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1 **Development of a Rapid LC/DAD/FLD Method for the Simultaneous Determination of**
2 **Auxins and Abscisic Acid in Plant Extracts.**

3

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13

14 **ABSTRACT**

15 Plant hormones play a crucial role in controlling plant growth and development. These groups
16 of naturally occurring substances trigger physiological processes at very low concentrations, which
17 mandate sensitive techniques for their quantitation. This paper describes a method to quantify
18 endogenous (\pm)-2-*cis*-4-*trans*-abscisic acid, indole-3-acetic acid, indole-3-propionic acid, and
19 indole-3-butyric acid. The method combines high-performance liquid chromatography (HPLC) with
20 diode array and fluorescence detection in a single run. Hybrid Tea rose ‘Monferrato’ matrices
21 (leaves, petals, roots, seeds, androecium, gynoecium, and pollen) were used as references. Rose
22 samples were separated and suspended in extracting methanol, after which (\pm)-2-*cis*-4-*trans*-
23 abscisic acid and auxins were extracted by solvent extraction. Sample solutions were added first to
24 cation SPE cartridges and the eluates to anion SPE cartridges. The acidic hormones were bound to
25 the last column and eluted with 5% phosphoric acid in methanol. Experimental results showed that
26 this approach can be successfully applied to real samples and that sample preparation and total time
27 for routine analysis can be greatly reduced.

28

29 **KEYWORDS:** abscisic acid; auxins; indole-3-acetic acid; indole-3-butyric acid, indole-3-propionic
30 acid, HPLC; fluorescence detection; diode array detection; SPE; *Rosa*

31

32 INTRODUCTION

33 Plant hormones are of vital importance for normal plant functioning. Their minute quantities
34 trigger basic processes that mediate endogenous developmental programs¹ and integrate
35 extracellular signals to regulate and optimize plant growth and performance. They control the
36 balanced response of plants to adverse environmental conditions or biological threats.

37 To achieve precise regulation of these essential processes, the biosynthetic and catabolic
38 pathways of the different hormonal groups must be highly responsive and adaptable to changing
39 conditions. Comprehensive considerations on hormone biosynthesis, signalling, and control of gene
40 expression have been presented recently.² For example, it is not surprising that abscisic acid
41 signalling can act on a target shared with other response pathways for ethylene, jasmonates,
42 gibberellins, and auxins^{3,4} among others. These intimate relationships among phytohormones can
43 make accurate analysis complicated due mainly to the minute concentrations (less than 50 ng/g) of
44 secondary metabolites. Generally, traditional hormone determination methods have involved
45 multiple steps of intensive purification and large amounts of plant tissue.⁵

46 Various analytical techniques have been developed for endogenous and exogenous
47 phytohormone determination: gas chromatography mass spectrometry (GC-MS),⁶ liquid
48 chromatography-mass spectrometry (LC-MS),^{7,8} capillary electrophoresis with UV (CE-UV)⁹ or
49 fluorescence (CE-FLD) detection,¹⁰ high-performance liquid chromatography with UV (HPLC-
50 UV),¹¹⁻¹³ fluorescence (HPLC-FLD) or chemiluminescence (HPLC-CL)¹⁴ detection, and enzyme-
51 linked immunosorbent assay (ELISA).¹⁵ Although these methods have contributed greatly to
52 phytohormone analysis, some limitations persist in their application. For example, when GC or GC-
53 MS is employed, derivatization of phytohormones to more volatile methyl esters is required. The
54 ELISA method exhibits cross-reactivity with structurally-related compounds in the same sample.
55 The LC-MS method is too expensive for the scale needed for real samples. Several new analytical
56 techniques are emerging and are destined to play prominent future roles. At present the preferred
57 method of plant hormone separation and determination is still reversed-phase high performance

58 liquid chromatography (HPLC), even if complex sample preparation and high costs are limitations
59 for its application.¹⁶⁻¹⁹

60 To this end, the present work aimed to develop an effective, selective, sensitive, and
61 inexpensive analytical method for simultaneous quantitation of (\pm)-2-*cis*-4-*trans*-abscisic acid (**1**),
62 indole-3-acetic acid (**2**), indole-3-propionic acid (**3**), and indole-3-butyric acid (**4**) based on solid
63 phase extraction (SPE) purification and HPLC analysis. Different matrices of Hybrid Tea rose
64 ‘Monferrato’ have been used to validate the methodology.

65

66 MATERIALS AND METHODS

67 **Reagents and Chemicals.** Analytical reagent grade chemicals were used unless otherwise
68 indicated. Water (conductivity less than 0.05 μ S/cm), methanol, and acetonitrile (Merck) were all of
69 HPLC grade. Acids: **1**, **2**, **3**, and **4** (**Figure 1**), cartridges Discovery[®] SPE DSC-MCAX (bed wt.
70 300 mg, volume 6 mL; Supelco[®] Analytical, Bellefonte PA, USA), and Supelclean[™] SPE LC-NH2
71 (bed wt. 300 mg, volume 6 mL; Supelco[®] Analytical, Bellefonte PA, USA) were all purchased from
72 Sigma-Aldrich (Milano, Italy).

73 **Stock and Working Solution Preparation.** Stock standard solutions (1 mg/mL) of **1**, **2**, **3**,
74 and **4** were prepared using methanol as the solvent. All other standard solutions were prepared by
75 dilution of the stock solution to obtain concentrations ranging between 0.0010 and 10 μ g/mL for **1**
76 and between 0.00010 and 10 μ g/mL for **2**, **3**, and **4**.

77 **Samples and Sample Preparation.** Completely opened leaves, unfolded petals, entire
78 roots, mature seeds, androecium and gynoecium in folded flowers, and fresh pollen of the rose
79 hybrid ‘Monferrato’ were collected in September 2012 (mean temperature of 18.5 °C, mean relative
80 humidity of 67.2 %) in the rose garden of the Experimental Centre of the Department of
81 Agricultural, Forest and Food Sciences of the University of Torino (Italy; 45°03’59.73” N,
82 7°35’24.72” E), immersed in liquid nitrogen (N₂), and maintained at -80 °C until analysis.

83 Rose samples were weighed, ground in liquid N₂ and 0.3 g of each homogenized sample was
84 transfer to a 10 mL glass tube, the suspension were extracted with 2 mL of 80% aqueous methanol
85 containing 10-20 mg/L of butylated hydroxytoluene for 16 h at 4 °C in darkness under magnetic
86 stirring.²⁰ The extract was diluted to 6 ml with water, the pH was adjusted to 2.5 with 1 M aqueous
87 HCl, after which the samples were filtered and eluted. The eluates were then added first to SPE
88 DSC-MCAX cartridges that had been previously washed with 2 mL 100% methanol and
89 equilibrated with 2 mL water. Next, the elutes (6 mL) were added to SPE LC-NH₂ cartridges, also
90 previously washed with 2 mL 100% methanol, and equilibrated with 2 mL water. The acidic
91 hormones were bound to the last column and eluted with 1 mL of 5% phosphoric acid in methanol.
92 A total of 20 μL of purified samples were injected into HPLC.

93 **LC/DAD/FLD Conditions.** The chromatographic analysis was performed on an Agilent
94 Model HPLC chromatographic system consisting of an HPLC series 1200 (Agilent Technologies,
95 Böblingen, Germany) comprised of the following modular components: a vacuum degassing unit, a
96 quaternary pump, an auto injector, a column oven, a diode array detector (DAD G1315D), and a
97 fluorescence detector (FLD G1321A). The column used was a 250 mm x 4.6 mm i.d., 5 μm, Zorbax
98 eclipse XDB-C18 (Agilent Technologies, Böblingen, Germany).

99 Throughout this study, the mobile phases was (A) acetonitrile and (B) aqueous phosphoric
100 acid solution of pH 3.2. The column was equilibrated and the column temperature was maintained
101 at 40 °C +/- 0.1 °C. Separation was carried out by gradient elution with a constant flow rate of 0.5
102 mL/min. The gradient program was as follows: 5-70% A (0-75 min). After the gradient separation,
103 the column was re-equilibrated with: 70-100% A (75-86 min) for 5 min and 100-5% A in 20 min.
104 An injection volume of 20 μL was used for each analysis.

105 The standard solution of each acid was prepared in methanol and chromatographed
106 separately to determine the retention time for each (**Figure 2**). An HPLC Agilent series 1200
107 fluorescence detector was placed in series with the diode array detector. The signal for **1** was
108 monitored at 265 nm; the excitation wavelength and emission wavelength of the **2**, **3**, and **4** were

109 measured via fluorimetric detection at 281 nm and 340 nm, respectively. The retention times of the
110 solutes were determined from three different injections. Peak identifications were based on
111 retention times and standard additions to the samples; hormones were quantified according to a
112 calibration curve that was constructed from the measurement data of the matrix matched calibration
113 standards.

114 **Validation.** Method validation was performed following the recommendations of the
115 International Conference on Harmonization for selectivity, linearity, extraction efficacy, precision,
116 and accuracy. The limits of detection (LODs) and quantitation (LOQs), as well as analyte stability
117 in the sample and standard solutions were also evaluated.

118

119 **RESULTS AND DISCUSSION**

120 **Method Development.** To optimize extraction, a representative quantity of each matrix was
121 used to better understand the process and the influence of sample constituents and secondary
122 metabolites. As a study first step, organic solvents of different polarities (methanol, ethanol, and
123 acetonitrile) were assessed for their relative extraction efficiency. At present, solvent extraction is
124 the most widely used method for plant hormone extraction. Many different procedures and solvents
125 (methanol, methanol/water mixture, acetone, acetone/water, propanol, propanol/water, and neutral
126 or acid buffers) have been developed and broadly used²¹⁻²³ for plant hormone extraction. The
127 polarity of the extraction solvent is chosen to closely match that of the target compound; thereby,
128 the ratio of organic solvent to water is defined according to the polarity of hormones. Non-polar
129 solvents such as ether are rarely used to extract plant hormones. Instead, methanol is the preferred
130 solvent as its small size and low molecular weight allow for efficient plant cell penetration during
131 extraction.²⁴⁻²⁸ As methanol produced the best recoveries, consequent investigations were
132 performed using methanol with differing proportions of ultra-pure water (80, 70, and 60%).

133 We found that using 80% methanol as the extraction solvent resulted in the maximum target
134 compound quantities. Butylated hydroxytoluene was added to the solution as an antioxidant.

135 The optimal efficacy of an extraction procedure is defined as the highest yield of analytes in
136 the shortest time. During this extraction procedure investigation, time values ranged from 1 to 24 h
137 (1, 2, 4, 16, and 24 h). We found that the maximum amount of each analyte was extracted from all
138 sample types at 16 h; additional extraction time showed no increase in extraction efficacy.

139 Another complication in the process is that some plant hormones are labile during
140 extraction. Auxins, for example, are readily oxidized or degraded when exposed to light, oxygen,
141 and high temperature. In some studies, to protect against degradation, antioxidants are added during
142 the extraction process.²⁹ The influence of solar light on the efficacy of analyte extraction and the
143 purification procedures was observed in this study. To avoid analyte degradation, all analytical
144 steps of the extraction procedures were performed in the dark at 4 °C with amber glassware and
145 artificial light.

146 SPE column decision choices should take into account several factors: the sample matrix,
147 analyte physicochemical properties, and the nature of the bonded phase. Combinations of two or
148 more different SPE column types are often employed in plant hormone analysis. Generally, anion-
149 exchange columns and cation-exchange columns are used to extract acidic analytes and basic
150 analytes, respectively.³⁰⁻³² The interconversion between the conjugate bases of phytohormones and
151 their acid form is important for anionic SPE-NH₂ detention. We used different acids (acetic acid,
152 formic acid, phosphoric acid, and hydrochloric acid) to lower the pH of the sample to 2.5; the best
153 results were obtained with 1 M HCl in H₂O with the SPE DSC-MCAX previously used for
154 preliminary matrix purification. Various mobile phase compositions were tested to identify the
155 optimal chromatographic condition. Each column provided a different combination of
156 hydrophobicity and analyte interaction. The shortest analysis with good resolution and peak shapes
157 with no tailing was observed using Zorbax eclipse XDB-C18.

158 To obtain good separation and resolution across all analytes, we tested various isocratic
159 solvent systems: methanol/water; methanol/water both to 1 mmol H₃PO₄; acetonitrile/water both to
160 1 mmol H₃PO₄; and acetonitrile/water with 1 mmol H₃PO₄ only in water and brought to pH 3.2 with

161 NaOH 1 molar.³³⁻³⁵ As total analysis time was protracted, gradient elution was employed. The effect
162 of column temperature on analyte separation for the range from 25 to 45 °C was investigated. Good
163 resolution and peak shapes without tailing resulted at 40 °C.

164 HPLC, which can directly analyze polar compounds, is more suitable for most plant
165 hormones when using a UV detector rather than derivatization methods. The main drawback of
166 using a UV detector is its inferior sensitivity.^{36,37} Compared to a UV detector, the sensitivity of a
167 fluorescence detector (FLD) is about 2–3 orders of magnitude higher, making it far more suitable
168 for plant hormone detection.³⁸ Initially, we expected to use UV detection for all analyte
169 determinations, but auxin levels in selected samples were anticipated as too low for quantitation by
170 the method. Therefore, fluorescence detection was adopted for auxin determination given its
171 superior selectivity and sensitivity. Indole derivatives are known to usually contain the key structural
172 characteristics of fluorescent substances: rigid planar structures and a big π -conjugated system.
173 Therefore, **2**, **3** and **4**, all have natural fluorescence properties.

174 Representative chromatograms of the different rose matrices using DAD are shown in
175 **Figure 3**; the same is displayed in **Figure 4** using FLD. The retention times of **1**, **2**, **3**, and **4** were
176 39.30 ± 0.01 , 35.85 ± 0.01 , 42.07 ± 0.01 , and 46.79 ± 0.01 min, respectively.

177 Optimized conditions yielded symmetrical and sharp peaks for all four analytes with peak
178 purities higher than 999.1. The peak purities were calculated on the base of the usual standard
179 addition method. Furthermore, the resulting chromatograms revealed that, in spite of the complex
180 matrix of the samples, almost no other components were co-eluted with the compounds of interest.

181 **Method Validation.**

182 *Extraction efficacy.* The extraction efficacy and procedure reproducibility for **1** ($5 \mu\text{g/mL}$)
183 and **2**, **3**, and **4** ($1 \mu\text{g/mL}$) were determined for each representative sample type by comparing the
184 responses from samples spiked before extraction with those from samples extracted and spiked after
185 extraction. All extraction recoveries were relatively high (90–97%), which can be explained by the
186 simple and effective sample preparation procedure. Also of note, is that despite the wide variability

187 of matrix type and composition, adequate extraction recoveries for all four analytes were achieved
188 in all sample types.

189 *Linearity, LOD, and LOQ.* Linearity of **1** was tested in the range of 0.01 to 50 $\mu\text{g/mL}$, while
190 for **2**, **3**, and **4** linearity was tested in the range from 0.001 to 10 $\mu\text{g/mL}$. At least nine concentration
191 levels were used in all calibration curves. The obtained correlation coefficients were higher than
192 0.999, indicating satisfactory linearity of the developed method. The limits of detection and
193 quantitation were determined by injecting a series of dilute solutions with known concentrations.
194 LOD and LOQ were defined as a signal-to-noise ratio equal to 3:1 and 10:1, respectively. Limits of
195 **1** using UV detection were quite low at 0.003 $\mu\text{g/mL}$ (LOD) and 0.010 $\mu\text{g/mL}$ (LOQ). In the case
196 of **2**, **3**, and **4** using fluorimetric detection, the LOD and LOQ values obtained were even lower at
197 0.0003 $\mu\text{g/mL}$ and 0.0009 $\mu\text{g/mL}$, respectively. This result highlighted the high sensitivity of the
198 presented method.

199 *Precision.* Method precision experiments were performed using spiked assays of each
200 representative sample type (5 $\mu\text{g/mL}$ for **1**; 1 $\mu\text{g/mL}$ for **2**, **3** and **4**). The sample preparation
201 procedure and analysis were repeated six times within the same day to obtain the intra-day
202 precision, while inter-day precision was assessed by three replicate analyses on three consecutive
203 days. The precision measurement values, expressed as relative standard deviations (RSDs), were
204 below 1.5% and 3.5%, for intra- and inter-day precision respectively. The data revealed that the
205 proposed method was reproducible.

206 *Accuracy.* Accuracy was assessed by the determination of recovery using the standard
207 addition method. Samples were prepared by spiking each representative sample type with three
208 different levels of each analyte, and the entire procedure was repeated three times. The recoveries
209 were calculated based on the ratio of added and obtained amounts. The results show the proposed
210 method to have satisfactory accuracy as the recoveries of all analytes ranged between 90 and 97%.

211 *Stability.* As part of method validation, data were also generated to ensure that all analytes
212 were stable at distinct times and temperatures. Stability tests were performed to assess the short-

213 term and long-term storage, and auto sampler stability. Analyte stability in the spiked representative
214 samples and in the standard solutions, were also analyzed. Short-term stability was assessed at room
215 temperature for 6 h, a time period expected to exceed that of a routine sample preparation. Long-
216 term stability was assessed at -20 °C for 10 days, and the auto sampler stability was tested by
217 storing samples at 4 °C for 24 h. The recovered analyte percentages from samples ranged between
218 94.4 and 96.6%, indicating that degradation of all analytes was not significant under the chosen
219 conditions.

220 **Analysis of Real Samples.** Plant matrix complexity complicates any analysis of
221 phytohormones. Accurate quantitation of trace amounts of these compounds requires robust
222 methods. This work presented and described a highly specific protocol for simultaneous
223 determination of **1**, **2**, **3**, and **4** in plant material. The analysis was conducted in different matrices
224 obtained from the Hybrid Tea rose ‘Monferrato’. All samples were collected on the same day and
225 subsequently placed at -80 °C until the day of analysis.

226 Results displayed in **Table 1** show that all studied phytohormones were present in the
227 collected matrices, which demonstrates that **1**, **2**, **3**, and **4** were quantified simultaneously. Having a
228 method to accurately and readily quantify phytohormones in plant components is highly useful. In
229 this study, **1** content in leaves, petals, roots, seeds, androecium, gynoecium, and pollen was
230 measured at 2.322, 0.670, 0.945, 0.160, 17.053, 6.086, and 3.901 $\mu\text{g/g}$, respectively. The resulting
231 values agreed with the ranges found in previous studies. The role of **1** in the regulation of seed
232 dormancy is well known.³⁹ Hormone values differ according to species and seed age. Bo et al.⁴⁰
233 detected 2.410 $\mu\text{g/g}$ **1** in Hybrid Tea rose ‘Crimson Glory’ and Yambe et al.⁴¹ 1.800 $\mu\text{g/g}$ in Hybrid
234 Tea rose ‘Inspiration’, while Ueda⁴² reported 2.700 $\mu\text{g/g}$ of **1** in the dormant seeds of *R. rugosa* and
235 0.400 $\mu\text{g/g}$ of **1** in *R. persica*.

236 Knowledge of **1** concentration in roots and leaves is of particular interest to understand plant
237 response to abiotic stresses. **1** is involved in the regulation of many stress-induced gene-expressions
238 and confers the plant with adaptability toward drought, salinity, cold, and other environmental

239 stresses. A recently conducted study in rose by Arve et al.⁴³ under dark/light conditions and
240 moderate/high humidity found **1** leaf quantitation levels ranging from ca. 1.00 $\mu\text{g/g}$ to ca. 5.00 $\mu\text{g/g}$
241 using UPLC-ES-MS/MS. An easy quantitation of the hormone in flower components would be
242 valuable to elucidate the role of **1** in flower senescence. Both Muller et al.⁴⁴ and Kumar et al.⁴⁵ have
243 observed a direct correlation between **1** and flower senescence; they found that during the stage
244 when petals were completely unfolded, **1** was 0.17 $\mu\text{g/g}$. Minimal knowledge exists on the role of **1**
245 in rose pollination and flower fertility. Bianco et al.⁴⁶ suggested that the androecium might be an **1**
246 source, particularly during pollination and subsequent petal senescence.

247 The effects of auxins in plants are extremely varied. Auxins commonly inhibit root
248 elongation, but high auxin concentrations promote initiation of secondary branches and roots, as
249 well as adventitious root formation on stems. Low levels of auxins in the organ have been correlated
250 with abscission, such that auxins have been used to prevent premature fruit drop. **2** is present in
251 lower and higher plants and is reported to represent between **1** and 100 ng/g of fresh weight in
252 plants, **3** and **4** have not been identified in every plant yet, and **4** occurs in only some higher plants
253 such as *Zea mays*.⁴⁷

254 In the present work we were able to quantify **2**, **3**, and **4** at the same time. The lowest
255 concentration of **2** was found in seeds (0.144 $\mu\text{g/g}$); for **3** and **4**, the lowest levels were found in
256 pollen (0.025 $\mu\text{g/g}$ and 0.012 $\mu\text{g/g}$, respectively). As expected a very high quantity of **2** (7.615
257 $\mu\text{g/g}$) was quantified in roots. Limited knowledge on endogenous auxin quantitation in the genus
258 *Rosa*, for comparison purposes, was found in literature. Tillberg⁴⁸ obtained a concentration of 0.08
259 $\mu\text{g/g}$ of **2** in mature seeds.

260 In conclusion, we have described a rapid, sensitive, and accurate method to determine the
261 amount of **1**, **2**, **3**, and **4** in different plant matrices based on SPE purification and HPLC separation,
262 coupled with diode array and fluorescence detection. The described procedure allows quantitation
263 of plant hormones in their natural states without any derivatization step prior to analysis. The
264 studied compounds are representatives of two different groups of acidic plant hormones ((\pm)-2-*cis*-

265 4-*trans*-abscisic acid and auxins) with several important biological properties. This method opens
266 the possibility to incorporate other plant hormones and related metabolites into a single analysis.
267 The simultaneous determination of phytohormones could support scientific community efforts in
268 precision agriculture, plant functional genomics, and hormone signal transduction.

269

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273 di rosa al fine di ottimizzare il lavoro di ibridazione e la costituzione varietale (FERTROS)”.

274

275 **ABBREVIATIONS USED**

276 **1**, (\pm)-2-*cis*-4-*trans*-abscisic acid; CE-FLD, capillary fluorescence detection; CE-UV,
277 capillary electrophoresis; DAD, diode array detection; ELISA, enzyme-linked immuno sorbent
278 assay; HPLC-CL, high-performance liquid chromatography with chemiluminescence, detection;
279 HPLC-FLD, high-performance liquid chromatography with fluorescence detection; **2**, indole-3-
280 acetic acid; **3**, indole-3-propionic acid; **4**, indole-3-butyric acid; LC-MS, liquid chromatography-
281 mass spectrometry; SPE, solid phase extraction.

282

283 **ASSOCIATED CONTENT**

284 **Supporting information**

285 **Table S1.** Extraction efficacy of the proposed sample preparation procedure.

286 **Table S2.** Method calibration data.

287 **Table S3.** Intra- (n = 6) and inter-assay (n = 18) precision of the proposed method.

288 **Table S4.** Accuracy (n = 3) of the proposed method.

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Figure legends

Figure 1. Chemical structures of (\pm)-2-*cis*-4-*trans*-abscisic acid (**1**), indole-3-acetic acid (**2**), indole-3-proponic acid (**3**), and indole-3-butyric acid (**4**).

Figure 2. Representative chromatograms (LC/DAD in A and LC/FLD in B) of standards of (\pm)-2-*cis*-4-*trans*-abscisic acid (**1**), indole-3-acetic acid (**2**), indole-3-proponic acid (**3**), and indole-3-butyric acid (**4**), 1.00 $\mu\text{g/g}$ each.

Figure 3. Representative LC/DAD chromatograms of the rose hybrid 'Monferrato' matrices (A leaves; B petals; C roots; D seeds; E androecium; F gynoecium; G pollen) analyzed with the developed method.

Figure 4. Representative LC/FLD chromatograms of the rose hybrid 'Monferrato' matrices (A leaves; B petals; C roots; D seeds; E androecium; F gynoecium; G pollen) analyzed with the developed method.

Table 1. Phytohormone Concentrations in the Different Investigated Matrices of the Rose Hybrid ‘Monferrato’.

Sample type	Sample (n.)	Phytohormones ($\mu\text{g/g}$)			
		1	2	3	4
Leaves	4	2.322 ± 0.44	0.847 ± 0.000	0.114 ± 0.000	0.059 ± 0.000
Petals	8	0.670 ± 0.23	1.081 ± 0.008	0.054 ± 0.000	0.023 ± 0.000
Roots	4	0.945 ± 0.51	7.615 ± 0.048	0.110 ± 0.001	0.027 ± 0.000
Seeds	4	0.160 ± 0.03	0.144 ± 0.000	0.035 ± 0.000	0.074 ± 0.000
Androecium	4	17.053 ± 0.77	7.091 ± 0.037	0.130 ± 0.000	0.032 ± 0.000
Gynoecium	4	6.086 ± 0.14	6.437 ± 0.001	0.078 ± 0.000	0.050 ± 0.000
Pollen	4	3.901 ± 0.03	0.583 ± 0.000	0.025 ± 0.000	0.012 ± 0.000

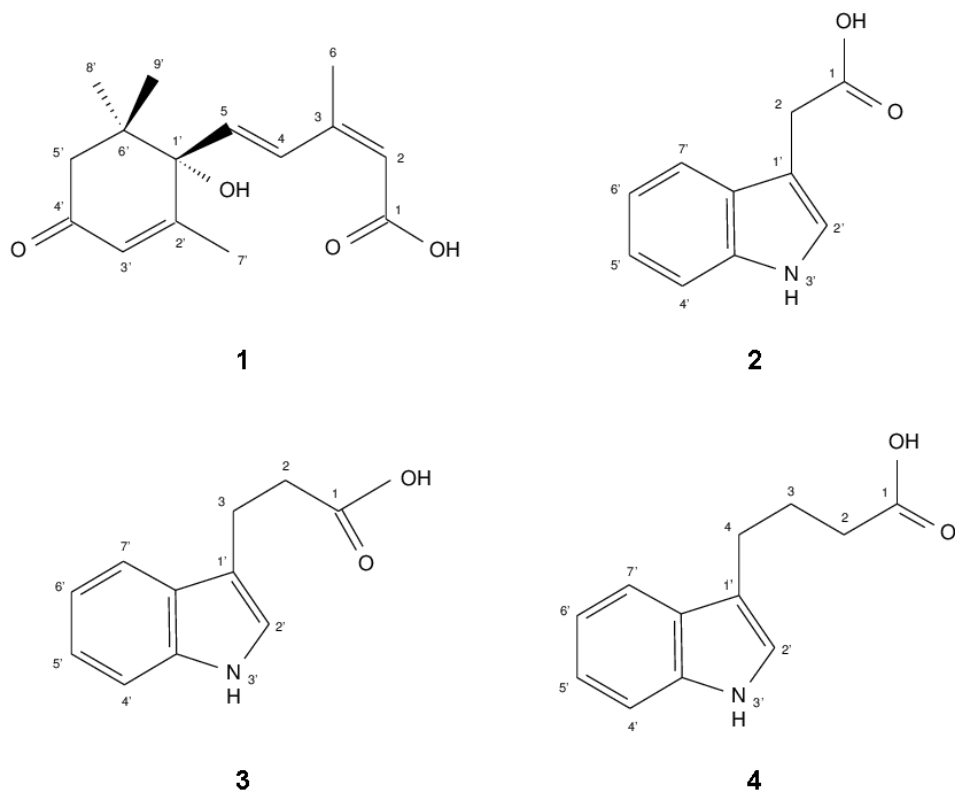


Figure 1

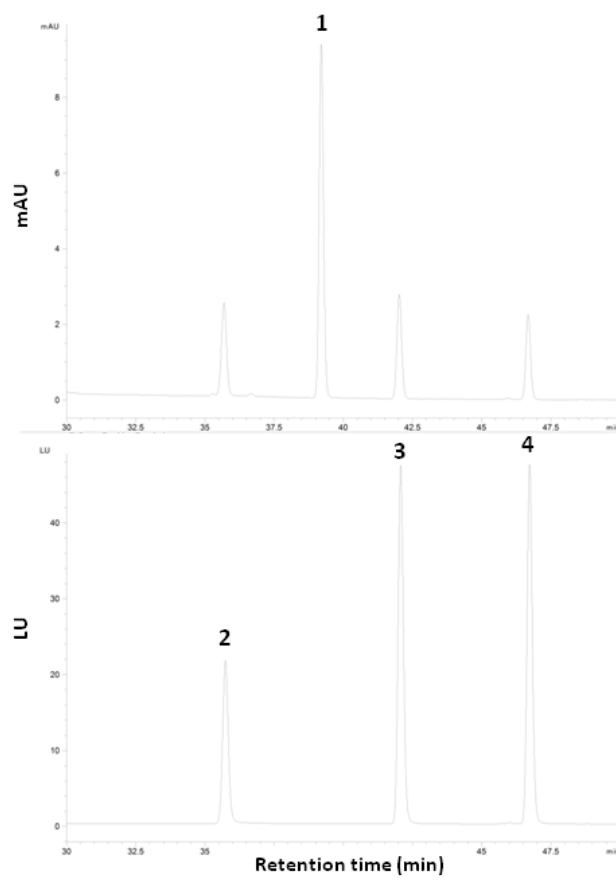


Figure 2

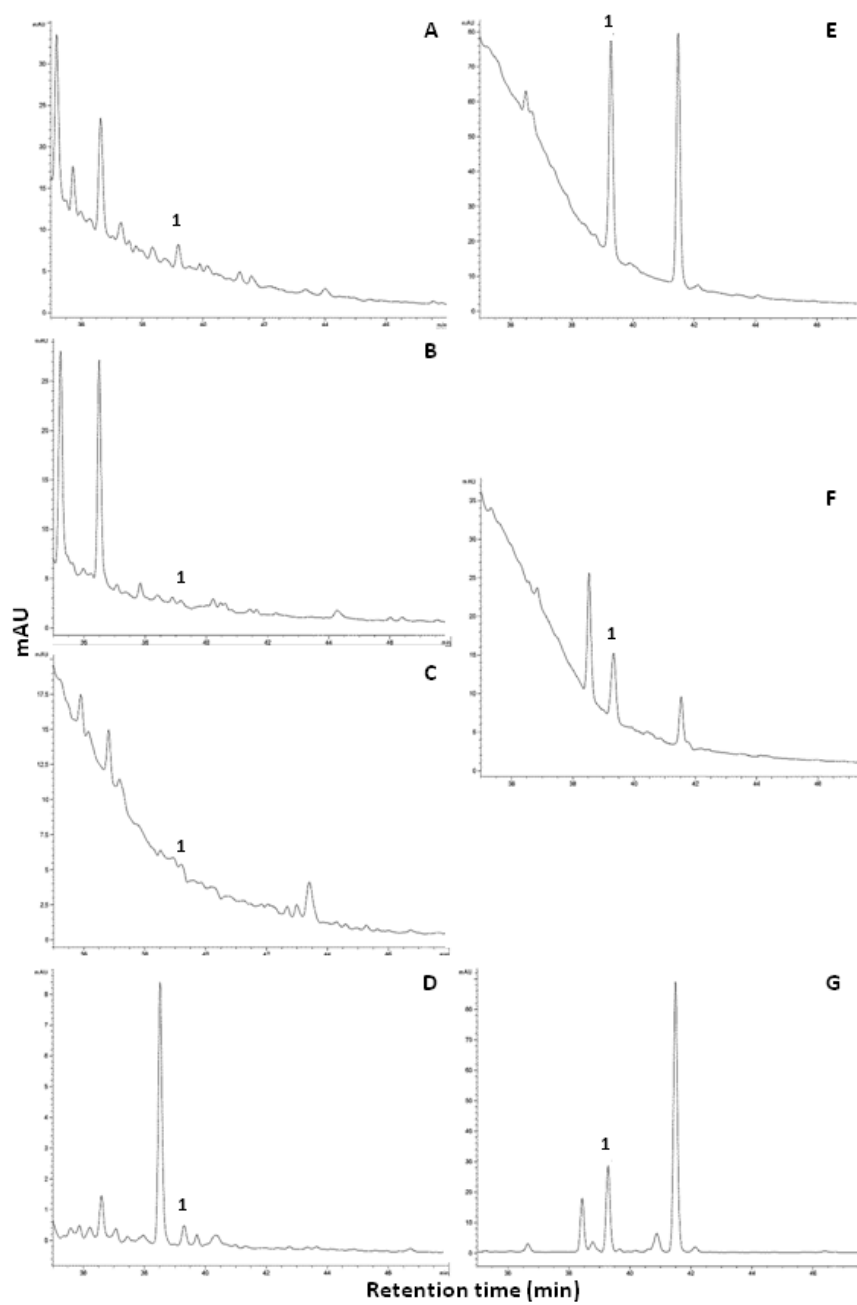


Figure 3

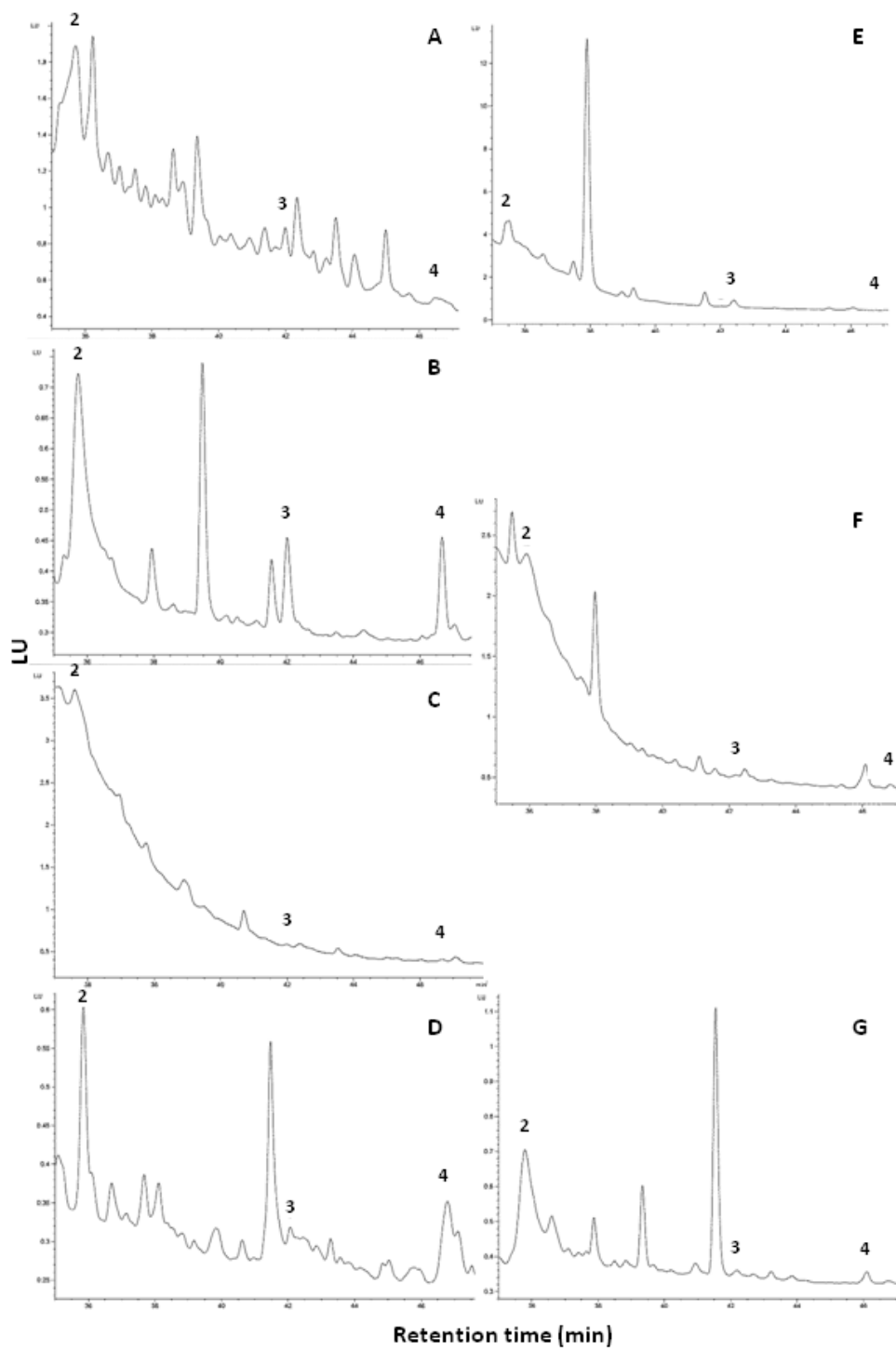


Figure 4