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(Article begins on next page)

1 **Exploiting the versatility of vacuum assisted headspace solid-phase microextraction in**
2 **combination with the selectivity of ionic liquids-based GC stationary phases to discriminate**
3 ***Boswellia* ssp. resins through their volatile and semi-volatile fractions**

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9 **Running title: Vac-HS-SPME and IL-GCMS for frankincense characterization**

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14 **Keywords**

15 *Boswellia* spp resins, fast GC, ionic liquid-based stationary phase, vacuum assisted headspace solid
16 phase microextraction

17

18 **List of abbreviations and acronyms**

19 HS-SPME: headspace solid phase microextraction, Reg-HS-SPME: HS-SPME under regular
20 conditions, Vac-HS-SPME: HS-SPME under reduced pressure, IL: ionic liquid

21

22

23 **Abstract**

24 The frankincense resins, secreted from *Boswellia* species, are an uncommon example of a natural raw
25 material where every class of terpenoids is present in similar proportions. Diterpenoids (serratol,
26 incensole, and incensole acetate) are used to discriminate samples from different species and origins.
27 Headspace solid phase microextraction (HS-SPME) has been used for frankincense analysis although
28 requires long sampling time for medium-to-low-volatility markers; HS-SPME under vacuum (Vac-
29 HS-SPME) can overcome this limit. GC is used for analysis but the separation of incensole and
30 serratol needs polar stationary phases. This study develops a method to discriminate frankincenses
31 based on Vac-HS-SPME combined with Fast GC-MS with ionic liquid-based stationary phases.
32 Optimized Vac-HS-SPME parameters for solid samples included a temperature during air-evacuation
33 that must be below 0°C, a sampling time of 15 minutes for pre-equilibrium and 15 minutes of
34 extraction, and a sample amount above 100mg to obtain significant recoveries of all classes of
35 markers. Fast GC provides the baseline separation of all markers in 20 minutes. The total analysis
36 time thus drops to about 50 minutes instead of 120 (60 minutes of sampling plus 60 minutes of
37 analysis) reported in the literature. The method was successfully applied to commercial frankincense
38 samples.

39

40 1. Introduction

41 The burning of incense is probably the oldest perfuming method in existence, and frankincense gum
42 oleoresins (also known as olibanum) are a central ingredient in many incense mixtures. Frankincense
43 resins are secreted from trees of the genus *Boswellia*, and typically appear as pea-to-thumb-size grains
44 with a translucent, whitish-yellow to dark brown color. About 30 species of *Boswellia* are known and
45 many of them are used to produce frankincense resins. The main commercial sources are: *Boswellia*
46 *sacra* Flueck. (Arabian Frankincense), which is the top quality product and native to Oman and
47 Somalia; *Boswellia serrata* Roxb. ex Colebr. (Indian Frankincense) from India and widely used in
48 Ayurvedic, Hindu and Buddhist medicines; *Boswellia papyrifera* (Caill. ex Delile) Hochst., which
49 grows in coastal regions of Sudan, Eritrea, Ethiopia and northern Somalia; and *Boswellia frereana*
50 Birdw., which is typically found in Somalia, but is of lower commercial interest [1]. Besides the most
51 commercially relevant plants, other rarer species are available, such as the endemic *Boswellia* species
52 from the island of Socotra, *B. ameero* Balf. f., *B. dioscoridis* Thulin, *B. elongata* Balf. f. and *B.*
53 *socotrana* Balf. f. [2].

54 Frankincense resin is an uncommon example of a natural raw material in which each terpenic class
55 (mono-, sesqui-, di-, and triterpenoids) is present in rather similar proportions. Boswellic acids, which
56 are pentacyclic triterpenic acids, are non-volatile markers and have been shown to possess anti-
57 inflammatory properties, while the essential oil from frankincense resins is a common raw material
58 in perfumery. The market value of frankincense is strongly influenced by differences in composition,
59 and approximately varies from 5 US\$ kg⁻¹ for *B. serrata* to 150 US\$ kg⁻¹ for *B. sacra* [3].

60 The volatile and semi-volatile fractions of *Boswellia* species are rather complex and differ notably in
61 composition and odor. In general, the resins mainly contain mono-, sesqui- and diterpenoids, with *B.*
62 *papyrifera*, in particular, being characterized by n-octyl acetate, n-octanol and the significant presence
63 of diterpenoids. These diterpenoids are cembrane derivatives; in particular serratol (also known as
64 cembrenol), incensole and incensole acetate (see Figure 1). They are present in most *Boswellia* resins
65 although in different amounts and proportions [2-6]. They can therefore be used to discriminate
66 between samples of different species and origins, and act as a complement to the fast screening
67 method, which is based on a quality evaluation of odor and a visual assessment of color, that is
68 commonly used.

69 The characterization of the frankincense volatile fraction is generally carried out via the direct use of
70 GC-MS on the essential oils that are obtained from the steam- or hydro-distillation of the resins. This,
71 however, is not a convenient approach to fast screening as it is time consuming and requires
72 considerable sample amounts.

73 Solid phase microextraction (SPME), and in particular when combined with headspace (HS)-SPME,
74 can well fit to this aim. HS-SPME is a solvent-free high-concentration-capacity sampling technique
75 [7] where target analytes are transferred from the matrix to a polymeric fibre coating via two
76 consecutive steps (matrix/headspace and headspace/fibre) and recovery is maximized when the
77 equilibrium of the full process is reached. The time needed to reach equilibrium depends on several
78 factors, such as the properties of the analytes, matrix and fibre coating. Although conventional HS-
79 SPME was already applied to characterize frankincense resins [6, 8], it shows obvious limits to
80 achieve equilibrium with semi-volatiles and implies long extraction times and high extraction
81 temperatures. These limits can be overcome by vacuum assisted HS-SPME (Vac-HS-SPME), a
82 technique where a low sampling pressure is applied during HS-SPME sampling. Vacuum facilitates
83 the volatilisation of semi-volatiles by reducing the resistance found the thin gas-film layer adjacent
84 to the sample-headspace interface. Analytes are transferred to the headspace faster and the time
85 needed to reach equilibrium is reduced. Sampling under vacuum greatly improves the extraction
86 kinetics (speed) of lower volatility compounds resulting in high extraction efficiency and sensitivity
87 over time [9, 10].

88 The complexity of the volatile fraction of frankincense resins means that it is mandatory that a GC
89 stationary phase (SP) with the appropriate selectivity to separate all olibanum components, in
90 particular the characterizing markers, is chosen for use. Most studies involve GC analyses carried out
91 with apolar columns because of their efficiency, stability at high temperature and high amount of
92 available data (i.e. retention indices) for analyte identification. Unfortunately, apolar SPs do not
93 separate two of the discriminating diterpenic markers (incensole and serratol), which often leads to
94 erroneous identification (i.e. the coelution peak has also been hypothesized to be isoincensole) [5].
95 Polar columns are therefore mandatory for the correct characterization of the resins. In this respect,
96 the introduction of ionic liquids (ILs) for use as SPs has opened up new possibilities in the separation
97 of critical pairs of compounds in natural matrices [11-15]. These SPs show peculiar selectivity and a
98 comparable, or higher, polarity than conventional polydimethylsiloxane- and polyethyleneglycol-
99 (PEG) based columns, and, at the same time, provide similar, or even higher, maximum allowable
100 operating temperatures and lower bleeding than most PEG-based SPs.

101 This article describes the development of a simple and fast method to sample volatiles and semi-
102 volatiles markers characteristic of *Boswellia* ssp. resins. The proposed method uses Vac-HS-SPME
103 combined with Fast GC-MS with narrow bore columns coated with IL SPs able to discriminate
104 between frankincenses and applicable to quality control that aims [16-19].

105

106 2. MATERIALS AND METHODS

107 2.1 Chemicals and Samples

108 Incensole, incensole acetate and serratol (cembrenol) were all provided by Prof. G. Appendino
109 (Università del Piemonte Orientale, Novara, Italy).

110 Standard solutions of incensole, incensole acetate and serratol were prepared in cyclohexane at a
111 concentration of 100 mg L⁻¹ and stored at 4°C.

112 Two authentic *Boswellia* spp. resin samples (one *Boswellia socotrana* Balf. f. and one *Boswellia*
113 *papyrifera* (Caill. ex Delile) Hochst) were provided by Prof. G. Appendino (Università del Piemonte
114 Orientale, Novara, Italy), while a further three commercial frankincense samples, labeled as *B. sacra*,
115 were bought from a local herbalist's shop and were called frankincense 1, 2 and 3. Each frankincense
116 sample was first frozen using liquid nitrogen, then pulverized with a mortar and pestle and finally
117 stored at -18°C.

118 The essential oils of the *B. socotrana* and *B. papyrifera* samples, obtained via hydrodistillation
119 according to the European Pharmacopoeia procedure [20], were also analyzed to optimize the
120 chromatographic method.

121 2.2 Vacuum-assisted HS-SPME and regular HS-SPME

122 The experimental set-up adopted to perform vacuum assisted (Vac-)HS-SPME experiments consisted
123 of a commercial headspace 20 mL vial hermetically sealed with a joint stainless-steel cap containing
124 a hole that could tightly accommodate a Thermogreen[®] LB-1 septum with half-hole (6 mm diameter
125 x 9 mm length; Merck-Sigma Aldrich). The stainless-steel gastight cap was provided by Professor
126 Eleftheria Psillakis [21]. The air-evacuation step and the HS-SPME sampling were carried out using
127 Thermogreen[®] LB-1 septa. The solid sample was placed inside the vial, the vial was then stored at
128 -18°C for one hour and finally air-evacuated. The air-evacuation step was carried out using a 22 gauge
129 hypodermic needle sealed to a 5 mL syringe that was tightly secured to the tubing of a N 820.3 FT.18
130 (7 mbar ultimate vacuum) pumping unit manufactured by KNF Lab (Milan, Italy). The needle was
131 then inserted through the septum and the vial was air-evacuated. Two air-evacuation times (45 and
132 120 seconds respectively) were tested. After air-evacuation the vial was directly transferred to the
133 autosampler. GC desorption lasted 10 minutes to avoid carry-over. To remove the cap, atmospheric
134 pressure was restored inside the vial by piercing the septum with a disposable syringe needle.

135 Regular (Reg-)HS-SPME experiments were performed with the previously described experimental
136 set-up, while omitting the air-evacuation step.

137 Divinyl benzene/carboxen/polydimethylsiloxane fibers (DVB/ CAR/PDMS 1: 2 cm long, d_f : 50/30
138 mm) were used for both Vac-HS-SPME and Reg-HS-SPME. The fibers were purchased from Supelco
139 Co. (Bellafonte, PA, USA) and conditioned before use as recommended by the manufacturer.

140 Three different sample amounts, 5 mg, 40 mg and 100 mg, and two sampling temperatures, 50°C and
141 80°C, were tested. Sampling-time profiles were obtained by sampling 100mg of the investigated
142 matrices at 80°C for 5, 15, 30 and 60 minutes. All extractions were run in triplicate. The HS-SPME
143 parameters were optimized using the frankincense resin from *B. socotrana*. All other frankincense
144 resins were sampled by adopting the optimized conditions (100 mg of matrix extracted for 15 minutes
145 at 80°C).

146 **2.3 Instrumental set-up**

147 Analyses were carried out using a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr,
148 Germany) installed on a Shimadzu GC-FID-MS system consisting of a Shimadzu GC 2010, equipped
149 with FID, in parallel with a Shimadzu QP2010-PLUS GC-MS system; data were processed and
150 elaborated using Shimadzu GCMS Solution 2.51 and GC Solution 2.53SU software (Shimadzu,
151 Milan, Italy).

152 **2.3.1 Columns**

153 GC analyses were carried out using two 30 m × 0.25 mm d_c , 0.25 μm d_f conventional columns coated
154 with 95% methyl-polysiloxane 5%-phenyl (SE-52) and autobondable nitroterephthalic-acid-modified
155 polyethylene glycol (FFAP-EXT) from Mega (Legano, Mi, Italy). A conventional IL-based SLB-
156 IL60i (30 m × 0.25 mm d_c , 0.25 μm d_f) column, and a narrow bore SLB-IL60 (15 m × 0.10 mm d_c ,
157 0.08 μm d_f) column from Supelco (Bellefonte, PA, USA) were also used.

158 **2.3.2 GC-FID-MS conditions**

159 Analyses were carried out under the following conditions: temperatures - injector, 250°C, transfer
160 line, 270°C, ion source, 200°C; carrier gas - He; flow control mode - constant linear velocity; flow
161 rate - 1 ml min⁻¹; injection mode - split; split ratio - 1:20. The MS was operated in electron ionization
162 mode (EI) at 70 eV, scan rate: 666 u/s, mass range: 35–350 m/z. FID temperature, 250°C; sampling
163 rate, 40 ms. Temperature programs: i) 50°C//5°C min⁻¹//250°C (5 min) for conventional SE-52,
164 FFAP-EXT, SLB-IL60i columns; ii) 40°C//10°C min⁻¹//180°C//15°C min⁻¹//230°C (2 min) for the
165 narrow bore SLB-IL60 column. The GC system was alternatively operated with MS or FID as
166 detectors. Identification was performed via comparisons of linear retention indices and mass spectra

167 either with those of authentic standards, or with data stored in commercial and in-house libraries and
168 the results were confirmed using those of previous publications [2, 4, 6].

169 **2.4 Data elaboration**

170 All elaboration was carried out using Excel (Microsoft) with the exception of the heat map, which
171 was created using Morpheus software (<https://software.broadinstitute.org/morpheus>).

172

173 **3. RESULTS AND DISCUSSION**

174 The development of a fast semiautomatic method entails the investigation of each analytical step. In
175 this study, two *Boswellia* essential oils (*B. socotrana* and *B. papyrifera*) were analyzed first on
176 different stationary phases (SPs) in order to find a column that could separate most frankincense
177 components, and, in particular, the diterpenoid markers. The second step dealt with the careful
178 optimization of sampling conditions (sample amount, sampling time and temperature) using *B.*
179 *socotrana* as the model sample. Vac-HS-SPME and regular HS-SPME (Reg-HS-SPME) were tested
180 in this step. Finally, the chromatographic method was sped-up to fast-GC by translating the analytical
181 conditions from conventional to narrow bore columns. The developed method was then validated on
182 a series of commercial frankincense samples.

183 **3.1 Choice of the GC stationary phase**

184 The choice of a SP that separates as many components as possible is, of course, a crucial step for the
185 effective characterization of a sample. The diterpenoid markers incensole, incensole acetate and
186 serratol, and the essential oils of *B. socotrana* and *B. papyrifera* were analyzed with a range of
187 commercially available columns to define the most appropriate SP for their analysis.

188 As has already been mentioned, apolar columns are not appropriate for the analyses of frankincense
189 resins because of the coelution of incensole and serratol (Figure 2a). Two polar columns were
190 therefore tested, the first was a polyethylene-glycol-based SP (FFAP-EXT) and the second was an
191 IL-based SP (SLB-IL60i). Figures 2b and 2c show that both columns separate diterpenoids with a
192 good resolution but the SLB-IL60i was chosen because of its higher resolution (22 with SLB-IL60i
193 vs. 14 with the FFAP-EXT column) and low bleeding. This column not only separates the diterpenic
194 markers but also the other characterizing components. Figures 3a and 3b report the GC-MS profiles,
195 using the SLB-IL60i column, of the *B. socotrana* and *B. papyrifera* frankincense resins, as sampled
196 by Vac-HS-SPME (see paragraph 3.2). The SLB-IL60i column was therefore used in this study. Table
197 S1 reports the list of the compounds identified, their retention times in the conventional and narrow-

198 bore IL-based columns, together with their molecular formula, molecular weight and main
199 physicochemical properties (LogP, boiling point and vapor pressure).

200 **3.2 Sample preparation - Optimization of the Vac-HS-SPME method**

201 The suitable characterization of frankincense resins by HS-SPME entails the optimization of the
202 sampling conditions to ensure that all classes of volatiles and semi-volatiles are recovered
203 (mono/sesqui/diterpenoidic compounds) with extraction times that are compatible with those of the
204 chromatographic run. In this section, the performance of the Reg-HS-SPME sampling of a *B.*
205 *socotrana* resin is compared to that of Vac-HS-SPME. Preliminary experiments showed that the 2
206 cm long DVB/CAR/PDMS fiber provided a complete picture of frankincense composition, and it was
207 therefore chosen for use in the following tests. The next experiments aimed to optimize the conditions
208 for the pre-equilibration of the frankincense with the headspace for the subsequent Reg- and Vac-HS-
209 SPME samplings. This step is critical because it influences the repeatability of the results, in particular
210 with Vac-HS-SPME. An equilibration time of 15 minutes was chosen as it was the minimum time for
211 which repeatability gave an RSD% of below 15% (except for compounds in traces) over five
212 experiments under any applied conditions (data not reported).

213 The sample amount was first optimized by sampling 5, 40 and 100 mg of frankincense for 15 min
214 with both Reg- and Vac-HS-SPME at a sampling temperature of 80°C [6, 8]. With Reg-HS-SPME,
215 the abundance of the high volatility analytes (mono and sesquiterpenoids) increased with sample
216 amount, while that of the diterpenoids was always very low and did not seem to be significantly
217 affected. The results with 5 mg of sample show that: i) for monoterpenoids, the performance of regular
218 sampling is about 50% higher than that of Vac; ii) for sesquiterpenoids, the difference is lower, but
219 Reg sampling is still more effective than Vac; while iii) for diterpenoids, the peak areas with Vac
220 sampling are double than those of Reg. Table 1 reports the relative analyte abundances obtained by
221 sampling with Vac-HS-SPME vs. Reg-HS-SPME. The poorer performance of Vac-HS-SPME
222 compared to Reg-HS-SPME with the most volatile components is due to the air evacuation step in
223 which they are significantly aspirated with air. This loss is not observed for the less volatile compounds
224 for which the reduced pressure of Vac-HS-SPME promoted vaporization to the headspace, although
225 with longer extraction times (i.e. closer to the equilibrium), the two techniques provide similar results
226 (data not reported).

227 Since suitable enrichment, in particular for the diterpenoids, cannot be achieved with 5 mg of resin,
228 the sample amount was increased to 40mg. The results show that some monoterpenoids are still lost
229 during the air evacuation step with Vac-HS-SPME, but that the medium volatility compounds show
230 a good improvement (Table 1).

231 Finally, 100mg of sample was evaluated and the results show: i) Reg-HS-SPME and Vac-HS-SPME
232 have comparable responses for the high volatility components; ii) slightly improved recovery of
233 medium volatility components (about 1.5 higher); and iii) drastic improvements in diterpenoids, with
234 peak areas being almost four times higher than Reg-HS-SPME. Further experiments were carried out
235 to exclude any discriminative loss from the headspace over time; air evacuation times of 45 and 120
236 sec were applied, resulting in perfectly overlapping patterns even for the most volatile compounds.
237 The results show that the slight peak-area differences between Reg-HS-SPME and Vac-HS-SPME
238 are probably related to competition with the adsorbent [10]. The sample amount was then fixed at
239 100mg.

240 The sampling time was then optimized by checking the behavior of the frankincense markers when
241 processed with the two investigated techniques with 5, 15, 30 and 60 minutes of sampling. The results
242 are summarized in Figure 4. α -Pinene and limonene (monoterpene hydrocarbons) were taken as a
243 reference for the most volatile frankincense components; these compounds reached equilibrium with
244 both Reg-HS-SPME and Vac-HS-SPME, but the longer extraction times necessary with Reg
245 sampling produce a decrease in the extraction efficiency, which is probably related to adsorption
246 competition. Similar behavior can also be observed for intermediate volatile compounds (*trans*- β -
247 caryophyllene, α -humulene and β -selinene), although the extraction performance of Vac-HS-SPME
248 was slightly better than that of Reg-HS-SPME. Finally, the extraction-time profiles of the diterpenoid
249 markers (serratol, incensole and cembrene A) show that sampling under reduced pressure permits
250 recovery to be sped-up even with short extraction times (15 min), although equilibrium could not be
251 reached, even over 60 minutes, by either technique. There were extreme differences in the extraction
252 times needed to reach equilibrium in the different classes of terpenoids with Reg-HS-SPME, and
253 these are in agreement with literature data [22]. The use of Vac-HS-SPME for 15 min was then chosen
254 for the following experiments as it provides the suitable recovery of all compound groups in a short
255 sampling time.

256 The possibility of decreasing the extraction temperature to 50°C was also explored, but a significant
257 decrease in the abundance of diterpenoids was observed (see Figure S1), meaning that higher
258 extraction temperatures were mandatory.

259 **3.3 Speed-up of the analysis step**

260 The third part of the study was focused on speeding-up the GC analysis to make it compatible with
261 the sampling time. The above chromatographic method was translated to a 15m \times 0.10 mm d_c , 0.08
262 μ m d_f column using the method translation approach [16, 17, 19]. The analysis time was thus reduced
263 to 19 minutes, while the separation of all the markers was maintained. Figure 5 reports the translated

264 GC-FID patterns, with the narrow-bore SLB-IL60 column, of the diterpenic markers and the *B.*
265 *socotrana* and *B. papyrifera* resins.

266 **3.4 Application of the optimized method to real-world frankincense samples**

267 The two authentic and the three commercial frankincense samples were analyzed with the optimized
268 Vac-HS-SPME-fast-GC-FID-MS method, and the resulting patterns were compared to those obtained
269 with Reg-HS-SPME. The optimal conditions adopted with Vac-HS-SPME were 100mg of
270 frankincense resin, sampled at 80°C for 15 min combined with 15 min of pre-equilibrium (total
271 sampling time 30 min). Tables S2 and S3 reports the mean peak area of each analyte in each sample
272 together with the repeatability (%RSD). The results show that Vac-HS-SPME is more repeatable than
273 Reg-HS-SPME reaching %RSD in general below 10% with the exception of traces. Due to the high
274 number of analytes, the results were summarized in a heat map (Figure 6) where the areas of each
275 analyte (row) is scaled in function of their abundance in each sample from minimum (blue) to
276 maximum (red). The samples were also hierarchically clustered by applying the one minus Pearson
277 correlation. The results of the heat map show that: i) the commercial frankincense 3 sample labelled
278 as *B. sacra*, was actually *B. papyrifera* as proved not only by the high abundance of markers such as
279 octyl acetate, octanol, incensole and incensole acetate, but also by its clustering with the authentic *B.*
280 *papyrifera*, ii) Vac-HS-SPME when compared to Reg-HS-SPME provides a drastic increase of
281 recovery of both hydrocarbons and oxygenated diterpenoids and most oxygenated sesquiterpenoids
282 as it is clear, for instance, from the difference in intensity of a) incensole, incensole acetate, cembrene,
283 cembrene A and the other diterpene hydrocarbons in *B. papyrifera* samples or b) caryophyllene oxide,
284 α and β -eudesmol and serratol in *B. socotrana* and in the commercial sample 2.

285

286 **4. CONCLUDING REMARKS**

287 A fast and versatile method has been optimized to discriminate *Boswellia* spp. resins using their
288 volatile and semi-volatile fractions.

289 The method is based on HS-SPME under reduced pressure (Vac-HS-SPME), which increases the
290 release of semi-volatile compounds (i.e. diterpenoidic compounds) in the headspace and enables their
291 sampling in 30 minutes. Vac-HS-SPME was successfully combined with fast GC, with columns
292 coated with an IL-based SP, providing the baseline separation of all markers in 20 minutes.

293 The latest geometry of Vac-HS-SPME sampling vials makes it possible to include it in robotic
294 autosamplers, i.e. to comprise it in inclusive platforms, the so-called “Total Analysis System” (TAS),

295 namely systems where the three main steps of an analytical procedure (sample preparation-analysis-
296 data elaboration processing) are on-line merged in a single step [23]. With an integrated platform,
297 after a preliminary air evacuation, it is possible to overlap sampling and analysis steps enabling to
298 analyze up to five samples of *Boswellia* resins in two hours that is the analysis time commonly
299 required for a single sample with conventional methods (60 min sampling and 60 min analysis). The
300 reported method is simple, fast, automated and compatible with the processing of a large number of
301 samples, as is required in a routine quality control laboratory.

302 This study shows, for the first time, that Vac-HS-SPME can successfully be applied to the analysis
303 of semi-volatiles in solid samples in the plant and natural products fields by applying a low
304 temperature (below 0°C) during the air-evacuation step, and a suitable sample amount (above 100
305 mg) for a suitable recovery of the most volatile analytes. Further studies are under way to optimize
306 the method for quantitation.

307

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314

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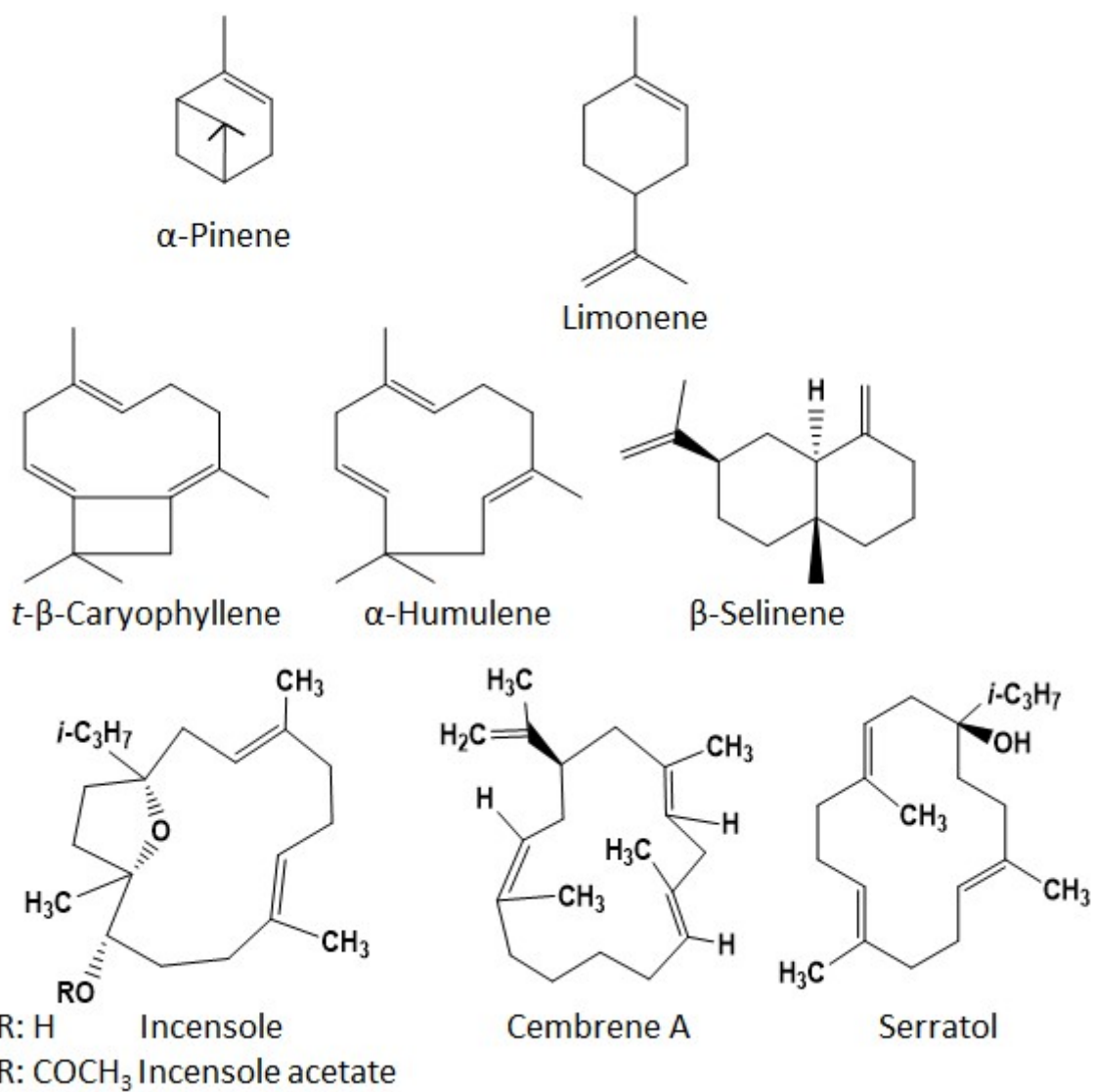
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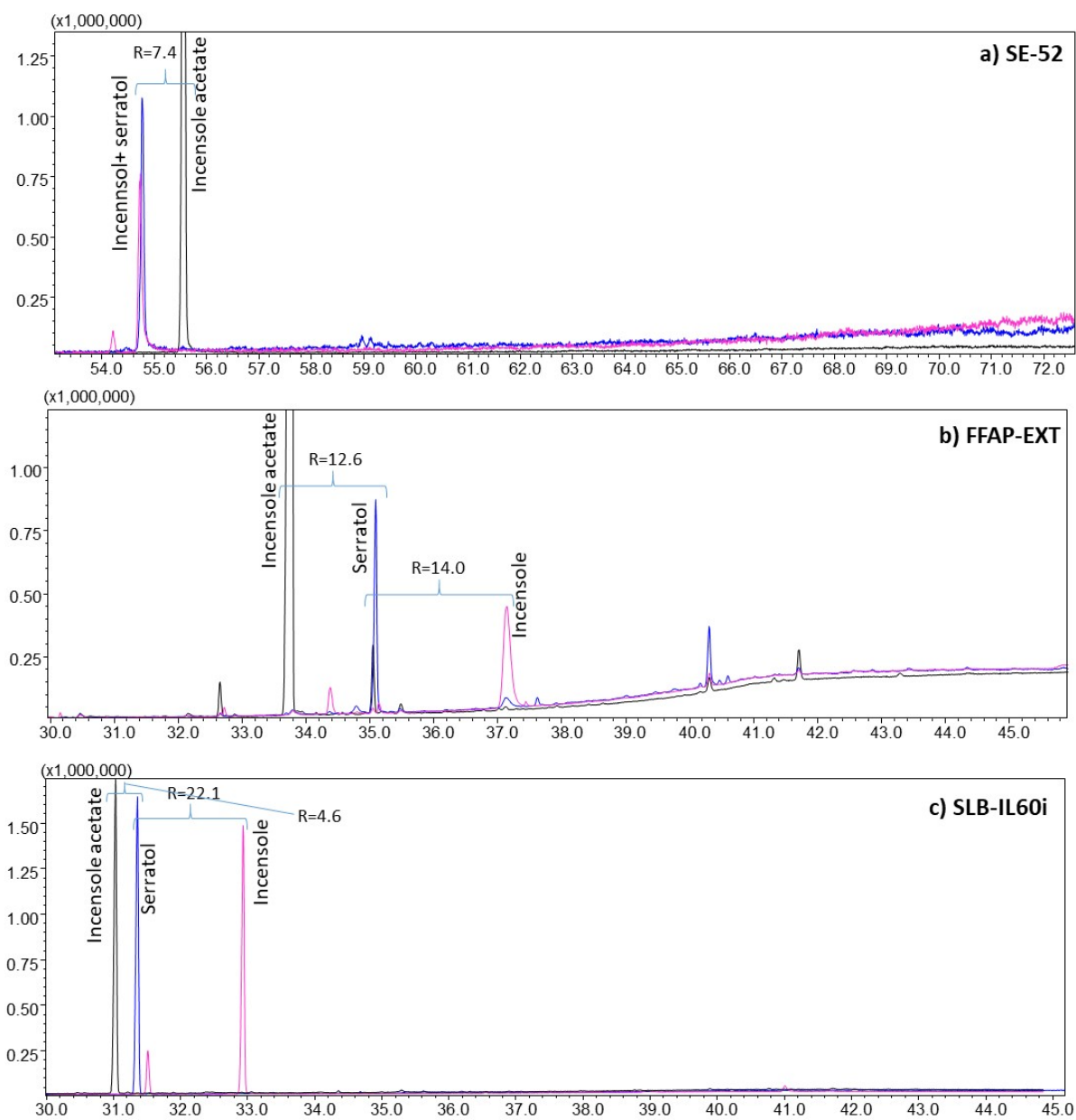
390 **Figure 1** – Chemical structures of the main markers of the frankincense resins.



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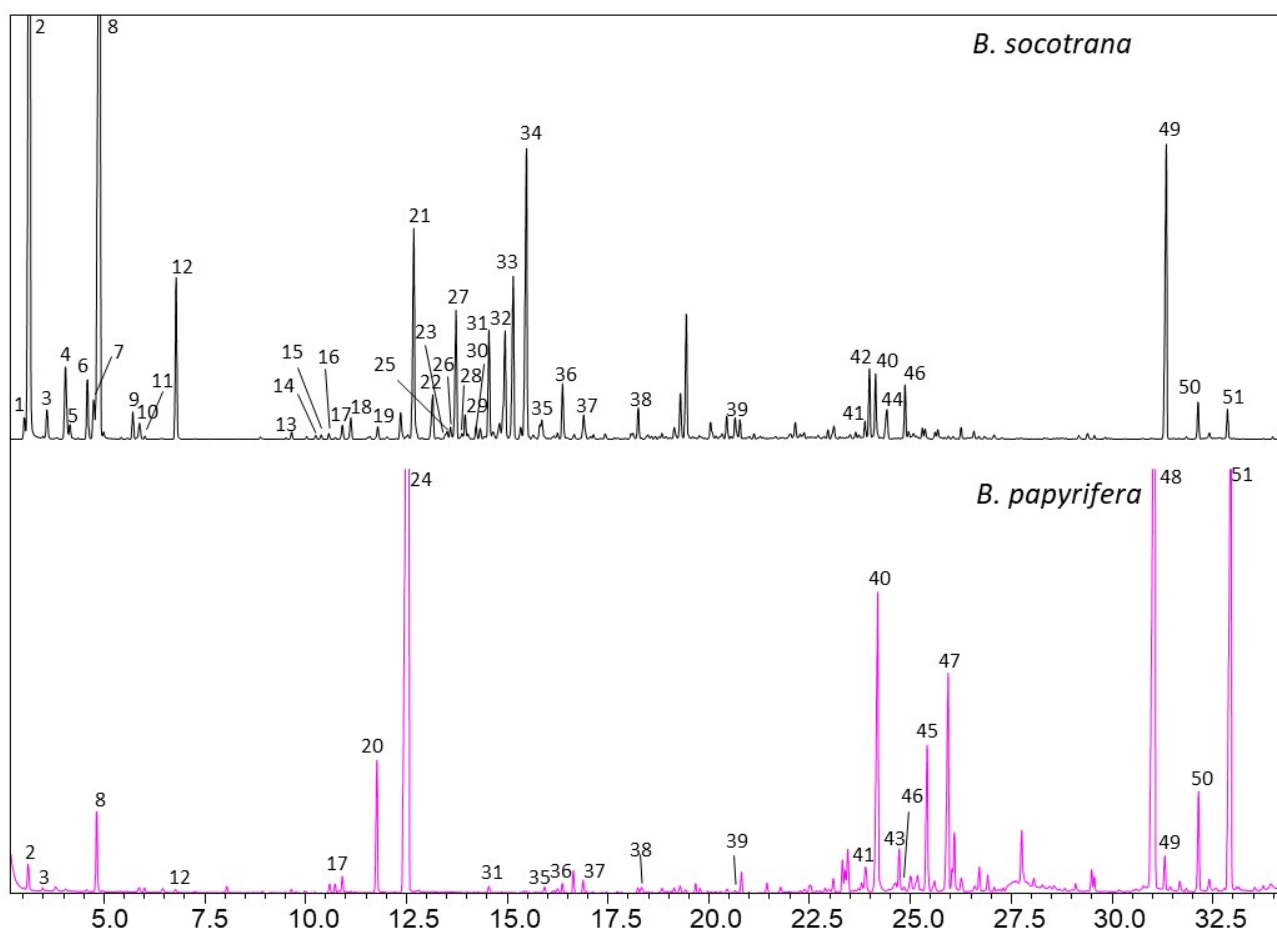
393 **Figure 2** – GC-MS profiles of incensole (pink), serratol (blue) and incensole acetate (black) on the
394 conventional SE-52 (a), FFAP-EXT (b) and SLB-IL60i (c) columns. Analysis conditions: see
395 experimental section.



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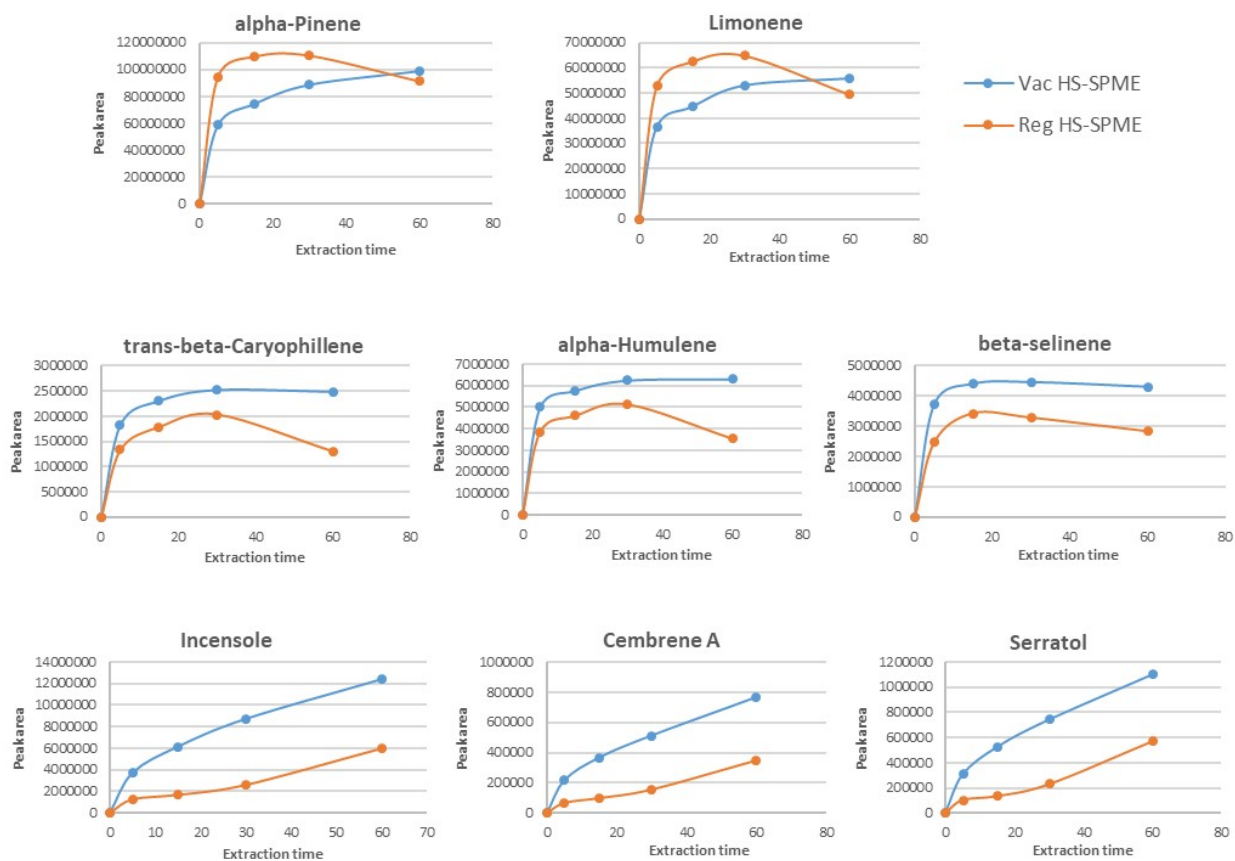
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398 **Figure 3** – Vac-HS-SPME GC-MS profiles of *B. socotrana* (black) and *B. papyrifera* (pink) obtained
 399 with the SLB-IL60i column. Analysis conditions: see experimental section. Legend: 1. α -Thujene, 2.
 400 α -Pinene, 3. Camphene, 4. β -Pinene, 5. Sabinene, 6. β -Myrcene, 7. α -Phellandrene, 8. Limonene, 9.
 401 β -Phellandrene, 10. 1,8 - Cineole, 11. *trans*- β -Ocimene, 12. *para*-Cymene, 13. *o*-Methylanisole, 14.
 402 α -Cubebene, 15. *cis*-Sabinene hydrate, 16. *p*-Cymenene, 17. α -Terpinolene, 18. α -Copaene, 19. β -
 403 Bourbonene, 20. Octanol, 21. β -Elemene, 22. *trans*-Pinocarveol, 23. *cis*-Verbenol, 24. Octyl acetate,
 404 25. 1,8-Menthadien-4-ol, 26. *trans*-Verbenol, 27. *trans*- β -Caryophyllene, 28. Aromadendrene, 29. γ -
 405 Selinene, 30. Germacrene D, 31. α -Fenchol, 32. α -Humulene, 33. α -Selinene, 34. β -Selinene, 35.
 406 Myrtenol, 36. *trans*-Carveol, 37. *cis*-Carveol, 38. Carvone, 39. Verbenone, 40. Cembrene, 41.
 407 Limonene-1,2-diol, 42. Caryophyllene oxide, 43. Hydrocarbon diterpene 1, 44. α -Eudesmol, 45.
 408 Hydrocarbon diterpene 2, 46. β -Eudesmol, 47. Hydrocarbon diterpene 3, 48. Incensole acetate, 49.
 409 Serratol, 50. Cembrene A, 51. Incensole.



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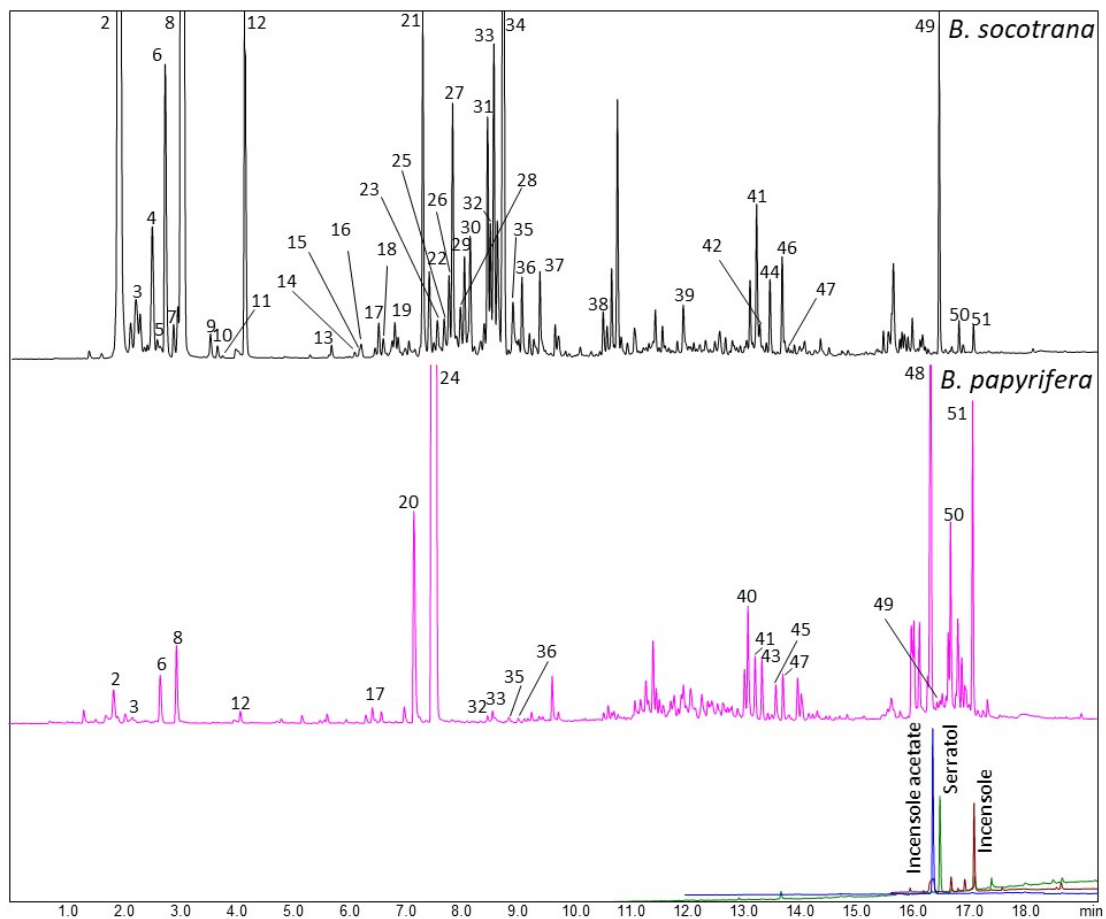
411 **Figure 4** – Extraction time profiles of α -pinene, limonene (monoterpenoids), *trans*- β -caryophyllene,
 412 α -humulene and β -selinene (sesquiterpenoids), serratol, incensole and cembrene A (diterpenoids)
 413 obtained with Vac-HS-SPME (blue) and Reg-HS-SPME (orange). Sampling amount: 100 mg,
 414 sampling temperature: 80°C.



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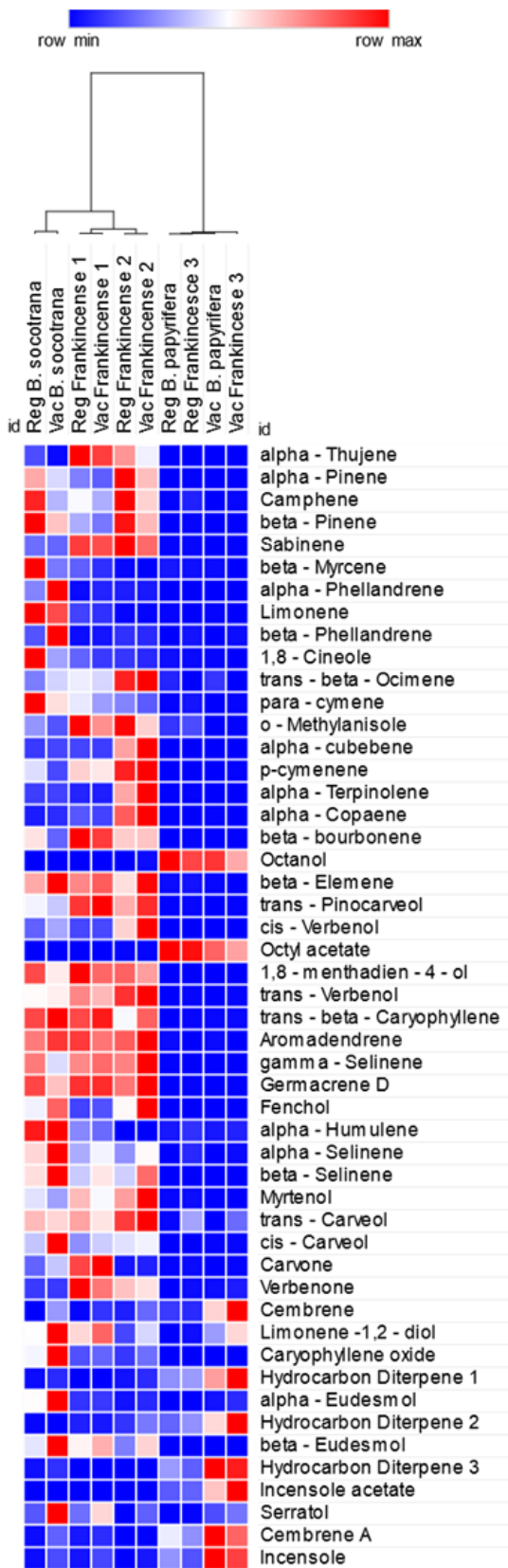
417 **Figure 5** – Vac-HS-SPME Fast-GC-MS profiles of *B. socotrana* (black), *B. papyrifera* (pink),
418 incensole (brown), serratol (green) and incensole acetate (blue) obtained with the SLB-IL60i column.
419 Analysis conditions: see experimental section. Legend: see caption to figure 2.



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422 **Figure 6** – Heat map on the investigated samples sampled by Reg-HS-SPME (Reg) and Vac-HS-
 423 SPME (Vac). Samples are hierarchically clustered by applying the one minus Pearson correlation.



424

425 **Table 1** – Relative analyte abundance (RAA) obtained by sampling 5, 40, 100mg of *B. socotrana*
 426 with Vac-HS-SPME vs. Reg-HS-SPME for 15 min at 80°C. Legend: red triangle (▼) RAA < 0.75,
 427 yellow line (—) 0.75 < RAA < 1.25, green triangle (▲) RAA > 1.25

Compound Name	5 mg	40 mg	100 mg
alpha-Tujene	▼0.66	▼0.60	—0.76
alpha-Pinene	▼0.68	▼0.69	—0.79
Camphene	▼0.70	▼0.54	—0.76
beta-Pinene	▼0.50	▼0.53	—0.82
Sabinene	▼0.41	▼0.50	—0.81
beta-Myrcene	▼0.60	▼0.59	—0.84
alpha-Phellandrene	▼0.59	▼0.67	—0.91
Limonene	▼0.50	▼0.60	—0.84
beta-Phellandrene	▼0.49	▼0.55	—0.90
1,8-Cineole	▼0.39	▼0.53	—1.02
p-Cymene	▼0.49	▼0.56	—0.84
o-Methyl-anisole	▼0.47	▼0.56	—0.77
p-Cymenene	▼0.54	▼0.67	—0.99
alpha-Terpinolene	▼0.37	▼0.67	—1.02
alpha-Copaene	▼0.33	—0.96	▲1.47
beta-Elementene	▼0.36	—1.01	▲1.43
trans-Pinocarveol	▼0.30	▼0.65	—1.00
trans-Caryophyllene	▼0.35	—1.00	▲1.37
Germacrene D	▼0.42	—1.01	▲1.40
alfa-Fenchol	▼0.37	—0.83	—1.04
alpha-Humulene	▼0.36	—1.12	▲1.34
alpha-Selinene	▼0.42	▲1.25	▲1.42
beta-Selinene	▼0.43	—1.21	▲1.41
trans-Carveol	▼0.43	—0.94	—1.12
cis-Carveol	▼0.43	—0.98	—1.16
Carvone	▼0.43	—0.83	—1.11
Limonene-1,2-diol	▼0.64	▲1.28	—1.10
Caryophyllene oxide	▼0.33	▲1.51	▲1.48
Cembrene	—1.19	▲3.31	▲2.92
beta-Eudesmol	—0.83	▲2.19	▲1.85
Serratol	▲2.32	▲3.07	▲3.66
Cembrene A	▲2.50	▲2.99	▲3.71
Benzylbenzoate	▲1.53	▲2.32	▲2.29
Incensole	▲2.06	▲2.70	▲3.93

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