
305 required with artificial degradation experiments. In another study where organic residues of six
306 artefacts from Sibhudu were compared with different tars and different production methods of
307 *Podocarpus* tar [26]. Based on the FTIR analysis and the resulting PCA ([26], Fig. 4)., the
308 archaeological samples were identified as *Podocarpus* tar produced via the condensation
309 method. However, both the archaeological samples and the condensation tar samples cluster
310 close to the origin in the PCA plot. This placement indicates that there is not a single variable that
311 explains the data more strongly than the others, and it makes it difficult to interpretate the PCA.
312 Furthermore, the GC-MS analysis was not able to detect diagnostic markers for the
313 archaeological samples, only a few *n*-alkanes and wax esters were detected. The diagnostic
314 markers of the archaeological samples could be systematic removed through long term
315 degradation processes or their concentration is below the limit of detection for GC-MS. The used
316 samples size (<1 mg) in the study is actually minimal for producing reliable GC-MS results [26].
317 The lack of diagnostic markers in the GC-MS makes it difficult to support the FTIR results in that
318 the archaeological sample is indeed a *Podocarpus* tar produced via the condensation method.

319 Both studies outlined above show that knowledge about degradation processes of South African
320 resins and tars is severely lacking and more investigation on the degradation pathways is needed
321 before confident identification is possible. Moreover, one study also indicates that there are
322 other possible adhesive sources native to South Africa, such as *Widdringtonia* resin*, Euphorbia*
323 *tirucalli* latex*, Aleo ferox* latex or the *Ficus sur* latex [26, 93] that also merit attention. To date, only
324 *Euphorbia tirucalli* has been identified in the archaeological record [51] (Table 1). Consequently,
325 our knowledge of the use of adhesives in South Africa is still limited and one may expect that a
326 broad range of different adhesives was employed. Several of which may not have preserved well
327 in the archaeological record.

328 In Europe, birch tar is the main adhesive argued to have been used during the Middle Palaeolithic
329 and later on. It has been shown that birch tar can be easily distinguished from other wood tars by

330 chemically stable markers [98]. However, the degradation pathways of those markers are
331 unknown, and very degraded adhesives may have one or two markers only. For instance, a very
332 degraded tar sample was recovered from an artefact that was found in beach sands dredged
333 from the North Sea by the Zandmotor (The Hague, the Netherlands) [21]. The Zandmotor is an
334 installation that dredges sand from the seafloor just off the coast of The Hague and has brought
335 numerous Palaeolithic artefacts to the surface, from what was once dry, human-inhabited land
336 [21]. This unique burial environment likely influenced the survival of the organic residues
337 differently in comparison to an artefact recovered from an inland location. The py-GC-MS
338 analysis of the black residue adhering to the stone tool revealed the presence of betulin, lupeol
339 and a series of long chain diacids, which was considered enough to identify the residue as birch
340 tar [21]. In addition, the absence of other markers was used to argue for a complex tar making
341 process. However, this interpretation overlooks the fact that the artefact is about 50 ka old and
342 that degradation has had a substantial impact on the preservation of analytes. It is therefore
343 more likely that the missing markers are a result of diagenetic alteration rather than evidence for
344 a specific tar production method (Lokker et al., submitted). Moreover, betulin and lupeol are
345 naturally present in birch bark and not unique to birch since they are also found in other tree
346 species from the *Betulaceae* family [98]. Strictly speaking, the adhesive from the Zandmotor
347 stone tool cannot be identified as birch tar, due to the lack of heating markers and distinct
348 markers for birch. Hence, the adhesive is labelled as a 'tar' based solely on visual appearance
349 rather than chemical evidence. Other birch tar samples in Europe, found in different burial
350 environments, have more birch tar markers preserved, including heating markers [1, 22]. Some
351 studies have tried to understand what method was used to produce the birch tar based on
352 comparisons of the archaeological samples with experimentally produced tar [45, 92]. However,
353 in the absence of knowledge on the degradation pathways of tar, it is impossible to draw
354 conclusions based on the absence of specific markers and such interpretations are therefore
355 unreliable.

356 The above review of the published literature illustrates how little we currently know about the
357 influence of taphonomic processes on the molecular composition of natural adhesives. In the
358 past ten years, a few short-term weathering experiments have been conducted with adhesive
359 samples in various settings and set-ups, with some being buried and others left on the surface
360 [29, 66, 99, 100]. These studies highlighted the complexity of the degradation processes and the
361 fact that these cannot be disregarded when analysing a residue. Interestingly, some adhesives,
362 like birch tar and pine resin, might be less susceptible to degradation than other adhesives, such
363 as animal glue [66]. This might explain the bias in adhesive identification towards birch tar, simply

364 because other adhesives are not preserved. Yet, none of these studies included chemical
365 analysis of the degraded adhesive with GC-MS, consequently, the influence on the molecular
366 composition is still not known. By focusing on just a few types of adhesives combined with poor
367 knowledge on degradation pathways other interesting but less visible residues on artefacts may
368 have been disregarded.

369 3.2. Compound identification with GC-MS

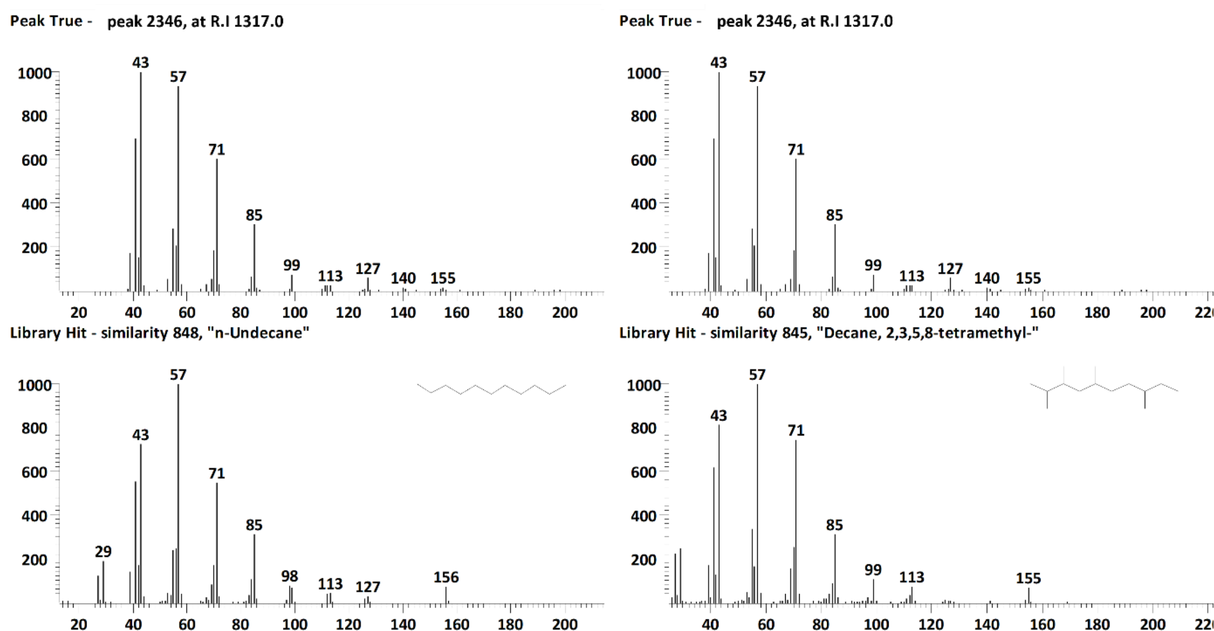
370 GC-MS allows to separate and identify the molecules present in a sample, however, its reliability
371 depends on correct instrumental usage and data analysis, which require experienced users. In
372 particular, user input is necessary for data processing and for the peak annotation of a GC-MS
373 chromatogram. For example, the library search of a GC-MS chromatogram will always return a
374 match with an entry for every mass spectrum, however, it is the user who decides if the match is
375 good or not. This is a fairly subjective decision and transparency on how this decision was made
376 is important to understand the quality of the reported annotation. Although awareness is
377 increasing of the importance of good chromatographic practices and transparent reporting in
378 research on Palaeolithic adhesives [18, 92, 101], essential elements in data treatment and
379 identification of the chromatogram peaks (e.g., minimum match threshold values, linear
380 retention indices proximities) are still often reported insufficiently; in the sections below, these
381 elements are discussed in more detail.

382 3.2.1. Library search

383 Currently, there are several commercially available databases, and they are often directly
384 incorporated into the GC software. This makes peak annotation easy with the help of a library
385 search. However, user input is still required to check the automatic search results. Therefore
386 basic knowledge on the practicalities involving peak annotation and mass spectral
387 fragmentation patterns is desired [102]. The most common MS library search method is a forward
388 search, which means that the acquired spectrum of the unknown compound is compared to
389 reference spectra. A library search algorithm returns a similarity score (ranging from 100 to 1000),
390 and a threshold of 650 or 700 is often implemented before a match is annotated [102,
391 103]. During a library search of the mass spectrum, a list of multiple possible match candidates
392 (a “hit list”) is generated. Most of the time, the first hit is not the best fit, the user must check for
393 this and select a better match candidate for the peak. This matching is based on user knowledge
394 of the sample properties and, if homologous series are present (e.g., n-alkanes), on elution
395 orders. Other scores are the reverse (how well the reference spectrum explains the unknown
396 spectrum) and the probability (likelihood of a unique spectrum), they are also often stated
397 alongside the similarity and help in deciding the best match [103, 104]. Providing a full peak list

398 with the similarity score will give other researchers the opportunity to check the data processing
399 and identify borderline matches. Other important data processing parameters, that are
400 preferably reported too, are: the used software, the background subtraction method, the signal-
401 to-noise (S/N) ratio and the similarity threshold values [105]. Inconsistent reporting of library
402 search values across studies hampers comparability, because two datasets may apply different
403 criteria for what constitutes a valid identification. Lack of standardisation complicates meta-
404 analyses, obscures true reproducibility and can give a misleading impression of the confidence
405 in reported compounds. This is important in a field as small as the one focusing on prehistoric
406 hafting adhesives, where most scientists rely on a few similar publications.

407 It should be emphasised that a library search alone is never sufficient to confidently identify
408 unknown compounds in a sample and that the peaks are only tentatively identified. The reason
409 for this is that for most of the compounds in a sample, a unique spectrum is impossible to obtain
410 with standard EI-MS instrumentation. The hard ionisation of the EI results in fragmentation of the
411 molecule, even though it helps to gather structural information, some information is also lost.
412 This is detrimental when there are (structural) isomers present in the sample, because those
413 fragmentation patterns are similar. An important class of structural isomers for the analysis of
414 adhesives is hydrocarbons, they come in numerous configurations as linear or branched forms.
415 Moreover, hydrocarbons are very easily fragmented with EI which results in the loss of the
416 molecular ion. The molecular ion is formed with the removal of 1 electron resulting in a positive
417 charged cation ($M^{+}\cdot$) and the m/z value corresponds to the molecular mass. With EI this is often
418 the least intensive peak in the mass spectrum or not present for molecules that are easily
419 fragmented. Consequently, a confident identification of the hydrocarbon chain length and
420 configuration is difficult with GC-EI-MS. This issue is illustrated in Fig. 4, the first library hit is a
421 linear n-alkane while the second hit is a branched alkane, both similarities are high. Furthermore,
422 the molecular ion is not present in the unknown spectrum. Hence, it is very difficult to correctly
423 annotate this peak based on only a library search. The linear retention index (LRI) (see section
424 3.2.2) of the peak gives only an indication on chain length. Nevertheless, in adhesives research,
425 hydrocarbons are often reported as n-alkanes (linear chain) structure. With EI-MS and a library
426 search based only on the similarity, peak annotation of hydrocarbons with the specific
427 configuration (linear vs. branched) is difficult. Alternative ionisation solutions exist (e.g., softer
428 ionisation methods like chemical ionisation (CI) and photo ionisation (PI)) but they have rarely
429 been used in the field so far. Their potential for the identification of branching of hydrocarbons is
430 however very promising [106].



431

432 Fig. 4. An MS library search of a hydrocarbon, on top is the 'unknown' spectrum and below a library hit. The
433 left side depicts the first match of an n-alkane, while the right-side gives the second match, a branched
434 alkane. Based on the similarity score, both hits are plausible. Note that the molecular ion is not present,
435 however, the retention time (stated as at R.I 1317, which means that the retention index is 1317, see 3.2.2
436 for an explanation of retention indices) indicates that the chain length should be C13 or C14.
437

438 ### 3.2.2. Linear retention index

439 As discussed above, peak annotation conducted with only a forward MS library search is
440 tentative and provides little confidence in the annotation. A way to easily increase the reliability
441 of a library search is to include an extra search parameter, namely, the retention time. However,
442 retention times are dependent on the column stationary phase, column degradation,
443 temperature program, instrumental drifts etc., and are therefore, not comparable between
444 different studies. Nevertheless, the retention time can be transformed into independent values
445 i.e. retention indices. The first retention indices were proposed in the late 1950s by Kováts [107]
446 under isothermal oven conditions and later a temperature-programmed extension was proposed
447 by Van den Dool [108], which is nowadays known as linear retention index (LRI) [109, 110]. LRI is
448 calculated after the injection of a standard, which is composed of a homologous series of *n*-
449 alkanes, which cover the full chromatographic space. This is used to transform the retention time
450 of an unknown compound to a value relative to its elution order between the two closest *n*-
451 alkanes. LRI gives an extra confidence in compound identification as most entries in an MS library
452 also have their LRI reported; a good match must have an LRI within a certain range (commonly
453 ±20) [110]. For this reason, a good LRI match increases the confidence of a library search, which
454 is especially useful for isomers [111]. Furthermore, the n-alkane standard can be used for the
455 distinction between linear n-alkanes and branched alkanes, as well as to identify the chain length

when the molecular ion is not present (Fig. 4). For more information on the practicalities of LRI in GC-MS, the reader is referred to the literature (e.g., [109, 112].

Nevertheless, isomers with a similar mass spectrum and retention time, and thus similar LRI, cannot be confidently identified with a library search only (Fig. 5). In this case, exact peak identification is only obtained by injection of a standard, of which the composition and concentration is exactly known. Co-injection of the sample and the standard should result in an increased peak height, if not, the unknown analyte differs from the standard. Unfortunately, co-injection with a standard is hardly done due to the lack of standards, and the extra effort of a second injection. Nonetheless, for birch tar there are a few standards commercially available and these are sometimes injected for exact identification of the markers [33, 79].

Peak annotation based after a library search is always tentative, this should be kept in mind when addressing the marker components. LRI provides an extra line of evidence and might help in the distinction between isomers. However, standard injection is required for the exact identification of the presences of the (targeted) marker compounds. If a compound has no commercially available standard, is it possible to synthesise the compound in-house. The configuration should first be checked with nuclear magnetic resonance (NMR). Exact identification of all compounds present in adhesives is impossible due to the vast amount of organic compounds. However, focusing on the terpenoid fraction will already greatly improve our understanding of the chemical composition of the adhesive and will help with better identifying biomarkers.

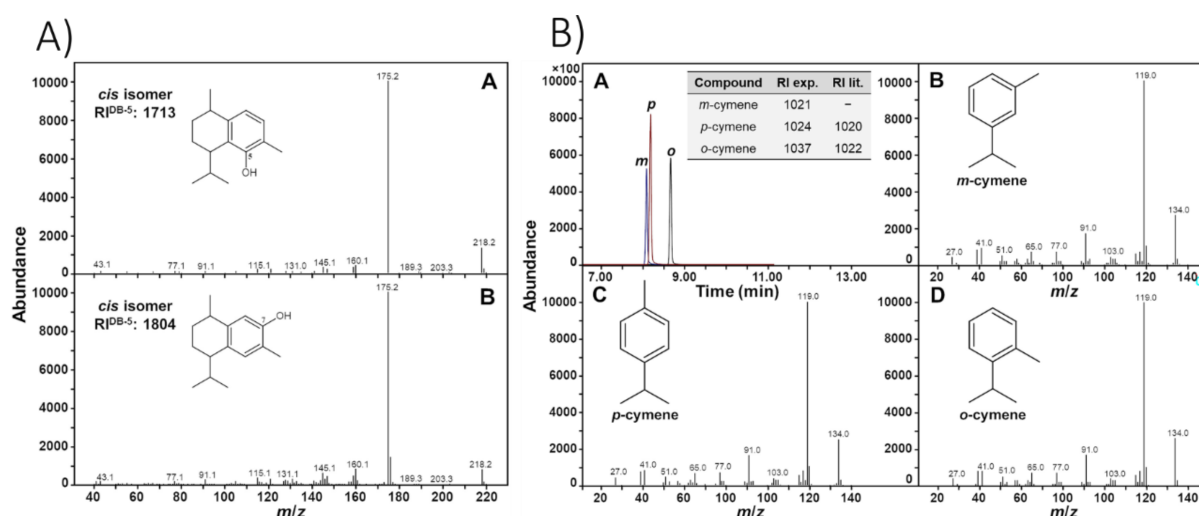


Fig. 5. An example of structural isomers which have A) a different retention time and thus a distinction with LRI can be made, or B) a similar retention time, then LRI cannot be used for the correct identification of the exact isomeric configuration. Both reproduced from Bizzo HR, Brilhante NS, Nolvachai Y, Marriott PJ. Use and abuse of retention indices in gas chromatography. J Chromatogr A 2023;1708:464376. https://doi.org/10.1016/j.chroma.2023.464376 (Fig. 2 and Fig. 3).

482
483 ### 3.2.3. Unknown compounds

484 The commercial MS libraries contain a massive number of compounds, however, it is still limited
485 compared to the molecules present in nature. In adhesive research this issue is most concerning
486 for birch tar, because several markers have been previously identified that are not present in any
487 library [79, 113]. The problem is that those compounds were identified with low-resolution MS
488 and only the most abundant m/z values are typically published. This is problematic because the
489 betulin related compounds all have highly similar mass spectra with the same most abundant
490 m/z values. Publishing only those values is not enough to enable other researchers to identify
491 those ‘unknown’ compounds in their birch tar chromatograms.

492 To further investigate ‘unknown’ compounds the analysis with a high-resolution MS will increase
493 the quality of the mass spectra and is able to reveal distinct mass spectra between the betulin-
494 related compounds [114]. However, for structure elucidation, these compounds must be
495 isolated and analysed with NMR, which is the only analytical instrument that is capable of
496 structure identification [97]. Without these experiments, the identification of the birch tar
497 markers remains a complex process with low confidence in the peak annotation.

498 ## 3.3. Quality assurance and quality control practises in GC-MS

499 Quality assurance and quality control (QA/QC) are important practices to ensure accuracy,
500 precision and reproducibility of the analytical results [105]. Thus, it provides an indication of the
501 reliability of the reported results, and it aids in reproducing the research. However, reporting
502 QA/QC measurements is often overlooked and deemed unnecessary even though the
503 importance of proper reporting QA/QC measurements has been emphasised more recently
504 [105]. Notably, QA/QC is also crucial when analysing archaeological materials since these
505 samples are variable and very small due to issues of preservation and degradation [115].
506 Although QA/QC adds a few extra steps in the analytical workflow, it considerably increases the
507 quality of the analytical work [105].

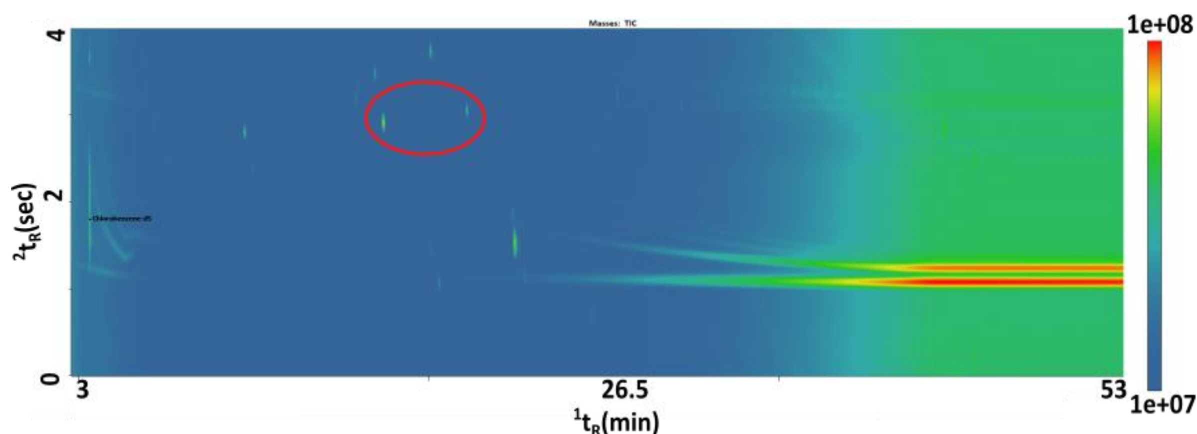
508 ### 3.3.1 Analysis of blanks

509 The main reason to analyse a blank is to detect contamination and carryover between the
510 samples. Two types of blanks are important to inject: a “solvent blank” or “true blank” and a
511 process blank. The first type of blank involves the injection of a dummy sample, which often
512 consists of the solvent in which the sample is dissolved. This blank identifies contaminations
513 from the system and sample carryover in a sequence. To ensure reliable monitoring, this blank
514 should be injected at the start, after every 5 to 10 sample runs and at the end of the sequence. A

515 process blank is required when samples undergo extensive preparation, such as the extraction
516 and derivatisation performed in adhesive analysis. This blank is again a dummy sample (solvent
517 only) that underwent the same steps as the other samples. The purpose of this blank is to identify
518 contaminations induced by the sample preparation process.

519 In the analysis of hafting adhesives, a third blank, the so-called "soil blank", might also be
520 included. This blank is the analysis of a soil sample taken from around the sampled artefacts or
521 from the same stratigraphic layer and this blank undergoes the same sample preparation steps.
522 Such a sample is not always possible, for instance, when the excavation was performed in the
523 past, but if available, it helps to identify contamination from the burial environment [18].

524 An example highlighting the importance of blanks—especially method and soil blanks—in
525 adhesive analysis is the frequent dominance of palmitic acid (C16:0) and stearic acid (C18:0)
526 (Fig. 6). These two FAs are not only widespread in nature but are also among the most common
527 FAs present on human skin [116, 117] and common laboratory contaminants. Contamination of
528 the stone artefacts with these FAs during e.g., excavation or sample preparation is therefore
529 entirely plausible. Yet, palmitic and stearic acid are often reported in the literature as the only
530 detected compounds in the sample (see [57] for an example) or as compounds that occur in
531 much higher concentrations than the other FAs (see [20] for an example). The detection of those
532 two FAs is suspicious in both cases, and it is more likely a result of contamination than traces of
533 an organic residue. The reporting of those FAs should be done with caution and contamination
534 of palmitic acid and stearic acid should be tested for with a method blank. Fig. 6 depicts a
535 comprehensive two-dimensional GC-MS (GC×GC-MS) contour plot of a method blank, produced
536 during the extraction process of experimentally made birch tar (Lokker et al., submitted). For
537 more information on GC×GC-MS the reader is referred to other excellent review papers which
538 explains the technique in detail [118–120]. In the method blank, only a few peaks appear with
539 palmitic and stearic acid among the most intense ones and are highlighted by the red circle. The
540 entire sample preparation was performed wearing gloves and using thoroughly cleaned
541 glassware; nevertheless, these FAs were still detected in the method blank. This indicates that,
542 at least a portion of the total FAs present in the analysed samples, likely originates from
543 contamination, adequately reporting these FAs is important.

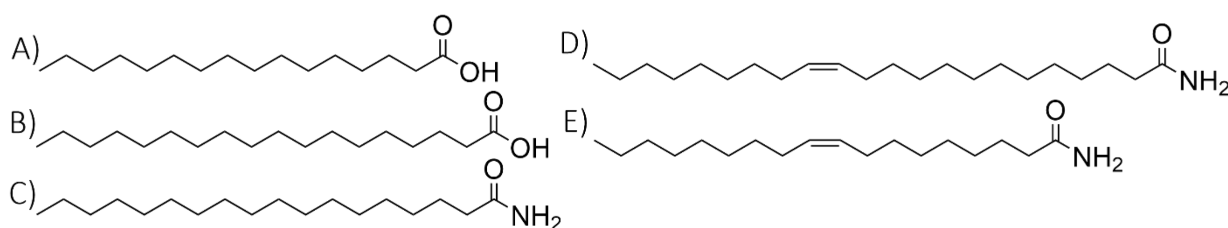


544 Fig. 6. A comprehensive 2D GC-MS contour plot of a method blank made during the sample preparation of
545 experimentally produced birch tar samples. The blank was prepared while wearing gloves and using
546 meticulously cleaned glassware. The two compounds present in the red circle are FA C16:0 and C18:0,
547 respectively.
548
549 Other lipids that can derive from contact with hands include cholesterol and squalene [116].
550 While these are not often reported as being present in residues interpreted as adhesives, they
551 are also more likely to represent contamination instead of actual compounds of the original
552 sample. Recognising plastic contamination, more precisely phthalates coming from the
553 laboratory gloves and/or the plastic bags used for storage, is more straightforward given that a
554 natural origin is less likely [45] .

555 Reporting compounds which are also found in the blanks is important and allows to verify that
556 the detected analytes are truly present in the sample and do not result from contamination. Best
557 practices for reporting, minimally include what type of blank has been used (solvent, method or
558 soil), the place in the injection sequence and how the blanks were used during data analysis. For
559 instance, were the blanks processed alongside the sample injections and what steps were
560 undertaken when a compound was also found in the blank, i.e., was the compound removed
561 from the reported peak table or not [105]?

562 In recent years, there has been growing awareness that contamination on stone tools may
563 interfere with OM and SEM-EDX analyses [67, 69, 72, 121]. Good practices with regard to sample
564 collection and storage conditions minimise possible contamination of the artefacts and increase
565 the confidence of the GC-MS analysis. These practices include, for example, artefact handling
566 with nitrile gloves only, storage of individual artefacts in plastic bags (e.g. Ziploc®), and no direct
567 markings on the stone tool. However, a recent study has shown that stearamide contamination
568 from these bags can occur on stone tools. Stearamide is a waxy solid compound commonly used
569 as a release agent; it is applied to the surface of plastic bags to prevent them from sticking
570 together [72] (Fig. 7). Erucamide and oleamide are also known plastic contaminants which might
571 be detected during analysis (Fig. 7) [122, 123]. In spite of this problem, plastic bags remain the

572 better storage solution for small artefacts as these bags prevent contamination from the
573 environment and provide an easy and safe storage and transport medium. Only through an
574 increased awareness of possible contaminant sources, data sharing on how to handle these
575 contaminants, and transparency with regard to how the artefacts have been handled and stored
576 (also during previous analysis) can increase confidence in correctly detecting contamination.
577 Furthermore, strict laboratory protocols and the injections of blanks, as outlined above, are
578 required throughout the entire analytical sequence to avoid that possible contaminants are
579 misinterpreted.



580 Fig. 7. A) Palmitic acid, B) Stearic acid, C) Stearamide, D) Erucamide, E) Oleamide

581 3.3.2. System stability tests

582 Every instrument can exhibit drift over time, affecting both accuracy and precision of the results.
583 To detect and minimise the impact of the drift, frequent system stability testing (SST) should be
584 performed before and during the measuring sequence. SST before the start of the measuring
585 sequence establishes that the instrument is working properly. Such tests include tuning and
586 calibration of the MS and blank injection to detect any contamination present in the system [105].
587 During the GC-MS analysis of the samples, SST best includes injections of blanks, quality control
588 (QC) sample and (certified) reference material (RM) at regular intervals during the injection
589 sequence. On top of that, the samples themselves should be injected in replicates and in random
590 order. Blank injections have already been discussed in section 3.3.1, the others are discussed in
591 more detail below.

592 A QC sample is used to track retention time drifts, intensity and mass accuracy over time [105,
593 115]. Often, a pooled QC sample is used for this, which is composed of an aliquot of each of the
594 unknown samples in the measurements. The pooled QC is popular because of its relative ease
595 of preparation, relevance to the samples and low cost [105]. However, archaeological samples
596 are generally too small to remove an aliquot to prepare a pooled QC. Instead of using precious
597 residue remains to make a pooled QC with the risk of not being able to conduct triplicate
598 injections, as having previously proposed by [115], a QC sample made with a standard solution
599 composed of similar compounds could also be used. Commercially available standards that can
600 be used as QC samples are the Grobmix, and the Philips mix, since they cover a wide range of
601 different compounds and polarity. Another option is the century mix which has even more

602 compounds, however this standard is not yet commercially available [114, 124, 125]. RM is a
603 generic term for a sample that is well characterised, homogenous and stable and can also be
604 used to check for system drifts. A CRM is metrologically validated and comes with an
605 accompanying certificate that also states the acceptable measurement uncertainty [105].

606 Besides the injection of the blanks and QC samples, the injection sequence should be designed
607 to randomise the order of samples and replicate runs of all samples. Randomising the sample
608 injection is important to minimise systematic biases introduced by instrumental drift, carryover
609 and gradual changes over time. Replicate injections of the sample are done to measure the
610 reproducibility of the analysis, which is usually expressed as the relative standard deviation
611 (RSD). Both quantitative and qualitative analysis will benefit from replicate injections, and it
612 should be standard practice to perform replicates. Although, a high number of replicates
613 improves the reliability of the detection, this is not always practical, and a commonly
614 recommended minimum is three injections [105]. Triplicate injections also provide a margin of
615 safety in case one injection fails due to instrumental error, the reproducibility is then checked
616 with the duplicates. In the case that the duplicates do not show good reproducibility (i.e. high
617 RSD values), is it better to reinject the sample.

618 Designing the injection sequence to include blank measurements, QC samples, replicates and
619 to randomise the order of the samples ensures that the observed patterns reflect true chemical
620 differences rather than analytical artefacts. This, in turn, enhances the reliability and
621 comparability of the resulting data. Reporting the randomising strategies alongside the number
622 of replicates injected is considered a key element of good analytical practices in complex mixture
623 analysis [105].

624 SST help to verify that the instrument's performance remains within predefined limits and that
625 any deviation can be corrected or accounted for before data analysis of the samples.

626 ### 3.3.3. Internal standards

627 Internal standards (ISTD) are added to the sample during the sample preparation. There are two
628 different ISTDs, the main important difference is the timing of addition and the reason. The first
629 reason is to identify instrumental changes and can be used to normalise the peak areas between
630 samples in the same run sequence. This standard is added to the sample after the sample
631 preparation so that the exact concentration is the same for every sample. The second reason is
632 to calculate the sample recovery and losses during sample preparation. In this case an ISTD is
633 added before the sample preparation. The second ISTD is mostly important when there is an
634 extensive sample preparation (such as the extraction and derivatisation of adhesives) and

635 quantification is desired or to track the derivatisation efficiency [18, 97]. A good ISTD has
636 chromatographic properties similar to those of the analytes in the sample, does not co-elute,
637 and produces a Gaussian peak that is fully resolved under the chromatographic conditions used
638 in the study. In adhesive research, the use of hexadecane and tridecanoic acid as ISTD is
639 sometimes reported, with the latter often employed to monitor derivatisation efficiency [18, 20,
640 33]. However, these studies frequently do not report how well the instrument performed or how
641 well the derivatisation step performed, for example by providing RSD or recovery values of the
642 ISTD. Additionally, the chosen ISTDs often elute within the same chromatographic region as the
643 compounds of interest, and full resolution ($Rs \geq 2$) between the unknown compounds and the
644 ISTD is not always achieved. Hexadecane and tridecanoic acids are compounds that may
645 naturally occur in archaeological samples. For these reasons, the use of isotopically labelled
646 (deuterated or ^{13}C analogous) ISTD is generally recommended to avoid overlap with endogenous
647 compounds and to provide a more reliable assessment of derivatisation and instrument
648 performance. Isotopically labelled ISTD are already the standard approach in GC-MS analysis,
649 but their application has not yet been adopted in the analysis of prehistoric adhesives. A wide
650 range of isotopically labelled standards are commercially available, however, they are slightly
651 more expensive than not isotopically labelled standards.

652 3.4. Interdisciplinary challenges

653 Collaboration between chemists and archaeologists has proven vital for the identification of
654 hafting adhesives and to expand our understanding of prehistoric tool technology [67]. However,
655 working at the interface between disciplines is also marked by significant challenges. Chemists,
656 while fully trained in operating the different instruments and knowledgeable about the correct
657 reporting of the results, might not fully understand the analysed material and its context. This
658 might result in a misinterpretation of the data. Archaeologists by contrast, have full
659 understanding of the artefact, its burial environment and taphonomic challenges, but they may
660 not be able to correctly interpret the chemical results. Close interaction between both is thus
661 essential to avoid problems. Indeed, some studies publish strong archaeological conclusions
662 which are not backed up by the chemical data provided. A few examples are conclusions based
663 on the absence of marker compounds (discussed in section 3.1 [21, 45] or the identification of
664 the adhesive type with low precision instrumentation [42]. In addition, chemical identification of
665 weathered organic samples is extremely difficult because the degradation pathways are mostly
666 unknown, so results need to be interpreted with caution. On top of that, destructive analysis is
667 best avoided for rare archaeological samples. However, non-destructive spectroscopic
668 instrumentation like FTIR or Raman spectroscopy often cannot account for the molecular

669 modification induced by degradation and therefore fail to precisely identify the original residue
670 [29, 56]. Advances in the chromatography field might offer semi to non-destructive sample
671 introduction methods for the analysis of prehistoric adhesives, for example, headspace analysis
672 of the volatiles from the residue is a promising development [2].

673 Another problem is that GC-MS is sometimes the only instrumentation used to infer stone tool
674 hafting, exclusively based on the characterisation and location of the residue [18, 20, 49, 50, 94].
675 While GC-MS provides valuable chemical information, it can only identify the composition of the
676 residue, whereas stone tool use and/or hafting have to be identified based on other approaches,
677 more specifically microscopic use-wear analysis [61, 62]. Complementary analytical
678 approaches such as chemical identification by GC-MS can provide additional lines of evidence
679 that permit more confident interpretation of the residue and its origin.

680 The challenges outlined in this review and summarised in Table 2 underscore the importance of
681 intense interaction and discussion between scientists with different disciplinary backgrounds to
682 improve mutual understanding and avoid erroneous interpretations of the results. Reporting of
683 the analytical results should aim for a level of detail that is habitually part of standard good
684 analytical practices. At the same time, results from use-wear analysis should be integrated
685 alongside the chemical data to provide an interpretation that is backed up with both chemical
686 and microscopic wear data. Together, these approaches provide a more robust understanding
687 of stone tool function and deeper insights into prehistoric hafting technologies—insights that are
688 more difficult to obtain when chemical and use-wear analyses are considered in isolation.

689 Conclusion

690 The interest in the characterisation of hafting adhesives has stimulated collaboration between
691 scientists and archaeologists. This review has critically addressed the interdisciplinary research
692 conducted for the analysis and identification of prehistoric hafting adhesives. It has been shown
693 that analytical data is often inappropriately used or overinterpreted to validate certain
694 hypotheses on prehistoric technology or human behaviour, in particular in debates on
695 Neanderthals and early modern humans. The lack of good analytical practices and data
696 transparency reduce the reliability of the identifications and hinders true progress and innovation
697 in the analysis of hafting adhesives. On the other hand, studies that include results from
698 chemical analysis often lack corroborating evidence from use-wear analysis and only rely on the
699 localised presence of a residue. In reality, both types of data are required to understand tool use
700 and hafting and to identify a residue as an adhesive used in hafting. Indeed, chemical analysis
701 can identify the origin of the residue as, for example, a pine tree exudate, but it cannot evaluate

702 whether this exudate served as a hafting adhesive. Consequently, if research on hafting
703 adhesives is to be advanced, stronger collaboration between archaeologists and analytical
704 chemists is required, including investment in the reciprocal understanding of the other field to
705 ascertain meaningful interpretations of both the chemical and archaeological data. in this
706 review, we tried to pinpoint a number of pitfalls based on the existing literature and provided
707 suggestions how these problems could be avoided in the future. We also identified gaps in our
708 knowledge that need to be tackled urgently. In particular investigations into the degradation
709 pathways of modern adhesives to assure that chemical modifications induced by taphonomic
710 processes can be appreciated better. Table 2 lists the most prominent issues as identified in this
711 review and may serve as a resource for other researchers interested in the topic.

Table 2. Summary of the concerns identified in this review and possible solutions.

Concern	Reason	Solution	Section
Little knowledge on degradation pathways	Weathering has extensive influence on the chemical profile of weathered adhesives.	Conduct artificial degradation studies to understand the degradation pathways.	3.1.
Conclusion of adhesive type based on absence of markers.	Original composition of weathered adhesives is not known.	Conduct artificial degradation studies to understand the degradation pathways.	3.1.
Strong focus on a few adhesives.	The strong focus on specific adhesives creates a bias and exclude other possible types of adhesives.	Creation and sharing of an exhaustive database which includes a wide variety of possible adhesives.	3.1.
Inadequate use of MS library search.	Reliability of peak annotation cannot be checked by the readers.	Give full transparency on the library search parameters.	3.2.1.
	Reporting specific n-alkanes with only a forward library search, while the mass spectra are too similar to make a distinction.	Refrain from stating the detection of n-alkanes, unless it is confirmed by a standard or another ionisation technique.	3.2.1.
Limited use of extra search parameters.	Peaks are mostly annotation with only a forward library search, which can result in misidentifications.	Use LRI values with a library search to identify compounds based on their retention time and mass spectra.	3.2.2.
Limited use of standards.	Isomeric compounds with similar mass spectrum and retention time can be misidentified	If exact identification is desired, use a standard, otherwise refrain from stating the exact isomeric configuration.	3.2.3.
Limited information on the blank injections.	Contamination can be present on archaeological artefacts resulting from different sources (soil, handling, sample preparation).	Conduct blank injections of a true blank, method blank, and a soil blank. Report any compound in the blank.	3.3.1.
Reporting compounds which are known contaminants.	Compounds can have multiple origins, e.g., FAs from plants but also human skin.		3.3.1.
No replicate injections.	Replicates show the reproducibility and robustness of the analysis.	Use triplicate injection, report the RSD.	3.3.2.
No QC sample injected.	QC injections identify instrumental drifts during an analysis.	Use a QC sample to identify possible instrumental changes.	3.3.2.
No report on injection sequence.	Randomising the injection order reduce the risk of systematic biases introduced by carryover and instrumental drift.	Randomise the injection sequence and report that the sequence was randomised.	3.3.2.
Bad selection of ISTD.	ISTDs that are not fully resolved in the chromatogram cannot be used for peak area calculations.	Choose an ISTD which is absent in the sample but close in retention time, such as isotopic labelled standards.	3.3.3.
Lack of use-wear analysis.	Chemical analysis can only identify the plant species of the organic residue. Use-wear analysis is required to qualify the residue as a hafting adhesive.	Incorporate use-wear analysis with chemical analysis to provide a full identification of the artefact and the residues.	3.4.
Adhesive identification with low accuracy instrumentation.	Weathered adhesives underwent major chemical modification, often with loss of distinct chemical markers.	If no accurate identification with GC-MS is possible refrain from stating exact adhesives-types, e.g., tarry-like adhesives instead of birch tar.	3.4.

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Conflict of Interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Authors' contribution statement

Anika Lokker: Conceptualization, Methodology, Visualization, Writing – Original Draft **Pierre-Hugues Stefanuto**: Supervision, Writing – Review & Editing **Dries Cnuts**: Supervision, Writing – Review & Editing **Veerle Rots:** Conceptualization, Funding Acquisition, Resources, Supervision, Writing – Review & Editing **Jean-François Focant:** Conceptualization, Supervision, Resources, Writing – Review & Editing

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