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Profiling and classification of French propolis by combined multivariate data analysis of planar chromatograms and scanning direct analysis in real time mass spectra

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[1]These first two authors made equal contributions to this work.

Highlights

- Profiling of French propolis by PCA of HPTLC-FLD image and HPTLC-DART-MS data
- Improved quality control strategy for the classification of propolis samples
- Comparison of normal- and reversed-phase chromatography of propolis combined with PCA
- Identification of characteristic marker compounds for the classification of French propolis
- Confirmation of the presence of two botanical types of European poplar-based propolis

Abstract

Quality control of propolis is challenging, as it is a complex natural mixture of compounds, and thus, very difficult to analyze and standardize. Shown on the example of 30 French propolis samples, a strategy for an improved quality control was demonstrated in which high-performance thin-layer chromatography (HPTLC) fingerprints were evaluated in combination with selected mass signals obtained by desorption-based scanning mass spectrometry (MS). The French propolis sample extracts were separated by a newly developed reversed phase (RP)-HPTLC method. The fingerprints obtained by two different detection modes, *i. e.* after (1) derivatization and fluorescence detection (FLD) at UV 366 nm and (2) scanning direct analysis in real time (DART)-MS, were analyzed by multivariate data analysis. Thus, RP-HPTLC-FLD and RP-HPTLC-DART-MS fingerprints were explored and the best classification was obtained using both methods in combination with pattern recognition techniques, such as principal component analysis. All investigated French propolis samples were divided in two types and characteristic patterns were observed. Phenolic compounds such as caffeic acid, *p*-coumaric acid, chrysin, pinobanksin, pinobanksin-3-acetate, galangin, kaempferol, tectochrysin and pinocembrin were identified as characteristic marker compounds of French propolis samples. This study expanded the research on the European poplar type of propolis and confirmed the presence of two botanically different types of propolis, known as the blue and orange types.

Keywords

Propolis; HPTLC; Multivariate data analysis; DART-MS; Flavonoids; Phenolic acids

1. Introduction

Propolis is a natural resinous substance produced by honeybees (*Apis mellifera*) from various plant buds and exudates such as *Populus* spp. It is a soft and sticky material used by honeybees to protect their community from different predators and as sealing material to fill the cracks in the hive [1]. The color of propolis considerably varies from dark-brown, over yellow, to green or red, depending on its geographical origin and plant sources. The chemical composition of propolis depended on the sub-species of bees, botanical as well as geographical origin and collection season [2]. Due to the wide range of pharmacological properties such as antibacterial, anti-inflammatory, antioxidative, hepatoprotective and anti-tumor properties [3], propolis was recognized as beneficial natural product in the cosmetics, food supplement and nutraceuticals industry.

Propolis is a very complex natural mixture of compounds, such as flavonoids, phenolic acids and their derivates, and thus, very difficult to analyze and standardize. The common analytical strategy is to identify specific marker compounds to classify different propolis types. Separation techniques such as capillary electrophoresis, high performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), direct analysis in real time mass spectrometry (DART-MS) and gas chromatography hyphenated with structure elucidation techniques identified characteristic compounds in propolis [4,5]. So far, DART-MS has only been investigated using the DIP-it technique, generating a fingerprint of the whole sample with no separation of the compounds, in combination with HPTLC and principal component analysis (PCA) [5]. Recently, the poplar type of propolis was divided into two botanically different sorts, known as blue and orange types according to their planar chromatography fingerprint [5]. The poplar type of propolis, based on *Populus* L. plant material, is one of the most frequently and

thus most investigated type with origin in Europe, North America and Asia. As a complex natural material, this propolis type consisted of more than 420 compounds [6,7]. It mainly contained phenolic compounds such as aromatic (phenolic) acids and their derivates, flavonoids including flavones, flavanones, flavonols and dihydroflavonols, chalcones, dihydrochalcones and other organic compounds [8,9]. Different *Populus* hybrids (*P. alba*, *P. tremula* and *P. nigra*) have been reported as a primary source of propolis originating from temperate zones. *Quercus sp.*, *A. hippocastanum*, *Ulmus sp.*, *Picea sp.*, *Fraxinus sp.*, *B. pendula*, *Salix alba* and *Pinus sp.* were recognized as secondary sources of the poplar type propolis [2].

With regard to French propolis, only few scientific contributions were related to the chemical composition. Boisard *et al.* identified the chemical constituents of French poplar type propolis by HPLC coupled with a diode array detector and MS as well as nuclear magnetic resonance spectroscopy. Its antifungal activity was studied using three fungal strains and one filamentous fungus, while the antibacterial activity was investigated via Gram-positive and Gram-negative bacteria. Also, its polyphenol constituents, antioxidant potential and capability to decelerate aging processes were investigated [10,11].

Quality control of propolis gained in importance in the last decade due to the increasing use and demand of propolis added to various products. PCA was applied to obtain an objective classification of French propolis, while visual classification of HPTLC chromatograms is subjective and depends on the perception of the analyst. Hence, for profiling, fingerprinting and characterization of propolis, this study focused on the multivariate data analysis of normal phase (NP) and reversed phase (RP) planar chromatograms obtained by derivatization and subsequent fluorescence detection (FLD) and by scanning DART-MS. For classification of the propolis samples, NP-/RP-HPTLC-FLD and NP-/RP-HPTLC-DART-MS fingerprints were explored

solely and in combination. Pattern recognition techniques, such as principal component analysis, were applied for multivariate chemometric analysis.

2. Experimental

2.1. Reagents and chemicals

HPTLC plates silica gel 60 (NP) and HPTLC plates RP-18 water-wettable (W) were used, both with a layer thickness of ca. 200 µm (Merck, Darmstadt, Germany). A pre-washing step via chromatography with methanol – water (3:2, *v*/*v*) was performed for NP plates [12] and with methanol for RP plates. Both plate types were subsequently dried with a hair-dryer for 5 min. All solvents and acids (HPLC or analytical grade) were purchased from Sigma Aldrich, Schnelldorf, Germany, or Carl Roth, Karlsruhe, Germany. Neu's natural product reagent (ethanolamine diphenylborate, ≥98%) and polyethylene glycol (PEG 400) were delivered from Carl Roth and J. T. Baker, Avantor Performance Materials, Center Valley, PA, USA, respectively.

2.2. Sample preparation

All propolis samples were collected during summer 2015 at different geographical places (Fig. 1) and stored at -25 °C. After grinding for 20 s in an A11 basic analytical mill (IKA, Staufen, Germany) to avoid melting of the wax, 100 mg of each propolis sample were extracted with 4 mL ethanol in centrifuge tubes at 85 °C for 15 min. The suspension was centrifuged (3000 × *g*, 10 min), the supernatant was evaporated to dryness and the residue was dissolved in 5 mL ethyl acetate. The obtained solution was filtered through a disk filter (0.20 µm) prior to sample application.

2.3. HPTLC-FLD

The propolis extract solutions (5.0 µL) were applied as 8 mm bands at a band distance of 11 mm (20 mm for DART-MS) using the Automatic TLC Sampler 4 (CAMAG, Muttenz, Switzerland). The chromatographic separation on NP plates was performed according to Morlock *et al.* [5, 14] in a twin-trough chamber 20 × 10 cm (CAMAG) with a mixture of *n*-hexane – ethyl acetate – acetic acid (5:3:1, *v*/*v*/*v*) up to a solvent front position of 80 mm (measured from the lower plate edge). A similar mobile phase mixture was used for the RP plates, *i. e.* a mixture of *n*-hexane – toluene – ethyl acetate – formic acid – acetic acid (16:6:10:3:3, *v*/*v*/*v*/*v*/*v*) up to a solvent front position of 90 mm (measured from the lower plate edge) [13]. In both cases, 5 mL hydrochloric acid (37%) was applied on a filter paper in the second trough of the chamber to create an acidic vapor phase during the separation. Subsequent, the plate was dried under a stream of warm air for 5 min. For derivatization, the plate was dipped into the ethanolamine diphenylborate reagent using the Chromatogram Immersion Device (CAMAG), dried and dipped in PEG 400 for fluorescence enhancement and stabilization (both at an immersion time of 3 s and an immersion speed of 3.5 cm/s). The plate was illuminated at UV 366 nm using the TLC Visualizer (CAMAG) and image was captured by the built-in Baumer Optronic DXA252 digital camera with 12-bit per channel charge coupled device (CCD), providing a 100 µm spatial resolution and an image size of 1922 × 952 pixels. The capture settings were set to an exposure time of 1 s, a gain factor of 1 and noise reduction by averaging four images.

2.4. HPTLC-DART-MS

Prior to DART-MS scanning, the plates were cut with the SmartCut plate cutter (CAMAG) into 2 × 10 cm strips for each track. The strips were scanned with a substantially modified DART-SVPA interface (IonSense, Saugus, MA) for HPTLC-DART-MS [15] with an improved desorption/ionization functionality [16] coupled to the amaZon ETD ion trap mass spectrometer

(Bruker Daltonics, Bremen, Germany). A source cap with 1 mm inner diameter and 6.5 mm inner length at 5 mm distance to the sampling surface was used for scanning in the positive ionization mode at a speed of 0.2 mm/s. Helium (purity 5.0) was used at a gas temperature set to 500 °C and a gas flow of 3.0 L/min. The enhanced resolution mode of the trapControl (Bruker Daltonics) was used for the acquisition of the total ion current (TIC) chromatogram. The extracted ion current (EICs) chromatograms of the eight most abundant components in the propolis extract samples (Table 1) were extracted and processed with DataAnalysis version 4.0 (Bruker Daltonics).

2.5. PCA using data of HPTLC-FLD and HPTLC-DART-MS

The HPTLC fingerprints at UV 366 nm were exported from the winCATS software (CAMAG) and imported into the rTLC web application [17]. The software extracted the mean pixels (red, green and blue pixel channels) in the middle of each track for each propolis sample. The numerical data matrix obtained was used for preprocessing and statistical analysis. Dynamic time warping and mean-centering were applied as preprocessing steps to improve the quality of the PCA model [6]. In this study, the green channel showed the highest variations between the propolis samples, and thus, this channel was used for evaluation. A classification of each propolis sample was made according to the signal intensity of each compound along the sample track. With regard to the HPTLC-DART MS data, the EIC signal areas were exported to CSV-files and used as variables in multivariate statistical analysis via XLSTAT statistical software for Microsoft Excel (Redmond, WA, USA). Autoscaling was applied as preprocessing tool to improve the raw data quality of the PCA model.

3. Results and discussion

3.1. Profiling French propolis by NP-HPTLC *versus* RP-HPTLC

The thirty propolis samples collected from different geographical regions in France (Fig. 1) were extracted, separated, derivatized and documented at UV 366 nm. The NP-HPTLC fingerprinting [5,14] was applied as initial screening for the different phenolic compound patterns of French propolis. As the separation of propolis compounds was challenging due to the complexity of this natural product, the separation was also performed on RP-18 W phases. For transfer to these water-wettable reversed phases, the mobile phase was adjusted. The resulting HPTLC fingerprints of the phenolic compounds and its derivates revealed two main types of French propolis (orange and blue type), which was evident for the NP- as well as RP-HPTLC separation (Fig. 2). The elution power was slightly higher on the RP-18 W layer, if compared to the silica gel layer. Nevertheless, both separations led to a similar pattern, as mainly NP interactions were dominant during the separation on the RP-18 W layer. This was explained by the relatively apolar mobile phase (*n*-hexane – toluene – ethyl acetate – formic acid – acetic acid 16:6:10:3:3, *v*/*v*/*v*/*v*/*v*) with regard to the RP-18 W phase (reduced C18-chain coverage, if compared to a RP-18 phase) and its freely available residual silanol groups.

With regard to both separations (Fig. 2), the samples AT, CE1 and CE3 showed characteristic blue fluorescent bands and were classified as the blue type of propolis. The blue fluorescent compounds at mean hR_F values of 54, 70 and 81 on NP-HPTLC (Fig. 2a) and at hR_F values of 57, 65 and 77 on RP-HPTLC (Fig. 2b) were assigned to be caffeic acid, caffeic acid phenethyl ester (CAPE) and galangin, respectively. Samples such as AP, MD1, AU4, AU2, CE5 and AU3 had a similar pattern with two blue fluorescent bands of high intensity and hardly orange fluorescent bands.

Other samples (BR, RA1, FM, RA2, CE4, RA4, CE2, CE8, DR, RA5, AU1 and AU5) revealed a phenolic profile that was characteristic for the orange type, as the most dominant variety of French propolis. The orange fluorescent band at hR_F 41 (Fig. 2a) on NP-HPTLC and at hR_F 54 on RP-HPTLC (Fig. 2b) was identified as chrysin. Together with kaempferol at hR_F 27 (Fig. 2a) and quercetin at hR_F 54 (Fig. 2b), these are phenolic markers for the orange type of propolis from Germany and Serbia [5,18]. An intermediate subgroup of propolis samples (AU3, CE7, AU2, PO, CE6, AP, MD2, RA6, AU4, CE5, CS, CE10, CE9, MD1 and CO) contained orange and yellow as well as blue fluorescent bands of high intensity. This sample subgroup contained blue bands of high intensity for galangin, caffeic acid, and CAPE, while no significant amount of kaempferol and chrysin was detected (Figure 2a). Additionally, some intermediate subgroups contained lower amounts for compounds at hR_F 9 and 21, according to the RP system. By utilizing this system for the separation of samples of the orange subtype, the orange and yellow bands were separated much better, compared to the NP system. However, the intermediate subtype mainly contained blue bands, but only marginal visible orange bands. These bands were confirmed by PCA as characteristic markers for the differentiation between two subtypes of orange propolis. The afore-mentioned blue-type markers caffeic acid, CAPE and galangin can also be considered as characteristic markers of the orange propolis, though differing in intensity.

The NP- and RP-HPTLC classification of French propolis confirmed the two main botanically different types of European poplar-based propolis [7,14,19]. Although both, RP system and NP system, showed a similar fingerprint, the RP method revealed a better differentiation between the phenolic compounds, especially for caffeic acid, CAPE and galangin, and thus, a higher observable number of orange and blue fluorescent bands for both types of propolis. Additionally, the RP separation mitigated saturation effects during the color channel analysis of the

chromatogram image prior to PCA evaluation. These advantages of the RP method provided better input data for the classification of propolis samples using objective multivariate methods as discussed in detail as follows.

3.2. PCA of HPTLC-FLD fingerprints

PCA was applied on the data matrix obtained by image analysis of HPTLC-FLD chromatograms of 30 French propolis samples for both, the NP [5,14] and the newly applied RP separation. PCA reduced the multidimensionality of the data to classify objects, to identify important variables and to determine outliers. PCA was performed using the open source rTLC platform for a streamlined image evaluation and multivariate analysis of HPTLC chromatograms and is discussed in detail in another study [17]. The rTLC evaluation was much faster and more reliable, if compared to previous evaluations using a combination of ImageJ and MATLAB software [6].For the NP-HPTLC-FLD fingerprints, the first three principal components PC1, PC2 and PC3 accounted for 51.19%, 14.51% and 10.83%, respectively, and thus, to 76.54% of the total variability. In the 2D score plot of PC1 and PC2 (Fig. 3a), the blue type group was located in the lower right half, while the orange type was dominant in the left half and the intermediate type in the right half. The loading plots displayed the influence of phenolic compounds (characterized by the hR_F value) to the total variability, and hence, identified botanical markers of propolis, responsible for the classification (Fig. 3c and d). Phenolic compounds such as caffeic acid, CAPE and galangin with mean hR_F values of 54, 70 and 81 showed the highest positive impact along the PC1 direction, while a blue unknown compound at hR_F value of 24 and caffeic acid at hR_F 54 had the highest positive influence on PC2 (Fig. 3c). However, a clear differentiation of the groups was not evident.

The classification of the propolis samples obtained by RP-HPTLC-FLD analysis was superior to that of NP-HPTLC-FLD. Four principal components (PCs) described 75.02% of the total data variability. PC1 described (39.16%) of the variability, while PC2, PC3 and PC4 described 17.27%, 9.58% and 9.01%, respectively. The 2D score plot of PC1 and PC2 (Fig. 3b) revealed two distinct groups of French propolis samples alongside the PC1 direction. One group of propolis samples was positioned on the right bottom side of the PC score plot and mainly belonged to the blue type, while other samples positioned on the left side mainly belonged to the orange type (Fig. 3b). The intermediate subgroup of the propolis samples was mainly located in the upper right side of the 2D score plot. PC1 was most positively affected by caffeic acid, CAPE and galangin for NP- as well as RP-HPTLC-FLD-PCA. PC2 was positively affected by two unknown greenish-blue substance bands at hR_F values of 20 and 33 and negatively affected by galangin for RP-HPTLC-FLD-PCA (Fig. 3c and d). Thus, phenolic compounds were highlighted as most important for the two different types of propolis.

To conclude, the RP-HPTLC-FLD fingerprints were suited for classification of the French propolis samples and superior if compared to the NP-HPTLC-FLD fingerprints. Next, a combination of RP-HPTLC-FLD fingerprints with HPTLC-DART-MS data was investigated for any further potential improvement with regard to differentiation. Apart from classification according to the propolis types, there was no substantial difference in the chemical composition of the investigated samples according to their geographical origin (Fig. 1). This could be explained by the same botanical sources that the bees access for the propolis preparation in the investigated regions of France (similar flora).

3.3. Further characterization by RP-HPTLC-DART-MS

Further characteristic phenolic markers for differentiation of the propolis types were expected to be discovered by HPTLC-MS. HPTLC-DART-MS was selected due to its ease of operation, *i. e.* a simple scan along the chromatogram track using a substantially modified DART interface [15,16]. Scanning along a track took about 3 min for a 35 mm scan track (region of interest). The DART-MS scanning of the NP- and RP-chromatograms revealed eight compounds in the positive ionization mode that were dominant according to their abundance (Table 1). Phenolic acid compounds like caffeic acid were hardly detectable in the positive ionization mode. Nevertheless, this mode was preferred due to the more complex profile and thus orthogonal data set for PCA. The RP method showed a higher capability of detection with regard to scanning DART-MS recording, if compared to the NP method because the polar compounds were stronger adsorbed on the silica gel layer and thus their desorption rate was reduced. The eight compounds were identified by their MS spectra (Fig. 4) and their position in the sample track was aligned to their hR_F value. Thus, *p*-coumaric acid (*m/z* 163), chrysin (*m/z* 253), pinocembrin (*m/z* 255), tectochrysin (*m/z* 267), galangin (*m/z* 269), pinobanksin (*m/z* 271), kaempferol (*m/z* 285) and pinobanksin-3-acetate (*m/z* 313) were identified as dominant mass signals and chosen as variables for the subsequent PCA. The missing baseline resolution between adjacent zones was not critical in the case of HPTLC-DART-MS scanning, as the EIC chromatograms, extracted for the characteristic *m/z* ranges of the different components, were evaluated independently from any partially co-eluting compound.

3.4. PCA of the combined fingerprints of RP-HPTLC-FLD and RP-HPTLC-DART-MS

Finally, a classification of the investigated French propolis extracts was performed by PCA of both, the RP-HPTLC-FLD and RP-HPTLC-DART-MS data set (Fig. 5a). Thus, PCA was

performed on the combined data set composed of 30 RP-HPTLC-FLD image fingerprints and 30 times 8 EIC peak areas of their characteristic *m/z* signal, extracted from the RP-HPTLC-DART mass spectra (Fig. 6). The respective *m/z* value variables had been classified according to their contributions to PC1 and PC2 (Table 1). The PCA resulted in a two-component model explaining 76.73% of total variance. PC1 described 54.99%, whereas PC2 contributed to 21.74% of the total variability. The 2D score plot of PC1 and PC2 revealed two distinct groups of propolis samples; two types of propolis were separated along the PC2 axis. The orange type samples were localized on the left upper side of the 2D score plot and contained phenolic compounds such as *p*-coumaric acid (*m/z* 163) and tectochrysin (*m/z* 267) at a higher signal intensity than in other samples studied. The blue and the intermediate type of propolis were mainly localized in the lower half of the 2D score plot. These samples mainly contained flavonoids with *m/z* 271 and *m/z* 313, assigned to be pinobanksin and pinobanksin-3-acetate, respectively. Pinobanksin, pinobanksin-3-acetate, galangin and pinocembrin correlated positively with PC1 (Fig. 5b), while *p*-coumaric acid had the most negative impact on PC1, separating two botanically different types of French propolis. These phenolic compounds were recognized as characteristic markers of the French propolis samples. These results are in agreement with studies on Serbian and German propolis, in which galangin, chrysin and pinocembrin were recognized as markers for the blue and orange propolis types [5,18].

4. Conclusions

The combined use of RP-HPTLC-FLD fingerprints and RP-HPTLC-DART-MS data enabled a successful clustering and classification of 30 French poplar propolis samples according to their phenolic compound profile. Two main botanical different propolis groups (blue and orange propolis types) and one subgroup (intermediate type) were evident by PCA. The PCA of RP-

HPTLC-FLD fingerprints classified the samples according to their fluorescence signal profile after derivatization of the phenolic compounds. Caffeic acid, CAPE, galangin, chrysin and two blue unknown compounds at hR_F 14 and 25 were determined as botanical markers of French propolis. Complementary, PCA and classification based on RP-HPTLC-DART-MS exploited the EIC peak areas of eight selected compounds, *i. e. p*-coumaric acid, chrysin, pinobanksin, pinobanksin-3-acetate, galangin, kaempferol, tectochrysin and pinocembrin. Both classification methods provided similar results for clustering of the propolis samples and supported the reliability of the assignments. In combination, the classification of the 30 French propolis samples worked best. By using the streamlined open source rTLC platform [17], HPTLC image analysis combined and expanded by mass spectrometric analysis permitted a fast multivariate analysis of the 30 French propolis samples. *Inter alia* the presence of two botanically different propolis types of the European poplar was confirmed.

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Author contributions

The paper was written by all authors. TC did the sample preparation. TC and PR performed the HPTLC separations and PCA evaluations. TTH adapted the method for RP separation, did the HPTLC-DART-MS measurements and evaluations. All experiments were supervised by GEM.

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Table 1 The eight mass signals obtained by RP-HPTLC-DART-MS used as variables for PCA.

Variable (*m/z* value)	Impact of the variable on PC	Mean peak area [cps]	Assigned marker compounds	Characteristic for propolis type
163	negative (PC1) positive (PC2)	120907427	*p*-coumaric acid	blue and orange
253	positive (PC1) negative (PC2)	57591314	chrysin	blue and orange
255	positive (PC1)	166372733	pinocembrin	orange
267	positive (PC2)	6672543	tectochrysin	orange
269	positive (PC1)	17911179	galangin	blue and orange
271	positive (PC1)	49284345	pinobanksin	orange
285	positive (PC1) positive (PC2)	29987018	kaempferol	blue
313	positive (PC1)	186526321	pinobanksin-3-acetate	blue and orange

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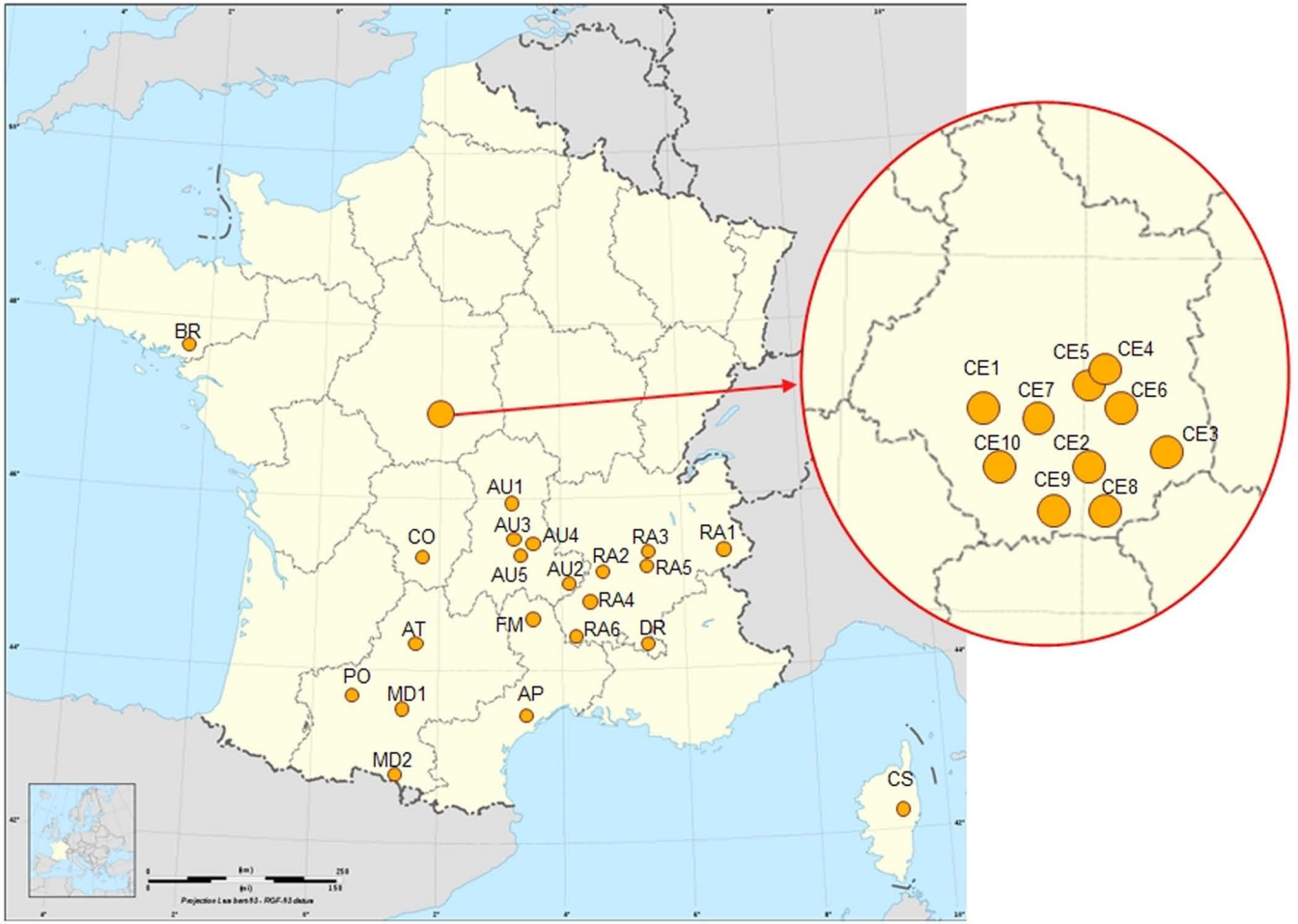
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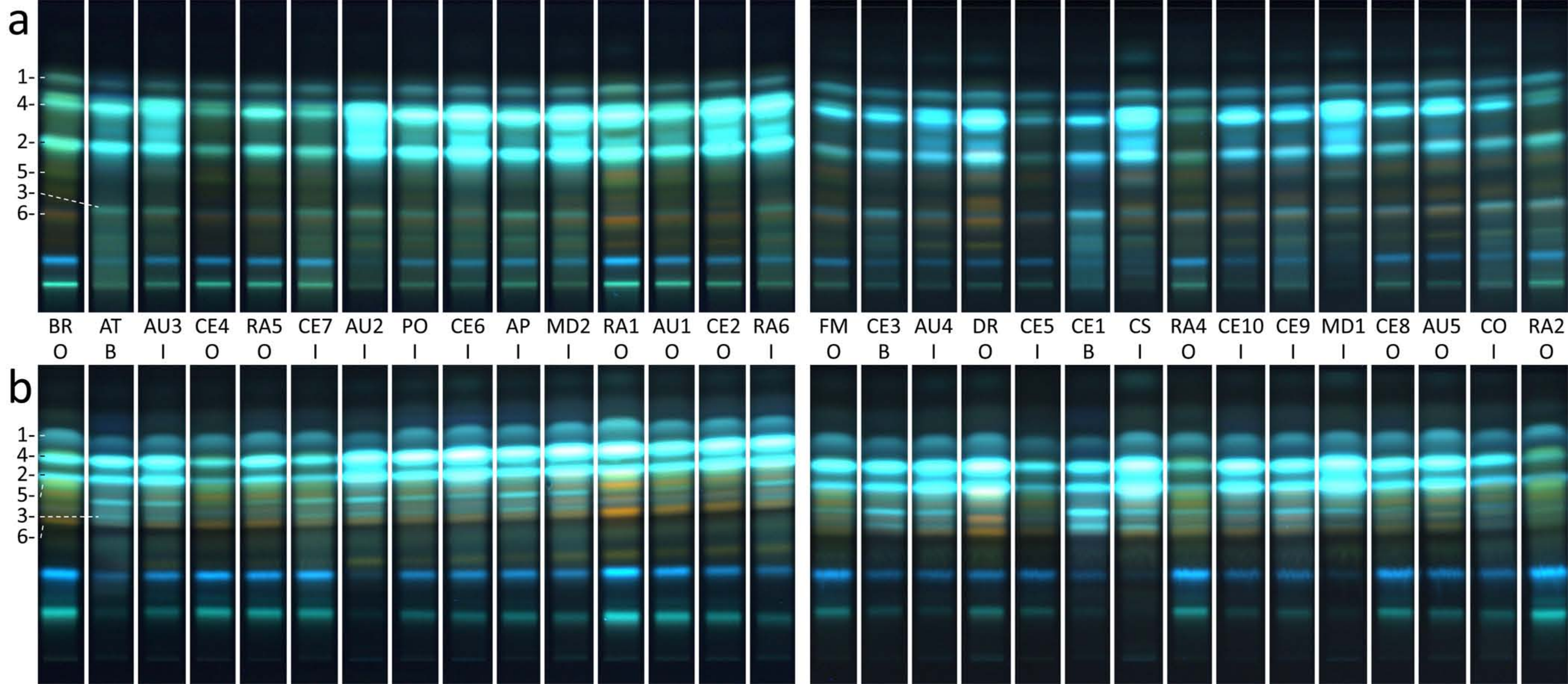
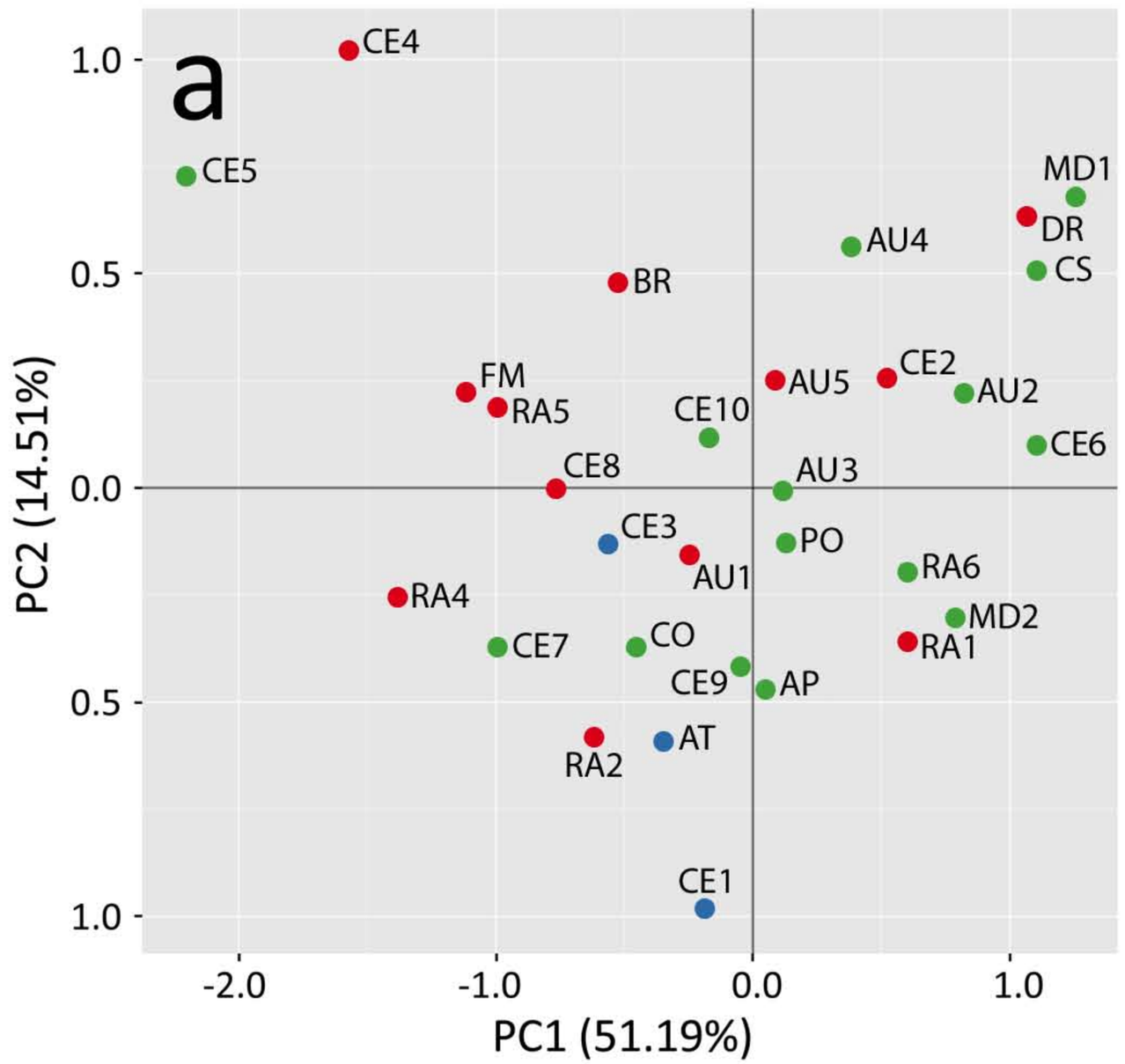
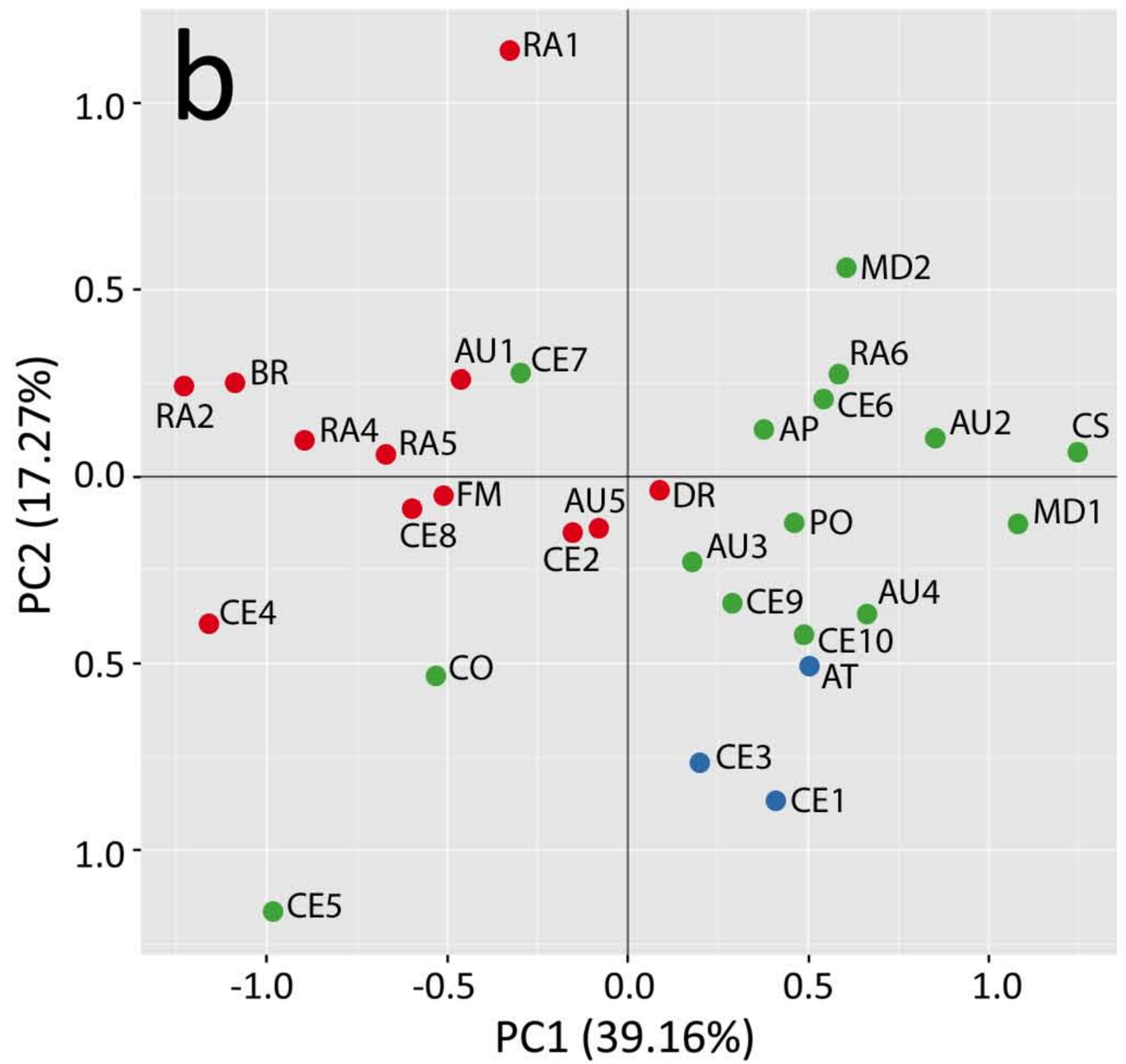
CE9
CE10

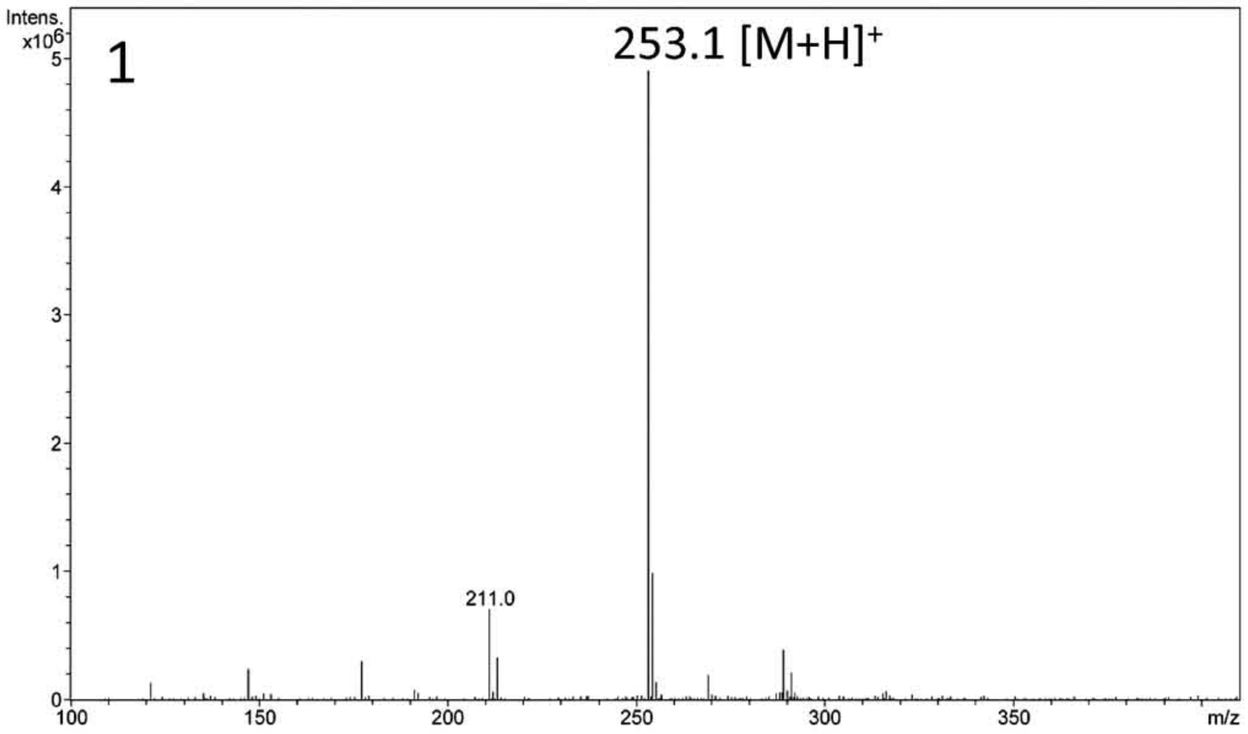

a
1-
4-
2-
5-
3-
6-
BR O
AT B
AU3 I
CE4 O
RA5 O
CE7 I
AU2 I
PO I
CE6 I
AP I
MD2 I
RA1 O
AU1 O
CE2 O
RA6 I
FM O
CE3 B
AU4 I
DR O
CE5 I
CE1 B
CS I
RA4 O
CE10 I
CE9 I
MD1 I
CE8 O
AU5 O
CO I
RA2 O
b
1-
4-
2-
5-
3-
6-

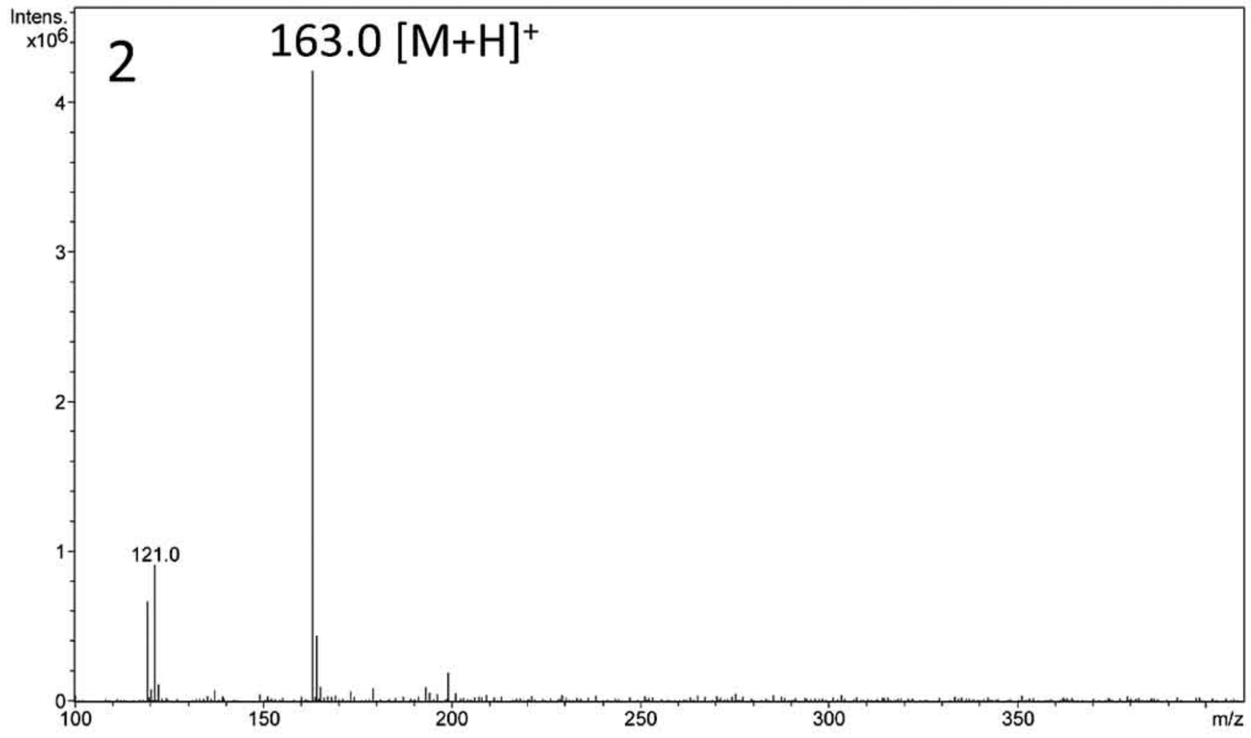

a
CE4
CE5
MD1
DR
CS
AU4
BR
FM
RA5
AU5
CE2
AU2
CE10
CE6
CE8
AU3
CE3
PO
AU1
RA6
RA4
MD2
CE7
CO
RA1
CE9
AP
AT
RA2
CE1
PC2 (14.51%)
PC1 (51.19%)
1.0
0.5
0.0
0.5
1.0
-2.0
-1.0
0.0
1.0

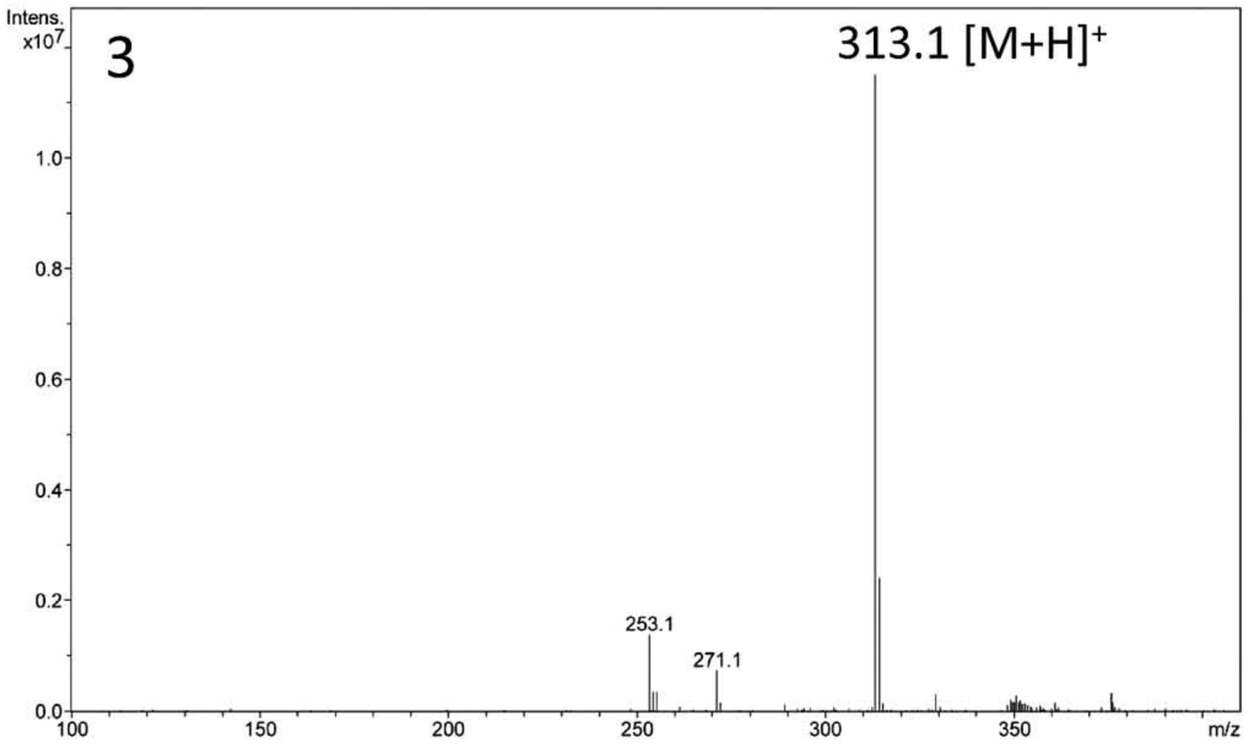

b
RA1
MD2
RA6
AU1
CE7
BR
CE6
RA2
AP
AU2
CS
RA4
RA5
FM
AU5
DR
CE8
PO
MD1
CE2
AU3
CE9
AU4
CE4
CE10
CO
AT
CE3
CE1
CE5
PC2 (17.27%)
PC1 (39.16%)
1.0
0.5
0.0
0.5
1.0
-1.0
-0.5
0.0
0.5
1.0

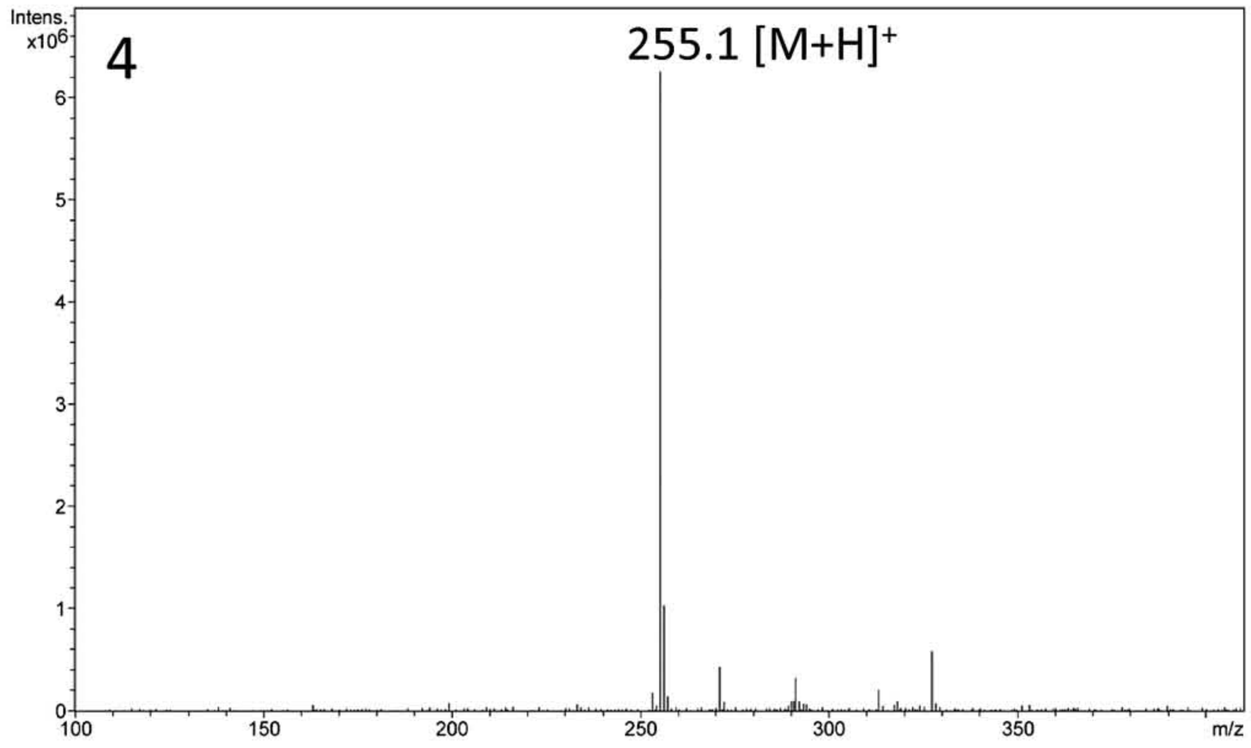
c
2
4
1
PC1 (51.19%)
0.2
0.1
0.0
PC2 (14.51%)
0.1
-0.1
-0.3
*hR_F 24
2
0
11
22
33
44
56
67
78
89
100
hR_F

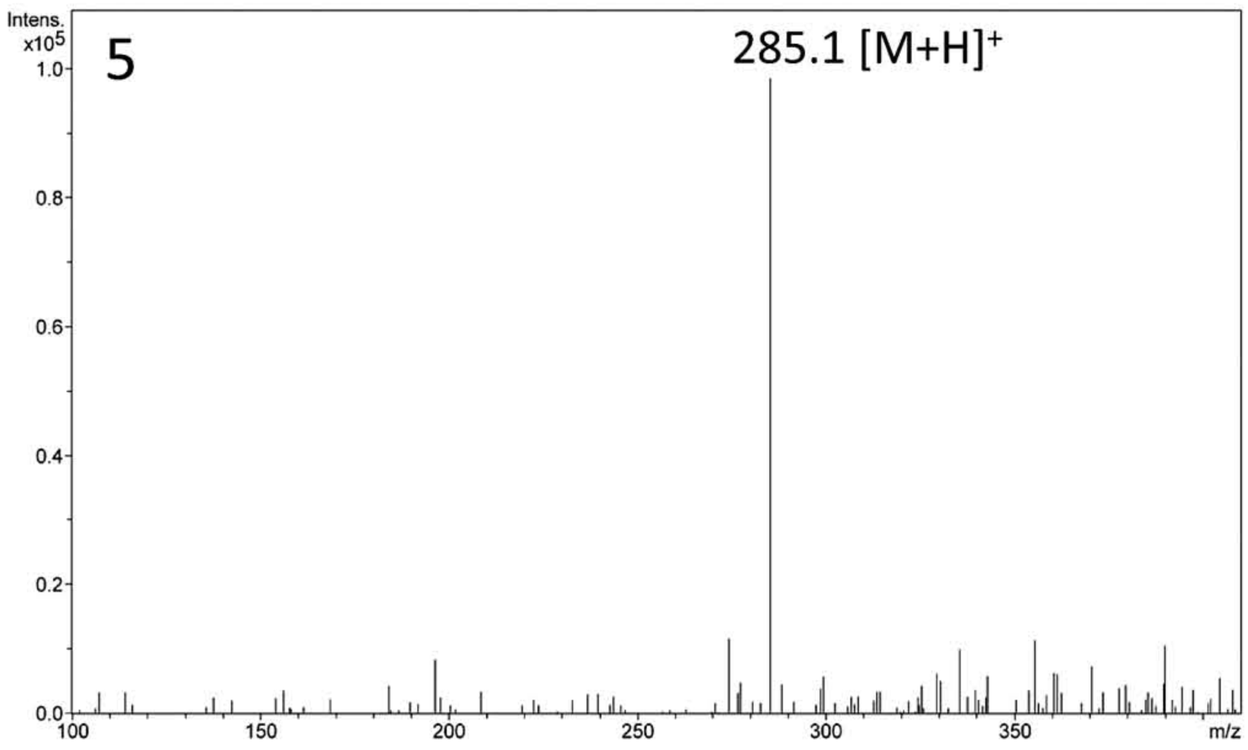
d
2
4
1
PC1 (39.16%)
0.2
0.1
0.0
-0.1
PC2 (17.27%)
0.3
0.1
-0.1
*hR_F 20
*hR_F 33
1
0
11
22
33
44
56
67
78
89
100
hR_F

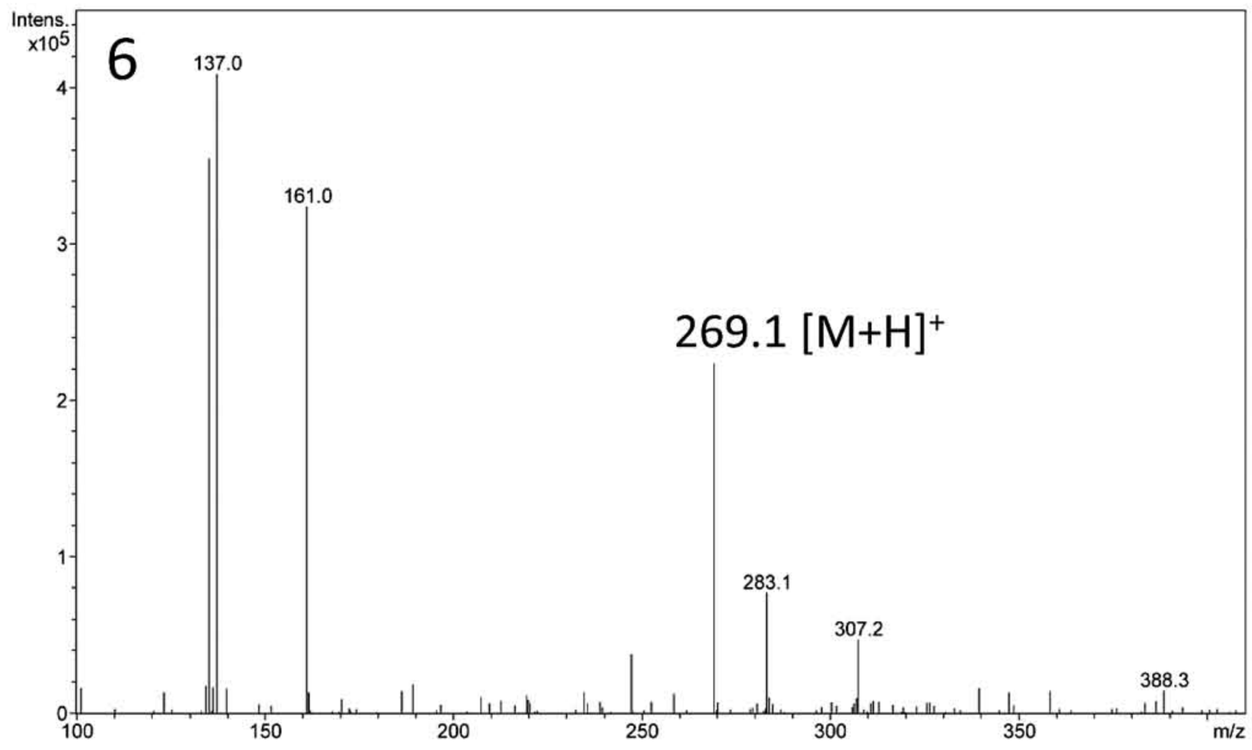

1
253.1 [M+H]+
211.0
Intens.
x10^6
m/z

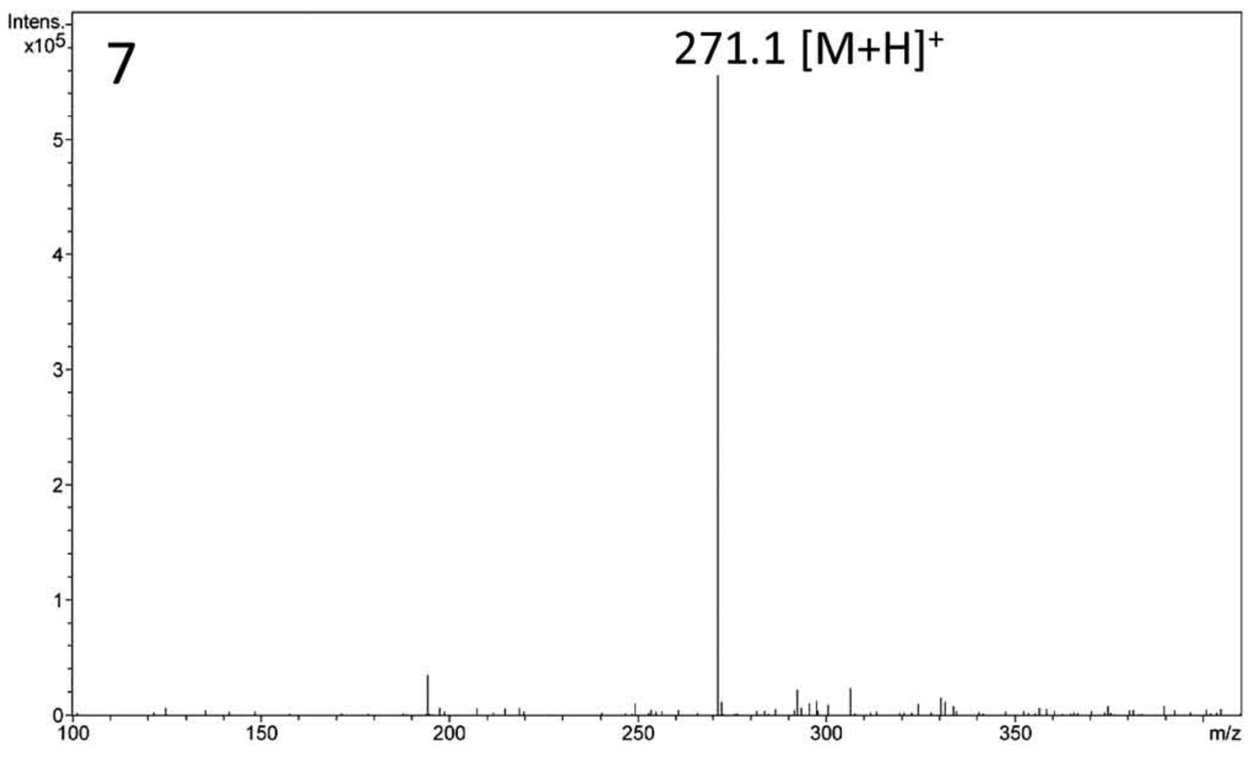

2
163.0 [M+H]+
121.0
Intens.
x10^6
m/z

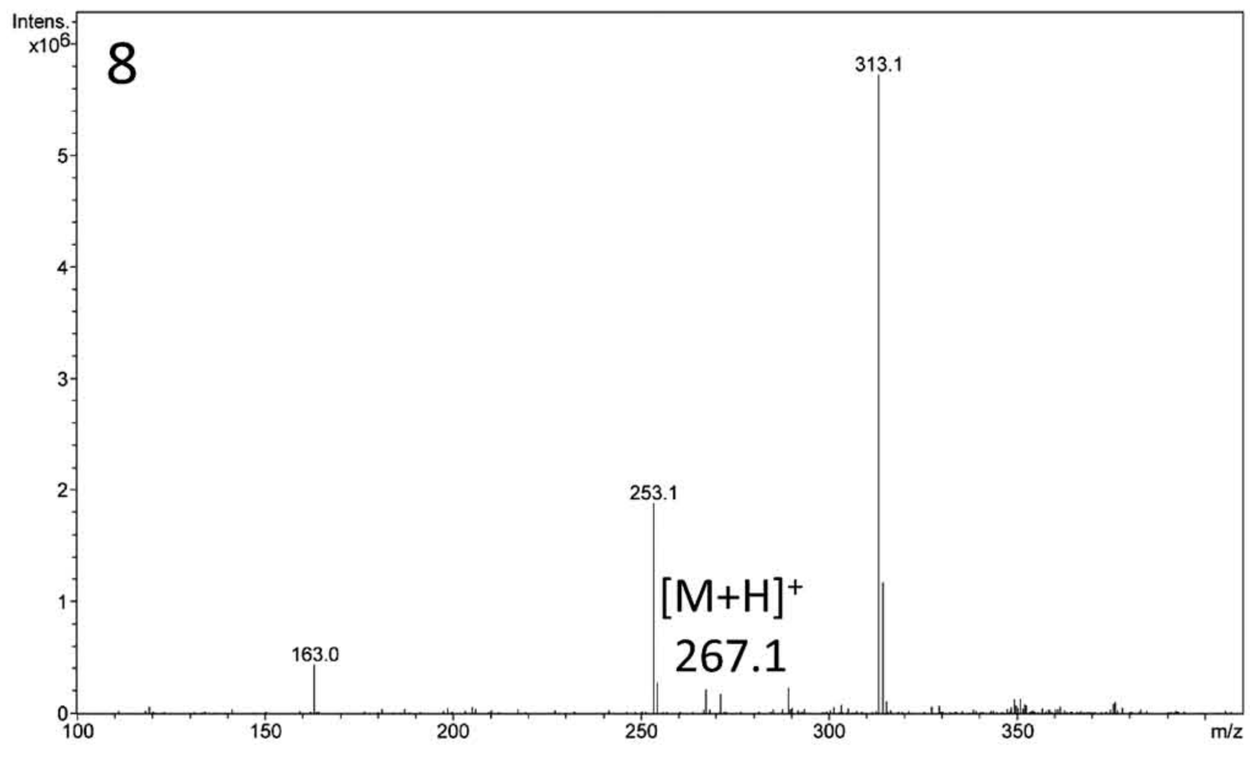

3
313.1 [M+H]+
253.1
271.1
Intens.
x10^7
m/z

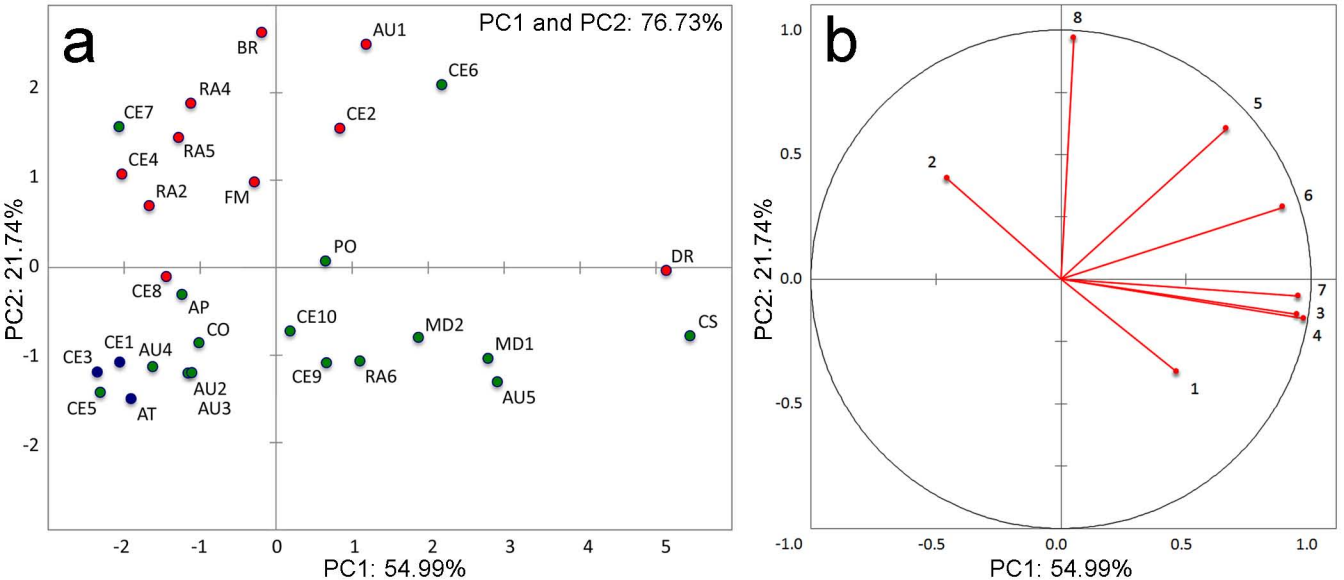

4
255.1 [M+H]+
Intens.
x10^6
m/z

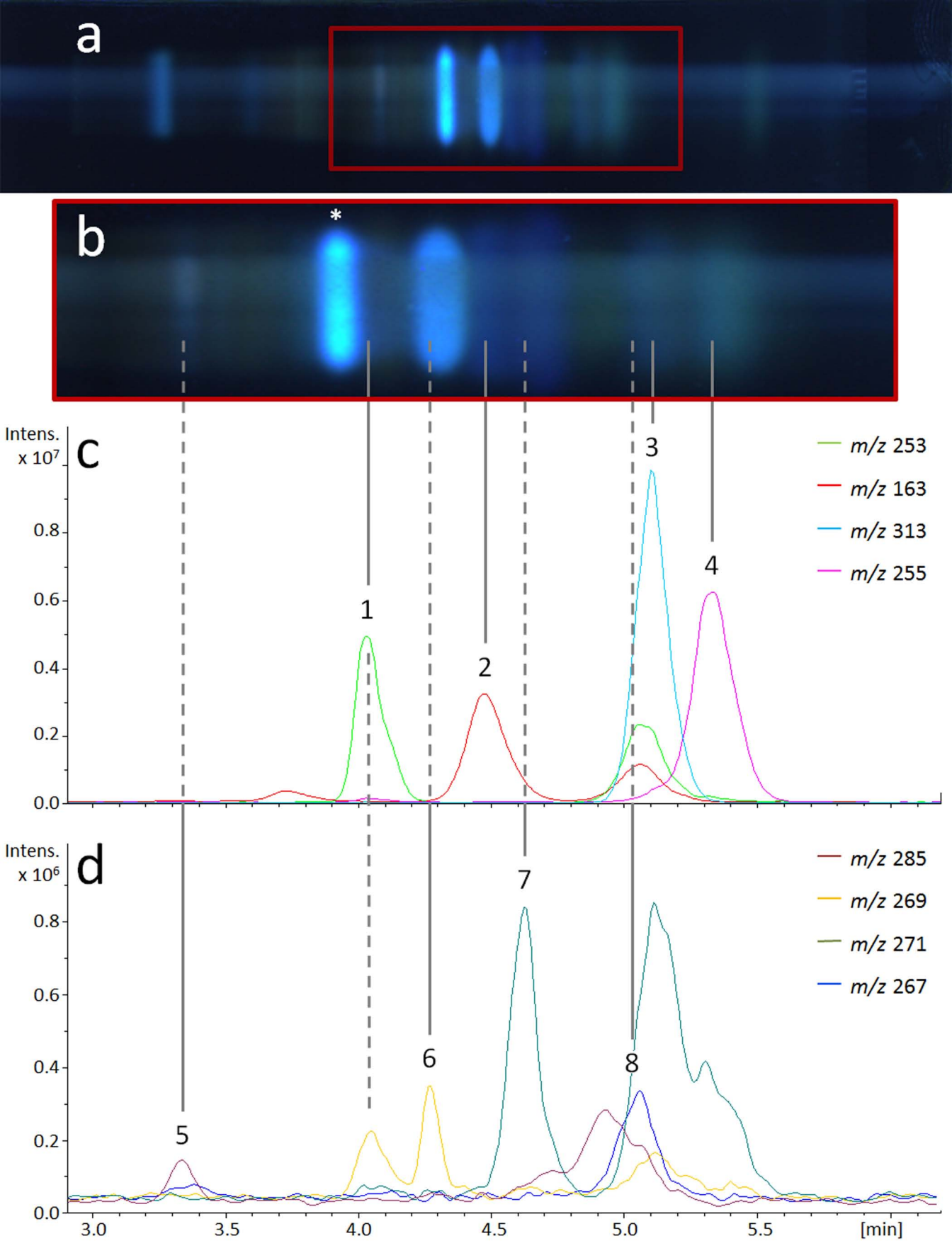

5
285.1 [M+H]+
Intens.
x10^5
m/z


6
137.0
161.0
269.1 [M+H]+
283.1
307.2
388.3
Intens.
x10^5
m/z


7
271.1 [M+H]+
Intens.
x10^5
m/z


8
313.1
253.1
[M+H]+
267.1
163.0
Intens.
x10^6
m/z


a
PC1 and PC2: 76.73%
BR
AU1
CE6
RA4
CE7
CE2
RA5
CE4
RA2
FM
PO
DR
CE8
AP
CE10
MD2
CS
CO
CE1
AU4
MD1
CE3
CE9
RA6
AU2
AU5
CE5
AT
AU3
PC2: 21.74%
PC1: 54.99%
b
8
5
2
6
7
3
4
1
PC2: 21.74%
PC1: 54.99%


a
b
*
c
Intens.
x 10^7
0.8
0.6
0.4
0.2
0.0
1
2
3
4
m/z 253
m/z 163
m/z 313
m/z 255
d
Intens.
x 10^6
0.8
0.6
0.4
0.2
0.0
5
6
7
8
m/z 285
m/z 269
m/z 271
m/z 267
3.0
3.5
4.0
4.5
5.0
5.5
[min]