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► To cite this version:

Elodie Mezzatesta, Annie Perraud, Cathy Vieillescazes, Carole Mathe. Analysis of balms taken from Egyptian human mummies using solid-phase extraction and gas chromatography–mass spectrometry. *Journal of Separation Science*, 2021, 44 (4), pp.850-859. 10.1002/jssc.202000746 . hal-03173550

HAL Id: hal-03173550

<https://hal.science/hal-03173550>

Submitted on 19 Apr 2021

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1 **Analysis of balms taken from Egyptian human mummies using Solid Phase**
2 **Extraction and GC-MS**

3
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11 Running title : Identification of balms taken from Egyptian human mummies

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16
17 N,O-Bis (trimethylsilyl)trifluoroacetamide : BSTFA; Dicarboxylic fatty acids : DC;
18 Dichloromethane : DCM; Diethylether : DEE; Dehydroabiatic acid : DHA;
19 Dehydroabiatic methyl ester : DHAM; Monocarboxylic fatty acids : MC;
20 Tetrahydrofuran : THF; Trimethylchlorosilane : TMCS

21
22 Keywords: *Balm; Bitumen; Beeswax; Resins, Mummies.*

23
24 **Abstract**

25 The aim of this paper is to establish a protocol by Solid Phase Extraction-Gas
26 Chromatography-Mass Spectrometry leading to a wide and fine qualitative chemical
27 characterization of the several natural substances present in human mummies'
28 balms, using a minimal quantity of samples. In this study, nine samples were
29 analyzed from mummies dating back from the Third Intermediate Period to the
30 Roman Period, and were provided by the Confluences Museum (Lyon, France).
31 Using Solid Phase Extraction, three fractions were examined in this protocol. The first
32 one, eluted with hexane, concerned chemical families of hydrocarbons of bitumen.
33 The second, eluted with ethanol, enabled terpenic compounds to be characterized
34 and beeswax. The last one, composed of diethyl ether with 2% of acetic acid,
35 extracted carboxylic acids with a long aliphatic chain (fatty matter) and glycerides.
36 This study also allowed the characterization of non-saponified compounds from
37 beeswax to be obtained while excluding the common saponification step. The
38 analyzed mummification balms were shown to contain fatty matter, beeswax,
39 bitumen and diterpenic resinous material. This one-pot Solid Phase Extraction-Gas
40 Chromatography-Mass Spectrometry method was efficient in reducing both the
41 number of analytical steps and the complexity of the archaeological balms
42 subsequently analyzed by GC-MS.

45 1. Introduction

46 Mummification was a fundamental part of funerary practice in ancient Egypt. The
47 Egyptians believed in immortality and rebirth in the afterlife and in order to be able to
48 access this afterlife, it was necessary to preserve the deceased body. The
49 application of balms was a key part of the mummification process in ensuring
50 successful and lasting preservation of the body [1]. Before applying these balms,
51 however, several steps of the complex process of mummification were carried out.
52 Firstly, an excerebration was performed, followed by evisceration of the thoraco-
53 abdominal cavity, before dehydrating the entire body using natron. The complete
54 process of mummification (including evisceration, dehydration and bandaging) took
55 70 days; within this period and after dehydration by natron, balms could be added
56 inside the eviscerated cavities (thorax, abdomen) and the skull, and then all over the
57 body which would subsequently be bandaged. However, the entire process could
58 vary for each mummy, depending on various factors. All these stages were described
59 by Herodotus (*L'Enquête*, II, 85-88) [2] and Diodorus Siculus (*Bibliothèque historique*,
60 I, 21) [3] and could differ according to the period and to the status of the deceased
61 individuals.

62 Many varieties of natural substances were used in the formulation. These substances
63 have certain pharmacological properties such as being hydrophobic, antibacterial,
64 antifungal and odoriferous. The most frequently used substances were plant oils or
65 animal fats, di- or triterpenic resins, beeswax, bitumen and spices [4–8]. The fatty
66 substances, composed of esters of fatty acids with long chain between 12 and 24
67 carbon atoms, were used for their hydrophobic properties, the most abundant
68 generally being palmitic (C16:0) and stearic (C18:0) acids. Sterols such as
69 cholesterol are considered as a marker of animal fat, and campesterol as a marker of
70 plant oils [9–11].

71 Plant resins were used in the formulation of balms for their antibacterial properties.
72 They are composed of terpenic compounds secreted by certain plants [5,12].

73 Using beeswax limited rehydration of the embalmed body. Beeswax is composed of
74 esters of palmitate with a very long carbon chain alcohols (from 22 to 34 carbon
75 atoms) [13,14]. Bitumen was used for its hydrophobic properties provided by three
76 different apolar chemical families corresponding to linear alkanes and triterpenoids
77 such as sterane and hopane compounds [15–17].

78 Identifying all of the compounds present in each balm plays an important role in the
79 understanding of archaeological data. Furthermore, such identification represents a
80 significant challenge because of the various chemical reactions such as oxidation
81 and hydrolysis, which can occur naturally over time (aging) or because of
82 anthropogenic factors, such as the heating temperature.

83 Compounds of alteration can play an important part in identifying plant species. The
84 molecular composition of samples can provide information on the nature of the
85 constituents, the state of conservation of the sample and the treatment undergone by
86 the material during the preparation and/or the formation of balms [18–20]. To identify
87 their composition, the most widespread analytical method described in the literature
88 is Gas Chromatography coupled to Mass Spectrometry (GC-MS) [21–28] because it
89 allows a large number of organic compounds from a complex matrix, even in traces,
90 to be separated and identified.

91 However, some chemical families, like bitumen, were difficult to accurately
92 characterize because of their low concentration and of the high proportion of fatty
93 acids present in the balms under study. Thus, because of the presence (i) of a

94 mixture of natural substances, (ii) of alteration processes, (iii) of a very low proportion
95 of some ingredients or, on the contrary, a high concentration of others, the use of
96 GC-MS is often preceded by several sample pre-treatments. A pre-treatment sample
97 was often necessary, such as saponification reactions for the detection of waxes
98 [29,30], dichloromethane or diethyl ether extractions for the detection of free fatty
99 acids and terpenic compounds, as well as fractionation for hydrocarbons
100 [6,26,29,31–35]. All of these pre-treatments enable a fine chemical characterization
101 of samples to be obtained, but pre-treatments also create the need for more
102 archaeological material mass, when the quantities available for analysis are generally
103 small.

104 The aim of this study therefore, was to develop a single and efficient protocol by
105 SPE-GC-MS. The main objective of this paper is to determine the fine chemical
106 composition of nine mummification balms and to reduce the number of experimental
107 steps, thus reducing the amount of archaeological sample used thanks to a *one-pot*
108 procedure.

109

110 2. Materials and Methods

111 2.1. Archaeological samples

112 Within the framework of a multidisciplinary research project entitled *Human Egyptian*
113 *Lyon Confluences Mummies (HELYCOM)-Mourir pour renaître*, mummification balms
114 of 9 human mummies were analyzed. In this research work, female and male
115 mummies from Upper Egypt (around Thebes) dating from 600 BC to the Roman
116 Period (~300 AD) were studied. In this study, a total of 9 samples belonging to the
117 Egyptian collection of the Confluences Museum in Lyon (France) were examined.
118 The samples referred to as 30000111 (34), 30000139 (15), 30000148 (18),
119 30000286 (11) and 90001951A (50) were mummified heads, and 90001169 (41),
120 90001258 (40), 90001259 (47) and 900015978 (66) were provided from complete
121 mummies. The mummies studied were those of men and women dating from the
122 Third Intermediate Period to the Roman Period and coming from different
123 necropolises such as Thebes, Esna, Kom-Ombo and Deir-el-Medineh. The weight of
124 the samples varied from 20 to 200 mg. They were collected at different location
125 points on the mummies: on the mummified skin, on the textile covered by balms, or
126 directly within the balm itself (Table 1).

127

128 2.2. Solvents and reagents

129 All solvents were of the highest purity grade. Hexane, tetrahydrofuran (THF) and
130 N,O-Bis (trimethylsilyl)trifluoroacetamide/Trimethylchlorosilane (BSTFA/TMCS) were
131 supplied by Sigma-Aldrich. Ethanol, dichloromethane (DCM) and diethylether (DEE)
132 were supplied by Merck.

133

134 2.3. Saponification

135 10 mg of sample was extracted with 3 x 1 mL of THF aided by sonification (5 min)
136 and then centrifuged at 6000 rpm (5 min). The supernatant was set aside to extract
137 the solid pellet again. The solvent extracts were combined and then 2 mL of a
138 solution of potassium hydroxide KOH 10% in MeOH/H₂O (9/1, v/v) were added. The
139 mixture was magnetically stirred and heated at 65°C during 1 hour. After evaporation
140 3 mL of pure water was added with 1 mL of HCl 5 M. The aqueous phase was

141 washed with 3 x 5 mL of diethyl ether. The organic phases were combined and dried
142 with anhydrous sodium sulphate, then filtered on filter paper. Excess reagent was
143 evaporated to dryness under a stream of nitrogen. Trimethylsilylation was applied.
144 After evaporation, the derivatized sample was solubilized in hexane/DCM mixture
145 (2/1, v/v) and filtered on PTFE cartridge before injection in GC-MS.
146

147 2.4. Solid Phase Extraction

148 Preliminary tests were realized to determine the best SPE conditions. In this study,
149 according to the apolar properties to the wide chemical compounds present in human
150 mummies' balms, three types of SPE columns were tested: C18, Cyano and Amino
151 phases associated to different solvents of elution (hexane, methanol, ethanol,
152 isopropanol, diethyl ether with 2% of acetic acid and dichloromethane). The NH₂ SPE
153 column associated to hexane, ethanol, diethyl ether with 2% acetic acid exhibited the
154 best results so this column was used in this study according to the following protocol.
155

156 10 mg of the sample were extracted with 1 mL of hexane/tetrahydrofuran (THF) (1/1)
157 using ultrasound for five minutes and then centrifuged at 6000 rpm for five minutes.
158 The supernatant was set aside to extract the solid pellet a second and third time. The
159 three fractions obtained were combined and evaporated to dryness under a stream of
160 nitrogen and the mixture was then dissolved in 500 µL hexane/THF (1/1, v/v). This
161 mixture was called the charge.

162 In parallel, an SPE cartridge strata NH₂ 200 mg/3 mL (Phenomenex) was conditioned
163 in an SPE vacuum manifold with 4 mL of hexane at a flow of 1.2 mL.min⁻¹. The
164 charge was deposited on the cartridge. Then a first elution was carried out with 4 mL
165 of hexane and collected (fraction 1). A second elution was performed with 4 mL of
166 ethanol and was collected (fraction 2). Finally, a last elution was carried out with 3
167 mL of diethyl ether (DEE) including 2% acetic acid (fraction 3). Each fraction was
168 evaporated to dryness under a stream of nitrogen and then trimethylsilylated with 200
169 µL of BSTFA/TMCS at 70°C during 30 min. Fractions 1 and 2 were solubilized with
170 60 µL hexane/DCM (2/1, v/v) and were injected into GC-MS at temperature gradient
171 No.1. Fraction 3 was solubilized with 1.5 mL hexane/DCM (2/1) and injected into GC-
172 MS by using gradient program No.2.

173 Each SPE parameter, namely the nature of the sorbent, the dimension of the
174 cartridge, the sampling flow rate, the sample volume, the eluents and the desorption
175 flow rate, was optimized.

176

177 2.5. GC-MS conditions

178 A Thermo Scientific Focus gas chromatographic system composed of a Thermo
179 Scientific AI 3000 auto-sampler coupled with an ITQ 700 ion trap mass spectrometer
180 was used (Thermo Fisher Scientific). The GC column was a fused silica capillary
181 column Thermo trace GOLD TG-5MS (5% diphenyl / 95% dimethylpolysiloxane, 30
182 m length × 0.25 mm i.d. × 0.25 µm film thickness). Helium was the carrier gas with a
183 constant flow of 1 mL.min⁻¹. 1 µL of each sample was injected with a splitless time of
184 1 min. The injector temperature was set at 250 °C. Mass spectra were recorded in
185 electron impact mode with an electron ionization voltage of 70 eV, an ionization time
186 of 25,000 µs and a mass range of 40–650 *m/z*. The transfer line, ion trap and
187 manifold temperatures were respectively set at 300 °C, 200 °C and 50 °C. Detected

188 compounds were identified from their retention time and interpretation of their mass
189 spectra in comparison with standard compounds and using NIST database. The oven
190 temperature was programmed as follows:

- 191 - Temperature gradient No.1: 160 °C, isothermal for one minute, then increased
192 by 10 °C.min⁻¹ up to 180 °C, constant for three minutes, followed by a second
193 increase of 3 °C.min⁻¹ up to 260°C, constant for five minutes, followed by a
194 third increase of 2.5°C.min⁻¹ up to 300°C and finally, a final increase of
195 10°C.min⁻¹ up to 320°C, the temperature of which was maintained for three
196 minutes. The total analysis time was fifty-nine minutes.
- 197 - Temperature gradient No.2: 115°C, constant for two minutes, then increased
198 by 15°C.min⁻¹ up to 220°C, followed by a second increase of 3°C.min⁻¹ up to
199 250°C and finally, a last increase of 10°C.min⁻¹ up to 320°C, the temperature
200 of which was maintained for three minutes. The total analysis time was twenty-
201 nine minutes.

202

203 3. Results and discussion

204

205 Owing to (i) the hypothetical occurrence of well-known natural substances, such as
206 fatty matter or plant resins and (ii) to the presence of alcohol and/or carboxylic
207 functional groups, the selected cartridge was the Strata NH₂. It effectively allows the
208 retention of compounds depending on the electrostatic attractions of the functional
209 groups of the compounds of interest to the NH₂ grouping, bound at the surface of the
210 SPE cartridge. The SPE protocol was carried out according to the schema described
211 in Figure 1. All of the molecules detected in the nine archaeological samples are
212 listed in Table 2.

213

214 3.1. Tetrahydrofuran solubilization

215 Solubilization of the sample is necessary beforehand, since depositing the solid
216 matter directly on the SPE cartridge percolates the cartridge and inhibits elution. The
217 solvent must facilitate the most exhaustive possible extraction of the compounds of
218 interest, both qualitatively and quantitatively. Under these experimental conditions,
219 the solvent should be moderately apolar in order to extract the compounds present in
220 the balms. Several tests were carried out, comparing them with solvents usually
221 described in the specialized literature, such as dichloromethane and diethyl ether.
222 Tetrahydrofuran/hexane (1/1) was chosen owing to a polarity close to that of
223 chloroform or dichloromethane, but it is a water-miscible solvent and it should
224 therefore have superior capacities for the solubilization of polar molecules [36].

225

226 3.2. Fraction 1

227 The first eluted fraction was carried out using hexane, which is the most apolar
228 solvent used in this study. This elution was start with an apolar solvent because the
229 stationary phase is a polar phase, moreover, hexane is also the conditioning solvent
230 of the cartridge. Hexane was able to elute the most apolar molecules present in the
231 studied balms, such as aliphatic molecules, linear, branched, cyclized alkanes or
232 polycyclic aromatic hydrocarbons (PAHs). An illustration of the obtained results in
233 Sample No.18 is presented in Figure 2. The detection of the three chemical families
234 of bitumen at *m/z* 57 (alkanes), *m/z* 191 (hopane) and *m/z* 217 (sterane) can be used
235 for the characterization of bitumen [9]. The chemical families of hopanes and

236 steranes existed in only a very low proportion in bitumen (5%), the characterization of
237 these chemical families thus usually requiring special treatment [37]. Identification of
238 archaeological bitumen was generally performed by isolating and splitting the several
239 compounds depending on their nature: saturated hydrocarbons, aromatic
240 hydrocarbons, and other compounds using column chromatography [38,39].

241 The GC-MS analyses of Fraction 1 of the samples referred to as 34, 15, 18, 41 and
242 50 resulted in the detection of occurrence of the three chemical families of bitumen:
243 alkanes, hopanes and steranes. These results confirmed the presence of bitumen in
244 the balms under study.

245 In addition to these results, a PAH was detected and identified to retene. An
246 anthropic degradation of Pinaceae resin such as thermal degradation can be
247 deduced from the detection of retene. This latter molecule was formed mainly under
248 very high temperatures [40] and its occurrence was observed in 5 balms and in a
249 significant proportion in the balm of Sample 11. Contrary to the other diterpenic
250 compounds eluted in the fraction 2, retene was identified in the fraction 1 because of
251 its apolar property.

252

253 3.3. Fraction 2

254 Fraction 2, eluted with ethanol enabled most polar compounds such as diterpenoids,
255 which are functionalized compounds, to be eluted, with the occurrence of alcohol,
256 carboxylic acid, ketone, or aldehyde functions [9].

257 Diterpenic resin was detected in 8 out of 9 samples (34, 15, 18, 11, 41, 40, 47 and
258 66). For example, the sample referred to as 18 had many diterpenoids, which are
259 characteristic of the use of Pinaceae resin, such as in the elution order:
260 dehydroabietic acid (DHA, $R_t=23.13$ min), 7-hydroxy-DHA ($R_t=24.5$ min) and 7-oxo-
261 DHA ($R_t=26.3$ min) (Figure 3). During the aging process, an initial predominant
262 isomerization produces dehydroabietic acid. A natural degradation of this acid could
263 occur the formation of products such as 7-oxo-DHA, 7-oxo-15-hydroxy-DHA [41–43].
264 Additionally, to the diterpenoids already identified, another compound, dehydroabietic
265 methyl ester (DHAM, $R_t=20.5$ min), was characterized in both of the samples
266 referred to as 18 and 11. This molecule is produced by methanolysis. In fact, during
267 heating at a very high temperature, resinous wood releases methanol, which reacts
268 with the carboxylic acids of diterpenoids and this reaction leads to the formation of
269 the corresponding methyl ester derivatives. DHAM allows wood tar to be
270 characterized and this compound is totally absent when resin alone is heated [44].

271 Moreover, palmitate esters corresponding to unsaponified beeswax molecules were
272 identified in this fraction ($R_t=55-58$ min). Beeswax does not necessarily undergo
273 hydrolysis with aging, and its molecules therefore can remain in their ester form [45].
274 In this eluted fraction, the presence of beeswax was directly characterized in samples
275 34, 15, 18, 66, 50 and 47. The characterization of beeswax is usually performed by
276 initially carrying out a pre-treatment of the samples corresponding to a reaction of
277 saponification [29]. With the purpose of confirming either presence or absence of
278 beeswax, saponification was performed as a preliminary step. Results obtained
279 through saponification are in accordance with aforementioned SPE-GC-MS data [36].
280 In order to interpret the elution phenomenon of these apolar compounds in ethanol, it
281 is necessary to consider the state of ionization of the several compounds and also to
282 consider the occurrence of free fatty acids in the mixture. In this solution, the
283 stationary amine phase was in the NH_3^+ form and free fatty acids were in the COO^-
284 form, enabling ionic bonds therefore to be formed between the two forms and thus
285 creating a type of semi-apolar grafted phase in the cartridge. Beeswax esters could

286 form Van der Waals bonds (London interactions) with the carbon chain of free fatty
287 acids, which would explain why they are not eluted with hexane in Fraction 1. The
288 subsequent addition of ethanol, a protic solvent, capable thus of generating hydrogen
289 bonds, could create an imbalance leading to the elution of esters.

290

291 3.4. Fraction 3

292 Fraction 3 was eluted with a mixture of diethyl ether with 2% acetic acid. In this
293 experimental condition, carboxylic acids presented COOH functions, and the amine
294 of the stationary phase was in NH_3^+ form. No ionic bond was formed between these
295 two chemical forms. For this reason, this fraction allowed fatty acids to be eluted. All
296 samples contained monocarboxylic fatty acids (MC) and/or dicarboxylic acids (DC).
297 An illustration of the obtained results in sample 18 is presented in Figure 4. In the
298 obtained GC-MS chromatograms, the relative proportion of MC represented on
299 average, approximately 50% of the total composition of balms and this percentage
300 could attain 90% depending on the archaeological sample studied. This relative
301 percentage was calculated from the peak area of each detected compound from
302 corresponding chromatograms. Monocarboxylic acids corresponded also to the main
303 chemical compounds present in all of the analyzed balms.

304 Saturated monocarboxylic acids cannot provide much information in the
305 archaeological context about the origin of the fat matter used. In fresh material, it is
306 possible to examine the peak area ratio of MC 16:0/MC 18:0 (palmitic acid/stearic
307 acid) to determine the nature of the fatty matter [9]. However, this method is not
308 applicable in an archaeological context because the proportions of these molecules
309 can change with the state of degradation of the material or can even be caused by a
310 mixture of oils and fats. This method is therefore not suitable for the analysis of
311 mummy balms. The same is true for the proportions of saturated fatty acids with a
312 short carbon chain. They fail to provide much information because these molecules
313 are certainly the result of fat/oil degradations.

314 In this study using GC-MS, therefore, the animal or plant nature of fats cannot be
315 accurately defined because biomarkers of plant oils and/or animal fats were not
316 detected. Owing to the use of GC-MS in archaeological context, the objective of this
317 paper was not to determine the nature or the origin of the fatty matter (i.e. fatty acids
318 and glycerides). Cholesterol was only detected in the samples referred to as 34, 11
319 and 40. The occurrence of this molecule did not allow animal origins to be identified
320 with certainty because the occurrence could result from an external contamination of
321 the deceased by capillarity or of a contemporary contamination during the transport
322 or the storage of the objects. Samples 15, 18, 41, 47 66 and 50 contained numerous
323 dicarboxylic acids. These types of acids were markers of degradation of fatty
324 substances. Unsaturated fatty acids can undergo oxidation and form hydroperoxide
325 intermediates to finally generate short monocarboxylic acids and dicarboxylic acids
326 [10].

327 4. Conclusion

328 The studied mummification balms contained fatty matter, beeswax, bitumen and
329 diterpenic resin. This efficient SPE-GC-MS protocol has thus enabled additional
330 substances to be identified, substances such as hopanes and steranes of bitumen
331 and palmitate esters of beeswax. It has also enabled markers of wood tar such as
332 DHAM compound to be identified and has reduced the number of experiments such
333 as the long pre-treatment of saponification or the splitting of the constituents of
334 bitumen. The quantity of samples has also been reduced, which is very interesting

335 from an archaeological point of view given the very small amount of material
336 available, in accordance with cultural heritage directives concerning preservation.
337 This developed SPE protocol as applied to human balms of mummies has enabled
338 each ingredient of the studied balms to be separated and concentrated. Moreover,
339 this multiple extraction/partial elution, carried out during a single experimentation, has
340 resulted in better pre-separation of the compounds deriving from a large panel of
341 natural substances used in Ancient Egypt. The SPE protocol described in this study
342 was also successfully applied and performed in the analyses of 61 samples taken
343 from 43 human mummies from Upper Egypt [36].

344

345 Acknowledgments

346 The authors acknowledge the anonymous reviewers for their careful reading of our
347 manuscript and their many insightful comments and suggestions. The authors wish to
348 thank the Museum of Confluences in Lyon and the LabEx ARCHIMEDE and Human
349 Egyptian LYon CONfluences Mummies (HELYCOM) - Mourir pour renaître project. A
350 part of this project was supported by LabEx ARCHIMEDE from the Investissement
351 d'Avenir program ANR-11-LABX-0032-01. The research has been funded by a PhD
352 grant given by The French Ministry of Higher Education and Research.

353

354 Conflict of interest

355 The authors declare that they have no conflict of interest.

356

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489

490 Figure captions

491 Figure 1: Schema of the final Solid Phase Extraction protocol developed with the
492 predominantly detected substances in each elution fraction.

493 Figure 2: Chromatograms of sample 18, Fraction 1 and extracted signal at m/z 57,
494 m/z 191 and m/z 217, respectively base peaks of alkanes, hopanes and steranes.

495 Figure 3: TIC chromatogram of sample 18, Fraction 2.

496 Figure 4: TIC chromatogram of sample 41, Fraction 3.

497

498

499 Figure 1: Schema of the final Solid Phase Extraction protocol developed with the
500 predominantly detected substances in each elution fraction.

501



5-10mg

Extraction US 10 min in 1mL THF/hexane 1/1 (X3)
Centrifugation 6500 rpm (5 min)

Supernatant deposited on the cartridge



Cartridge Strata NH₂
Conditioning hexane

Elution 1 : hexane 3_X 1mL

Unsaturated hydrocarbons

Elution 2 : ethanol 3_X 1mL

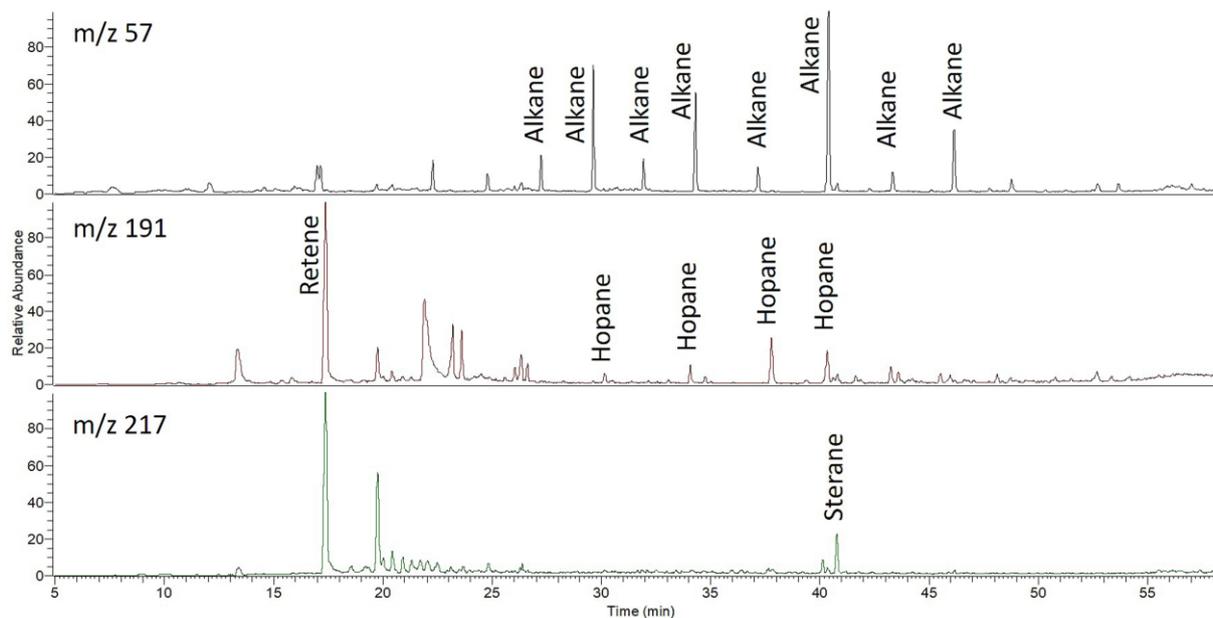
Diterpenoids and esters

Elution 3 : diethyl ether + 2% acetic acid 3_X 1mL

Fatty acids

512

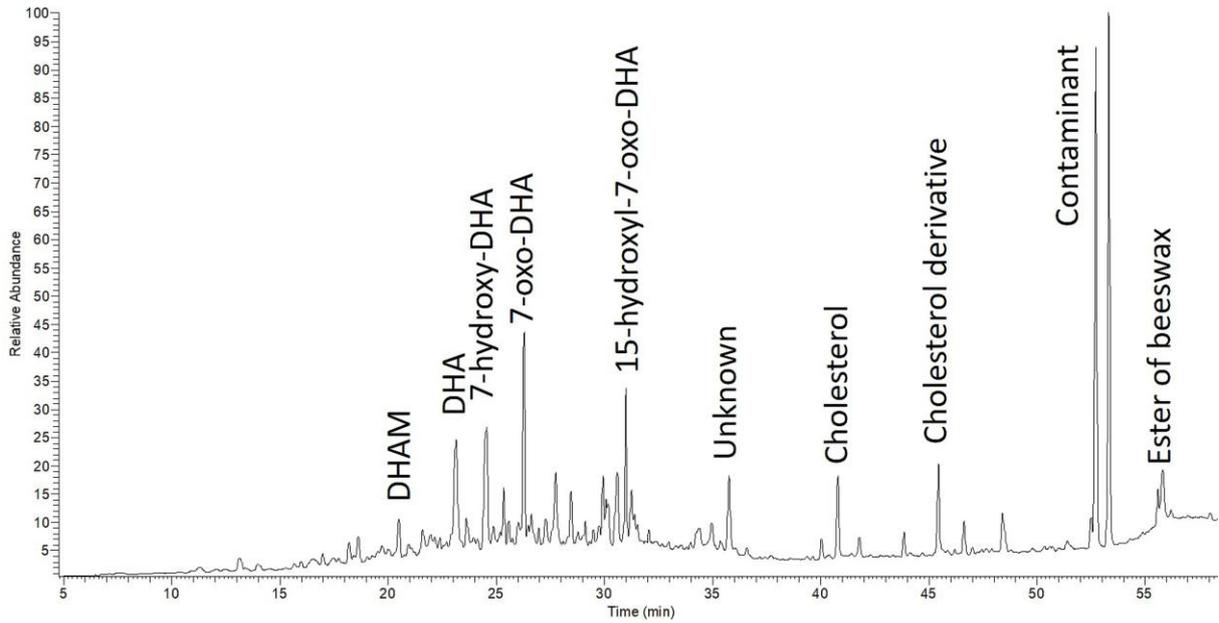
514 Figure 2: TIC partial chromatogram of sample 18, Fraction 1 and extracted signal at m/z 57,
515 m/z 191 and m/z 217, respectively base peaks of alkanes, hopanes and steranes.
516



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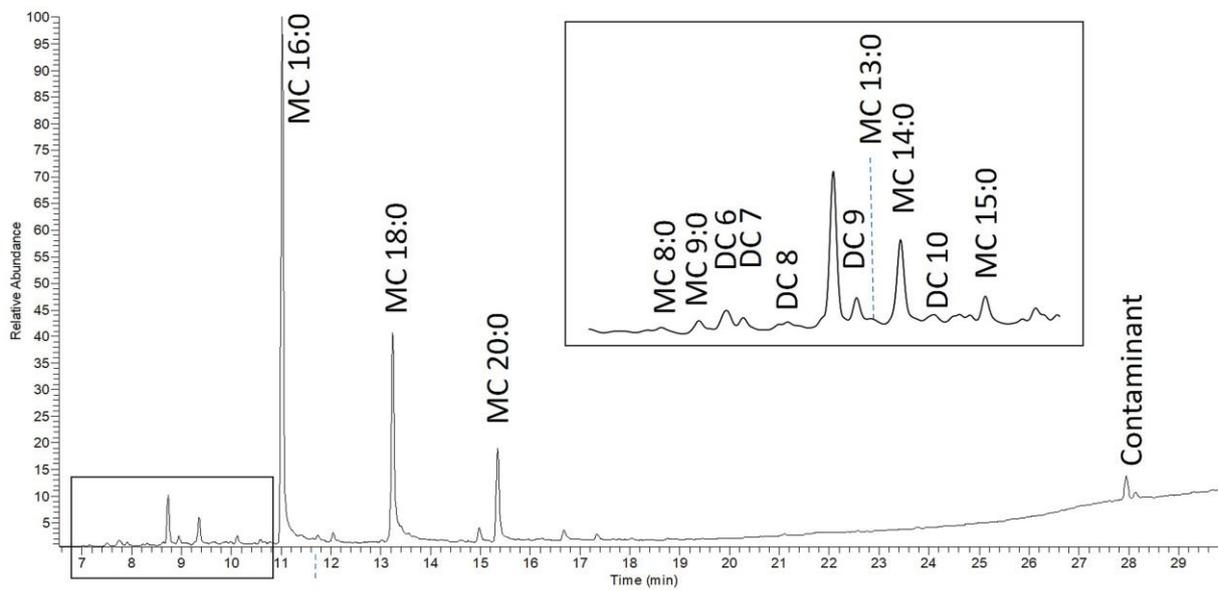
519 Figure 3: TIC Partial chromatogram of sample 18, Fraction 2.

520



523 Figure 4: TIC partial chromatogram of sample 41, Fraction 3

524



528 Table 1: Sample presentation

Inventory no.	Lab code	Sex and age	Site of excavation	Relative dating
30000111	34	Elderly male	Gournah	Graeco-Roman Period
30000139	15	Adult female	Gournah	Ptolemaic to Roman Period
30000148	18	Elderly male	Thebes	Unknown
30000286	11	Adult or elderly male	Esna	Third Intermediate Period to Late Period
90001951A	50	Young adult male	Thebes	Late Period to Ptolemaic Period
90001169	41	Adult male	Gournah	Graeco-Roman Period
90001258	40	Adult female	Kom-Ombo	Ptolemaic to Roman Period
90001259	47	Adult female	Unknown	Late Period to Ptolemaic Period
90001597	66	Adult	Gournah	Unknown

529

530

531 Table 2: Compounds detected by elution order in mummies' balms. "√": presence, "-": absence (MC: Monocarboxylic fatty acid, DC: Dicarboxylic
 532 fatty acid).

Compounds	Samples								
	34	15	18	11	41	40	47	66	50
MC 8:0	√	√	√	-	√	-	√	-	-
glycerol	√	√	√	√	-	√	-	-	-
DC 4	-	-	√	-	-	-	√	√	-
MC 9:0	√	√	√	-	√	√	√	√	√
DC 5	-	-	-	-	-	-	√	√	-
MC 10:0	√	√	√	-	-	-	√	-	-
DC 6	-	-	√	-	√	-	√	√	-
DC 7	-	√	√	-	√	-	√	√	√
MC 12:0	-	√	√	-	-	-	√	-	-
DC 8	-	√	√	-	√	-	√	√	√
MC 13:0	-	-	-	-	√	-	√	-	-
DC 9	√	√	√	-	√	√	√	√	√
MC 14:0	√	√	√	√	√	√	√	√	√
DC 10	-	√	√	-	√	-	-	√	√
MC 15:0	-	√	-	√	√	-	-	-	√
DC 11	-	-	-	-	-	-	-	√	-
MC 16:1	-	-	-	-	-	-	-	√	-
MC 16:0	√	√	√	√	√	√	√	√	√
DC 12	-	-	-	-	-	-	-	√	-
MC 17:0	-	-	-	-	-	-	-	-	√
MC 18:1	√	-	√	√	-	√	-	-	√
Retene	√	-	√	√	√	-	√	-	-
MC 18:0	√	√	√	√	√	√	√	√	√
Pimaric Ac.	-	-	-	-	-	√	-	-	-
Sandaracopimaric Ac.	-	-	-	-	-	√	-	-	-

Isopimaric Ac.	-	-	-	-	-	√	-	-	-
DHA	√	√	√	√	√	√	√	-	-
Abietic Ac.	-	-	-	-	-	√	-	-	-
3-hydroxy-DHA	-	-	-	-	-	√	-	-	-
7-hydroxy-DHA	-	-	√	-	-	√	-	-	-
15-hydroxy DHA	-	-	-	-	-	√	-	-	-
7-oxo-DHA	-	√	√	√	√	√	√	√	-
15-hydroxy-7-oxo DHA	-	-	-	-	√	-	√	√	-
MC 24:0	-	-	-	-	√	-	√	-	-
MC 26:0	-	-	-	-	√	-	√	-	-
Hopanes	√	√	√	-	√	-	-	-	√
Steranes	√	√	√	-	√	-	-	-	√
Cholesterol	√	-	-	√	-	√	-	-	-
Cholesta-3,5-dione	-	-	-	√	-	-	-	-	-
MC 28:0	-	-	-	-	√	-	√	-	-
MC 30:0	-	-	-	-	-	-	√	-	-
Unsaponified ester	√	√	√	-	-	-	√	√	√

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