

1 **Supporting Material**

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3 **NMR-based metabolomic for frauds detection and quality control of oregano samples**

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| Position | Number of integrated protons | ¹ H, δ, m, J (Hz) | ¹³ C HSQC | HMBC correlations | COSY correlations |
|-----------------|------------------------------|--|----------------------|---|----------------------|
| 1A | C | - | 124.70 | - | - |
| 2A | C | - | 124.77 | - | - |
| 3A | C | - | 142.11 | - | - |
| 4A | C | - | 146.66 | - | - |
| 5A | CH | 6.81, d, <i>J</i> = 8.54 | 117.05 | 4A; 6A | 6A |
| 6A | CH | 6.94, d, <i>J</i> = 8.54 | 122.43 | 1A; 3A; 5A; 7A | 5A |
| 7A | CH | 6.91, d, <i>J</i> = 16.27 | 142.59 | 1A; 6A; 8A; 9A | 8A |
| 8A | CH | 5.79, d, <i>J</i> = 16.27 | 115.52 | 7A | 7A |
| 9A | C | - | 168.05 | - | - |
| 1B | C | - | 129.90 | - | - |
| 2B | CH | 6.17, d, <i>J</i> = 2.33 | 116.74 | 2B; 3B; 7B _α ; 7B _β | 6B |
| 3B | C | - | 142.10 | - | - |
| 4B | C | - | 143.51 | - | - |
| 5B | CH | 6.35, d, <i>J</i> = 8.54 | 115.94 | 1B; 4B | 6B |
| 6B | CH | 6.00, d <i>J</i> 1 = 8.54; <i>J</i> 2 = 2.33 | 120.27 | 2B; 3B | 5B; 2B |
| 7B _α | CH | 2.92, dd, <i>J</i> 1 = 14.70; <i>J</i> 2 = 12.11 | 36.41 | 1B; 6B | 7B _β |
| 7B _β | CH | 2.49, dd, <i>J</i> 1 = 14.70; <i>J</i> 2 = 3.94 | 36.41 | 1B; 2B; 6B; 8B | 7B _α ; 8B |
| 8B | CH | 4.83, dd, <i>J</i> 1 = 12.11; <i>J</i> 2 = 3.94 | 77.99 | - | 8B _β |
| 1C | C | - | 123.16 | - | - |
| 2C | CH | 6.84, d, <i>J</i> = 2.33 | 113.03 | 4C; 6C; 7C | - |
| 3C | C | - | 146.92 | - | - |
| 4C | C | - | 143.80 | - | - |
| 5C | CH | 6.82, d, <i>J</i> = 8.54 | 116.93 | 1C; 3C | 6C |
| 6C | CH | 6.74, dd, <i>J</i> 1 = 8.54; <i>J</i> 2 = 2.33 | 117.65 | 2C; 4C; 7C | 5C |
| 7C | CH | 5.90, d, <i>J</i> = 5.82 | 56.74 | 3C; 8C; 9C | 8C |
| 8C | CH | 4.33, d, <i>J</i> = 5.82 | 86.90 | 2A; 9C | 7C |
| 9C | C | - | 172.47 | - | - |
| 1D | C | - | 130.47 | - | - |
| 2D | CH | 6.85, d, <i>J</i> = 2.33 | 116.77 | 4D; 6D | - |
| 3D | C | - | 143.94 | - | - |
| 4D | C | - | 142.93 | - | - |
| 5D | CH | 6.86, d <i>J</i> = 8.54 | 166.57 | 1D; 3D | 6D |
| 6D | CH | 6.70, dd, <i>J</i> 1 = 8.54; <i>J</i> 2 = 2.33 | 122.21 | 2D; 4D | 5D |
| 7D _α | CH | 3.04, dd, <i>J</i> 1 = 14.44; <i>J</i> 2 = 3.54 | 36.75 | 1D | 7D _β |

| | | | | | |
|------------|----|---|-------|----------------|------------------|
| 7D β | CH | 2.85, dd, $J_1 = 14.44$; $J_2 = 10.10$ | 36.75 | 1D; 2D; 6D; 8D | 7D α ; 8D |
| 8D | CH | 4.93, dd, $J_1 = 10.10$; $J_2 = 3.54$ | 76.64 | 1D | 7D β |

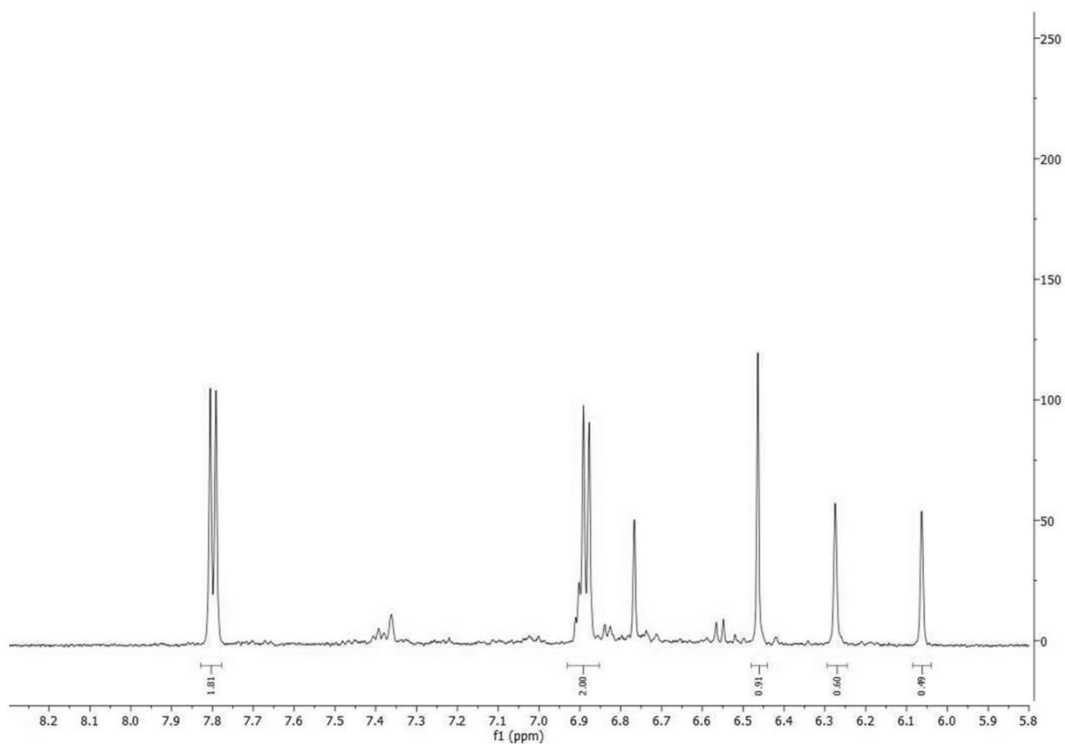
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22 **Table S1.** Summary of NMR experiments performed in order to elucidate the structure of salvianolic
 23 acid B. Sample was solubilized in CD₃OD. Table reports: number of integrated protons, chemical shift
 24 (δ), splitting pattern, coupling constants (in Hz), HSQC, HMBC and COSY correlations.

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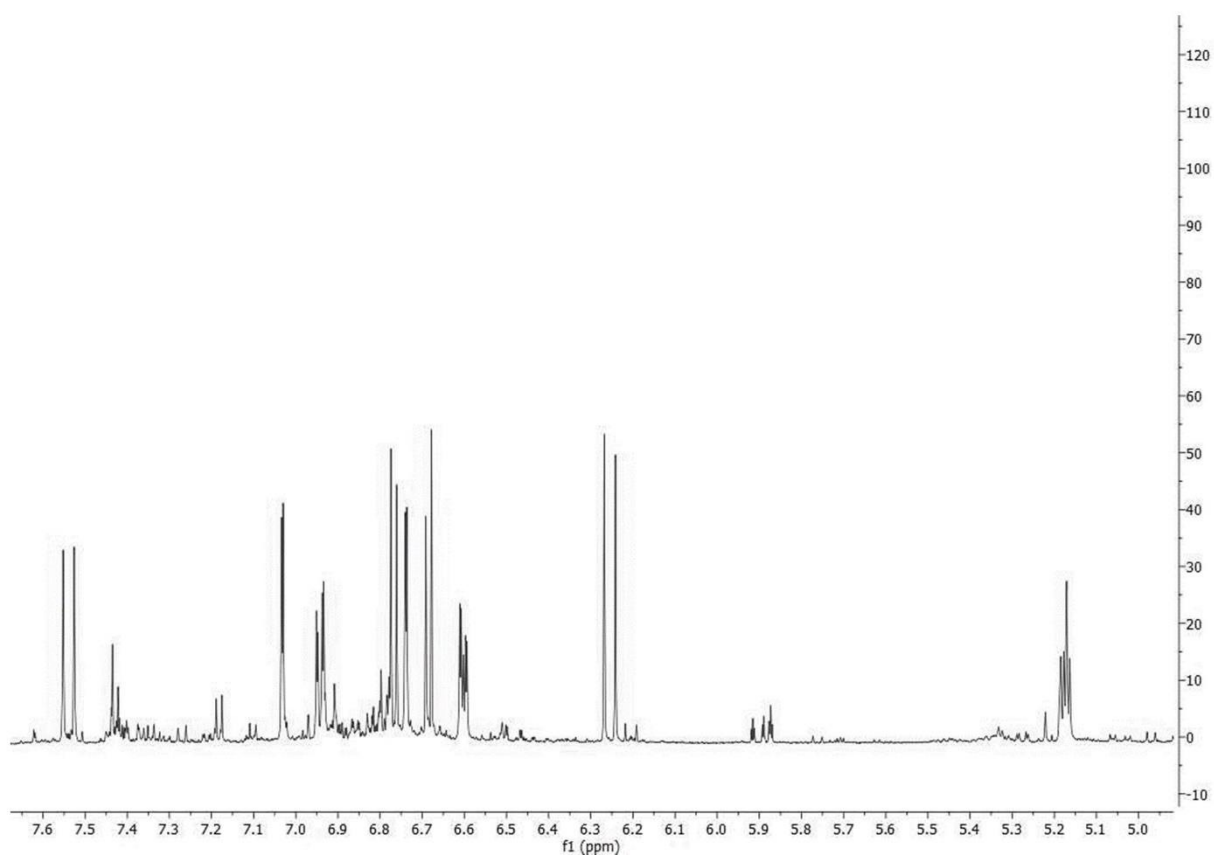
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29 **Fig. S1** ¹H NMR spectrum obtained for apigenin (in fraction 114) solubilized in CD₃OD.

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32 **Fig. S2.** ¹H NMR spectrum obtained for rosmarinic acid (found in EtOAc fraction from *Oregano*
33 *vulgare*) solubilized in CD₃OD.

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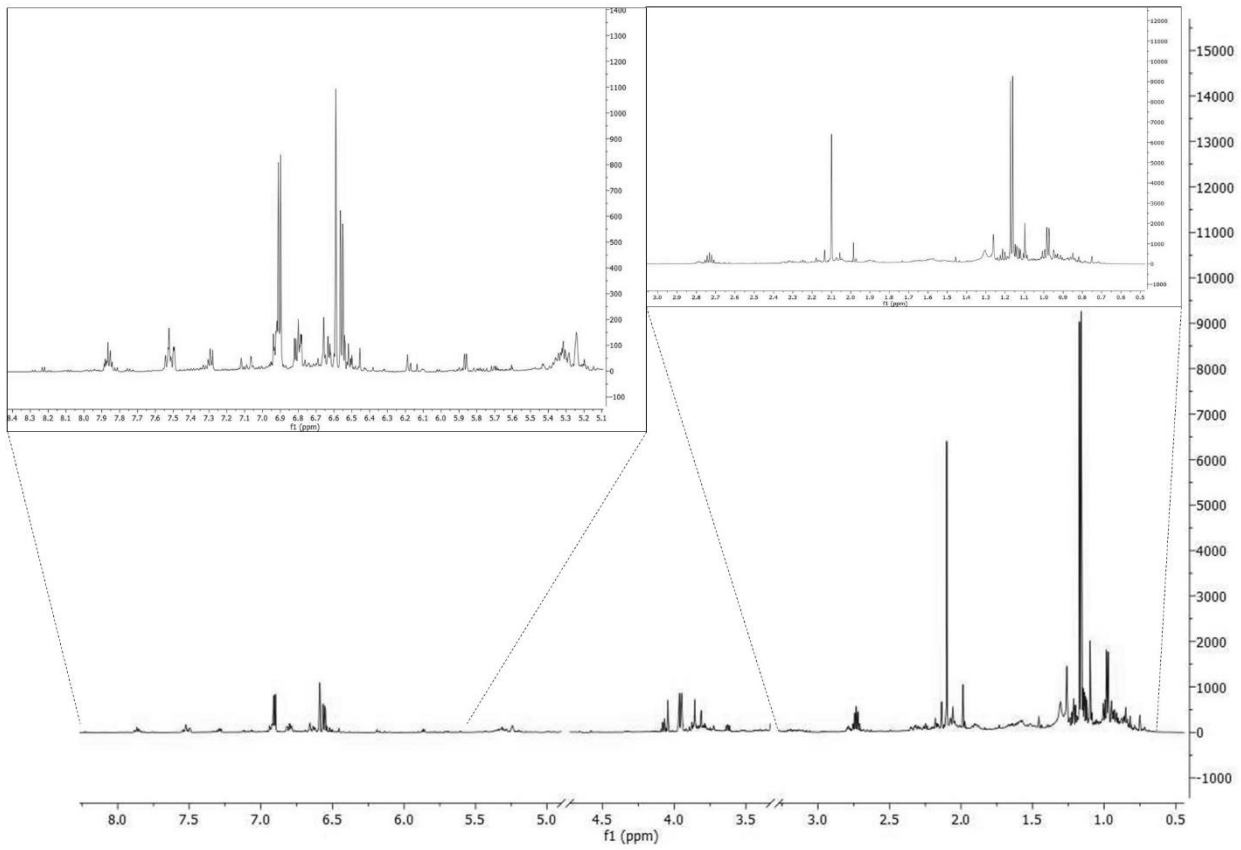
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46 **Fig. S3** ¹H NMR spectrum obtained for thymol, and p-cymene (found in CHCl₃ fraction from *Oregano*
47 *onites*) solubilized in CD₃OD.

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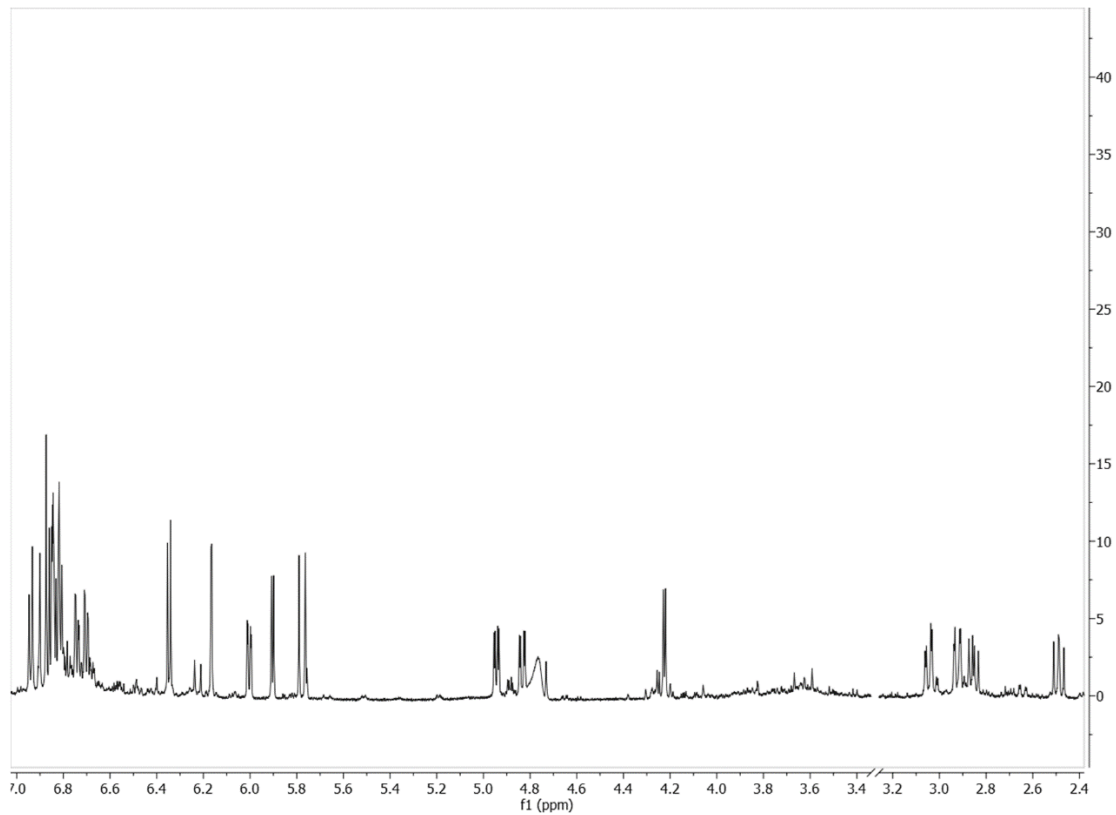
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61 **Fig. S4** ¹H NMR spectrum of salvianolic acid B solubilized in CD₃OD.

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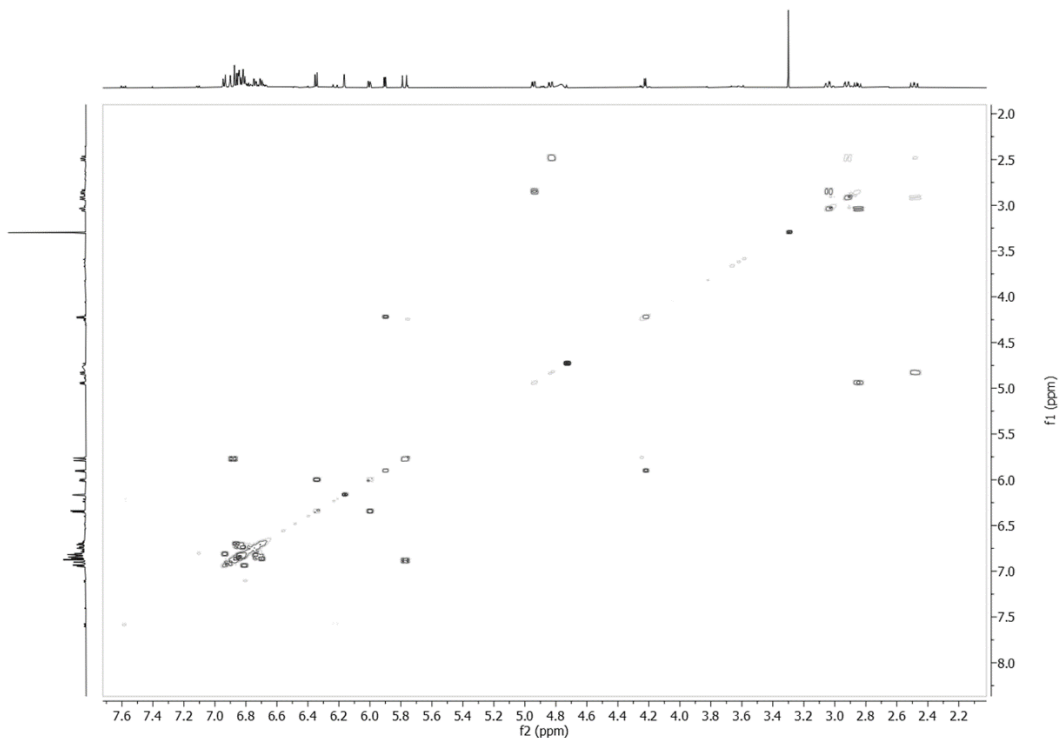
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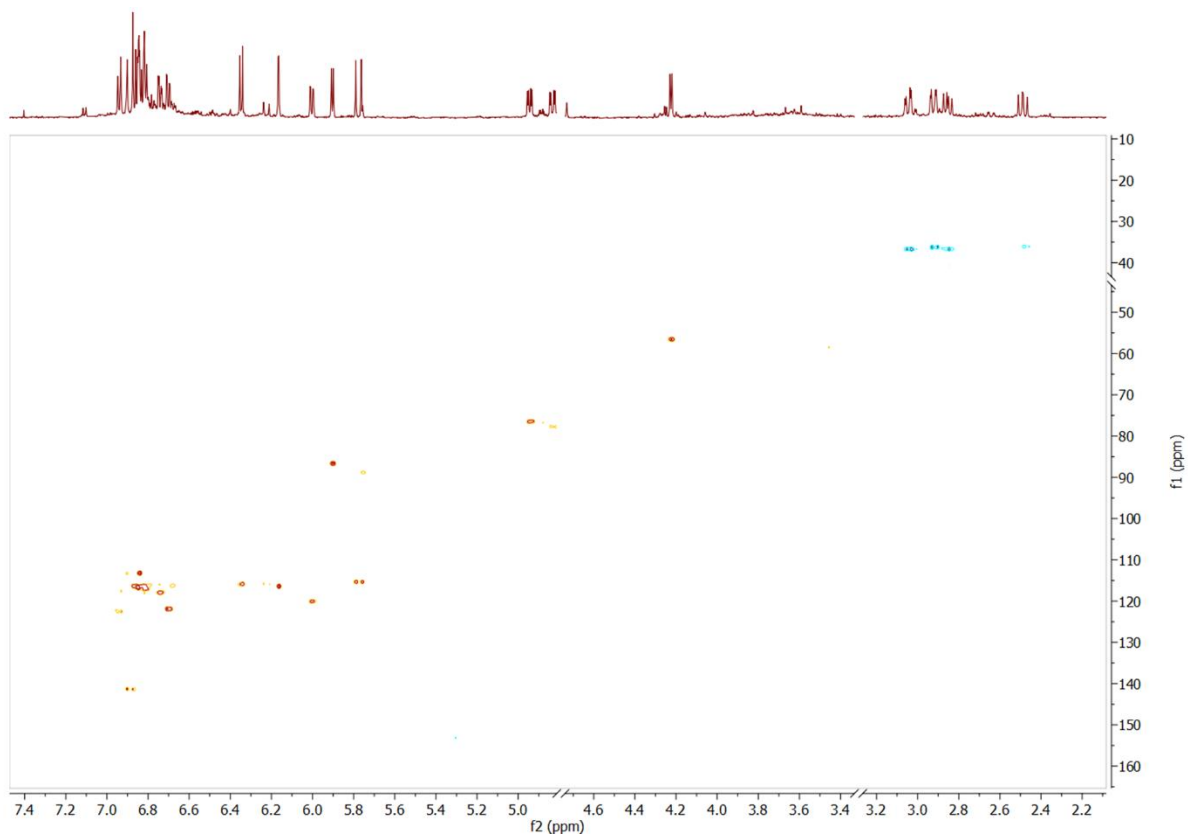


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69 **Fig. S5** NMR COSY spectrum of salvanolic acid B solubilized in CD₃OD.

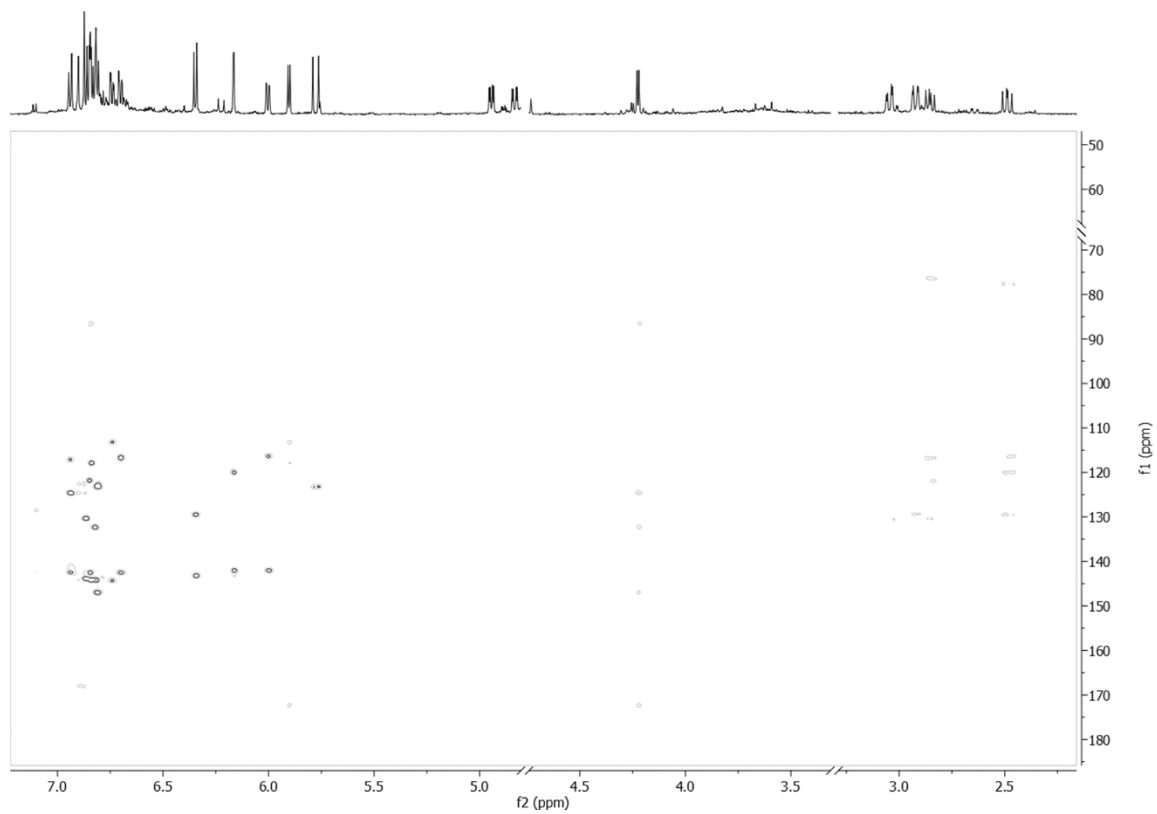
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73 **Fig. S6** NMR HSQC spectrum of salvianolic acid B solubilized in CD₃OD.

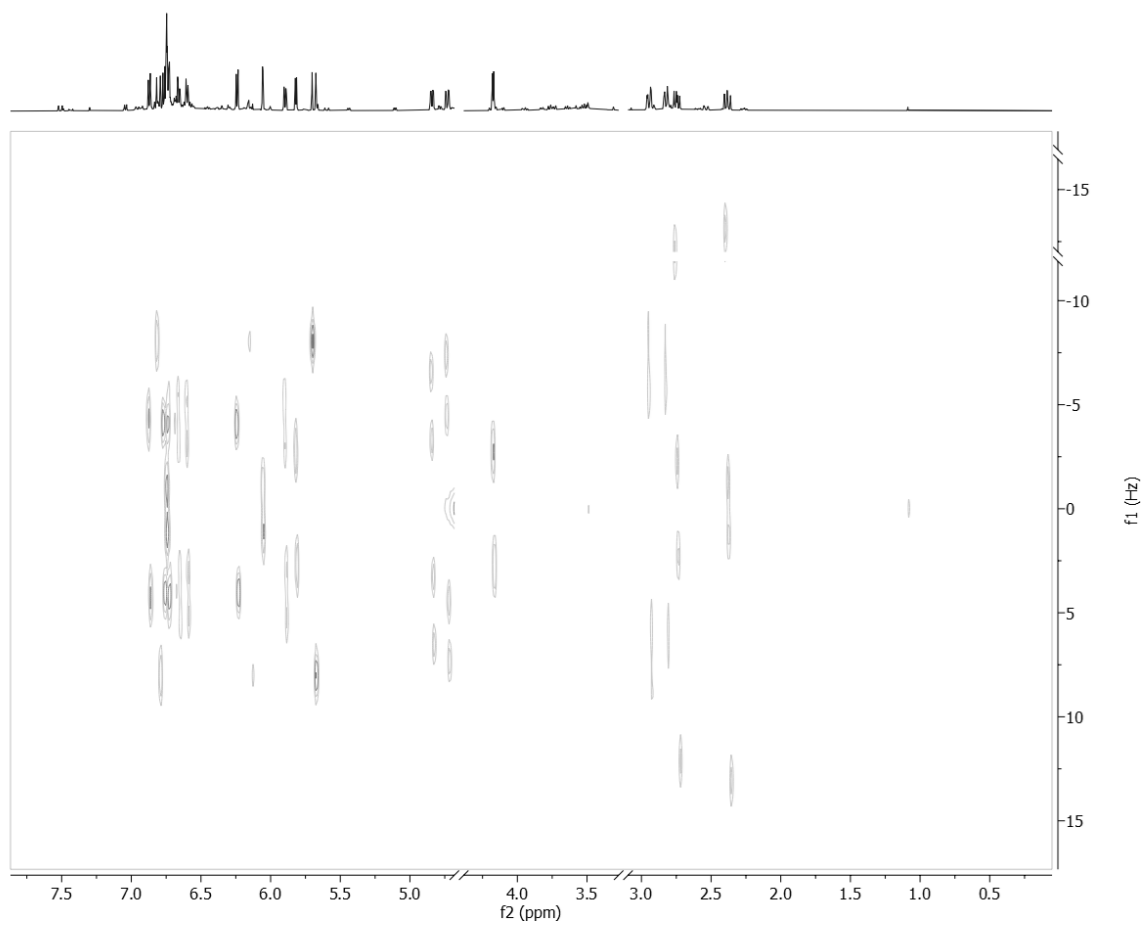
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79 **Fig. S7** NMR HMBC spectrum of salvianolic acid B solubilized in CD₃OD.

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82 **Fig. S8** NMR *J-res* spectrum of salvianolic acid B solubilized in CD₃OD.

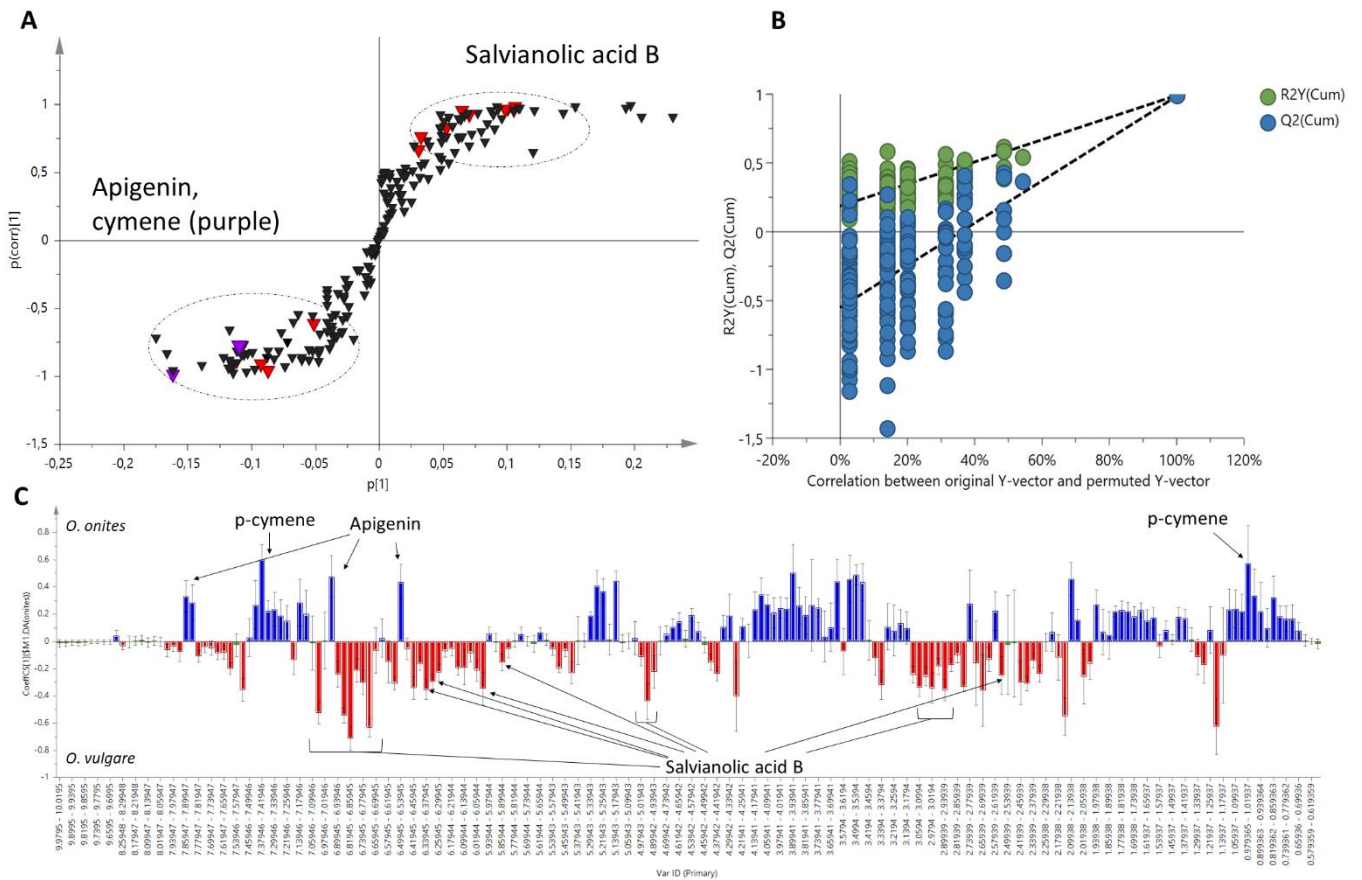
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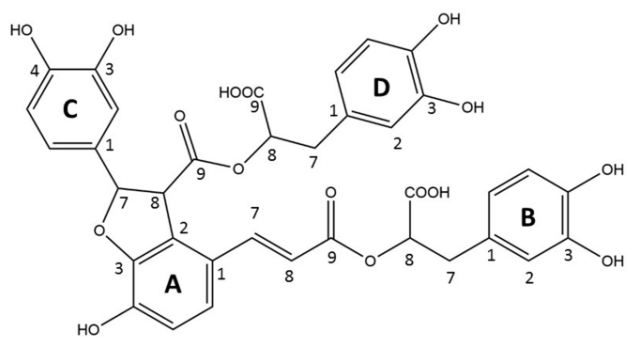


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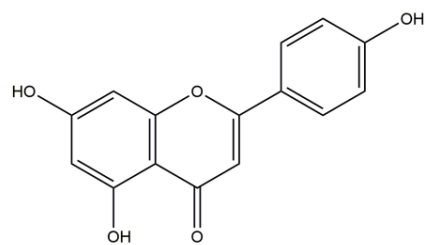
89 **Fig. S9 A)** S-plot from OPLS-DA model showing the most important spectral bins for the discrimination
 90 between the two commercial species of oregano. **B)** Result of the permutation test of the OPLS-DA
 91 model. **C)** Loading column plot from OPLS-DA model.

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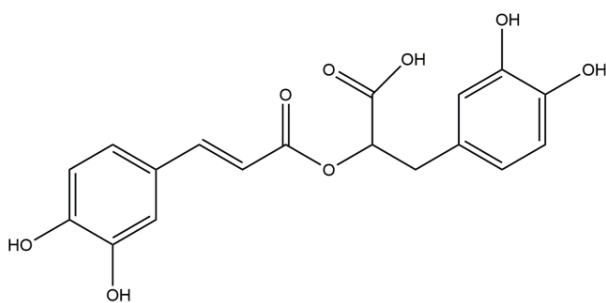
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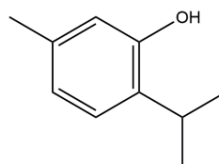
salvianolic acid B



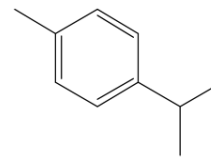
apigenin



rosmarinic acid



thymol



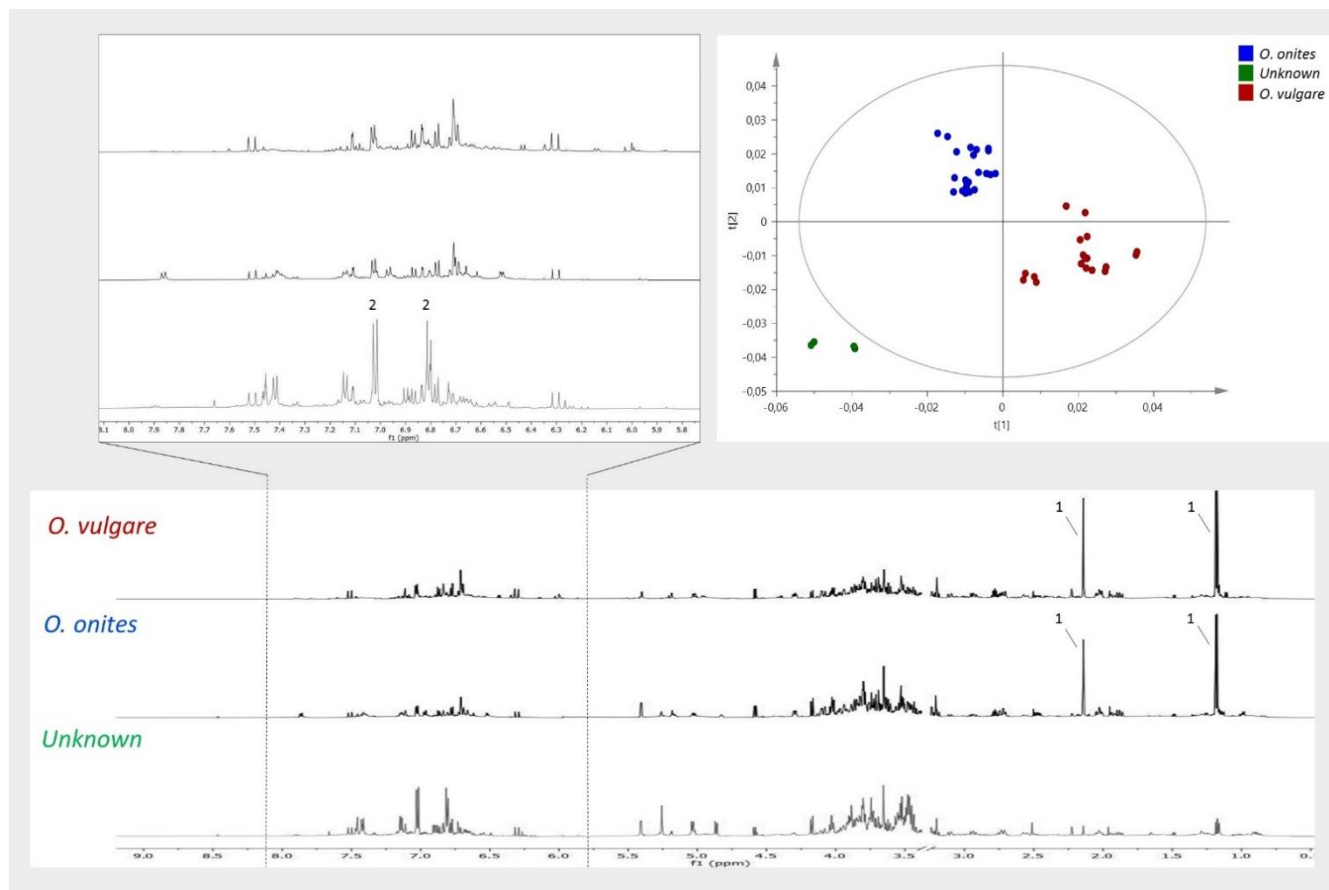
p-cymene

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96 **Fig. S10** Main biomarkers found in this work.

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100 **Fig. S11. PCA score scatter plot where unknown species (green dots) are distinguished from marketable**
 101 **oregano and placed as outliers by the model.** As showed by the ¹H NMR profiles, the lack of thymol (1) and
 102 other essential oil components and the high amount of tyrosine (2) were the main differences between these
 103 samples and the marketable oregano species.

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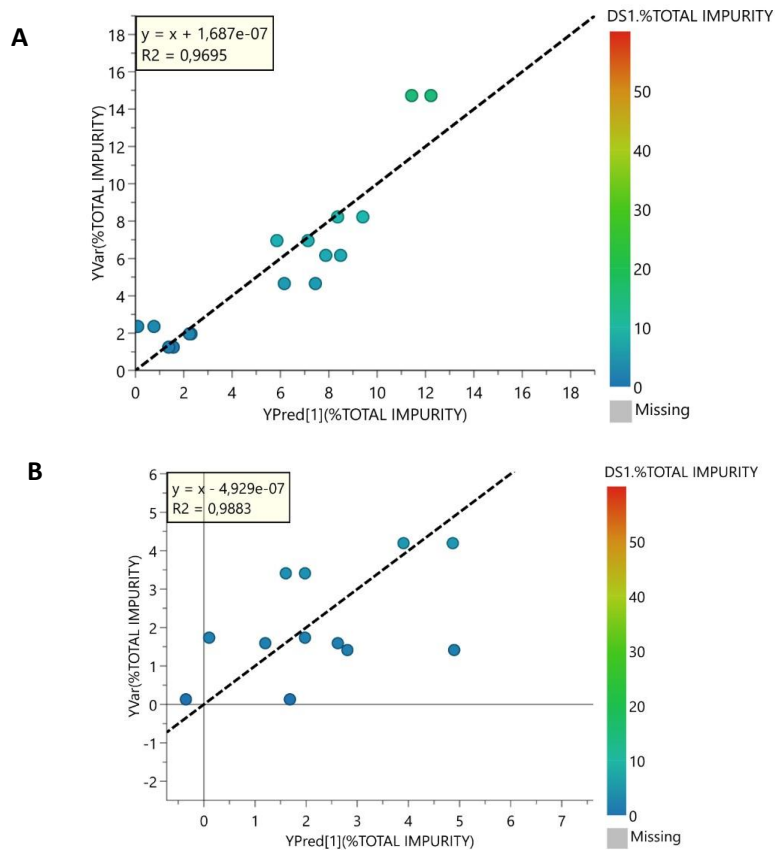
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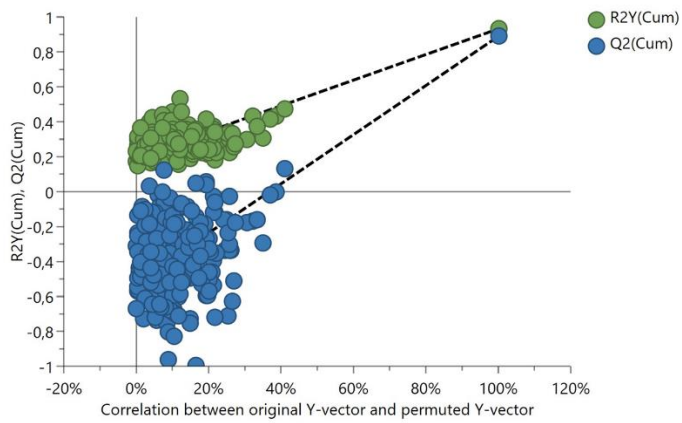
111 **Fig. S12** Extended regions of observed vs predicted plots from OPLS models ($y = \%$ of total impurity)
 112 of **A) *Origanum vulgare*** **B) *Origanum onites***

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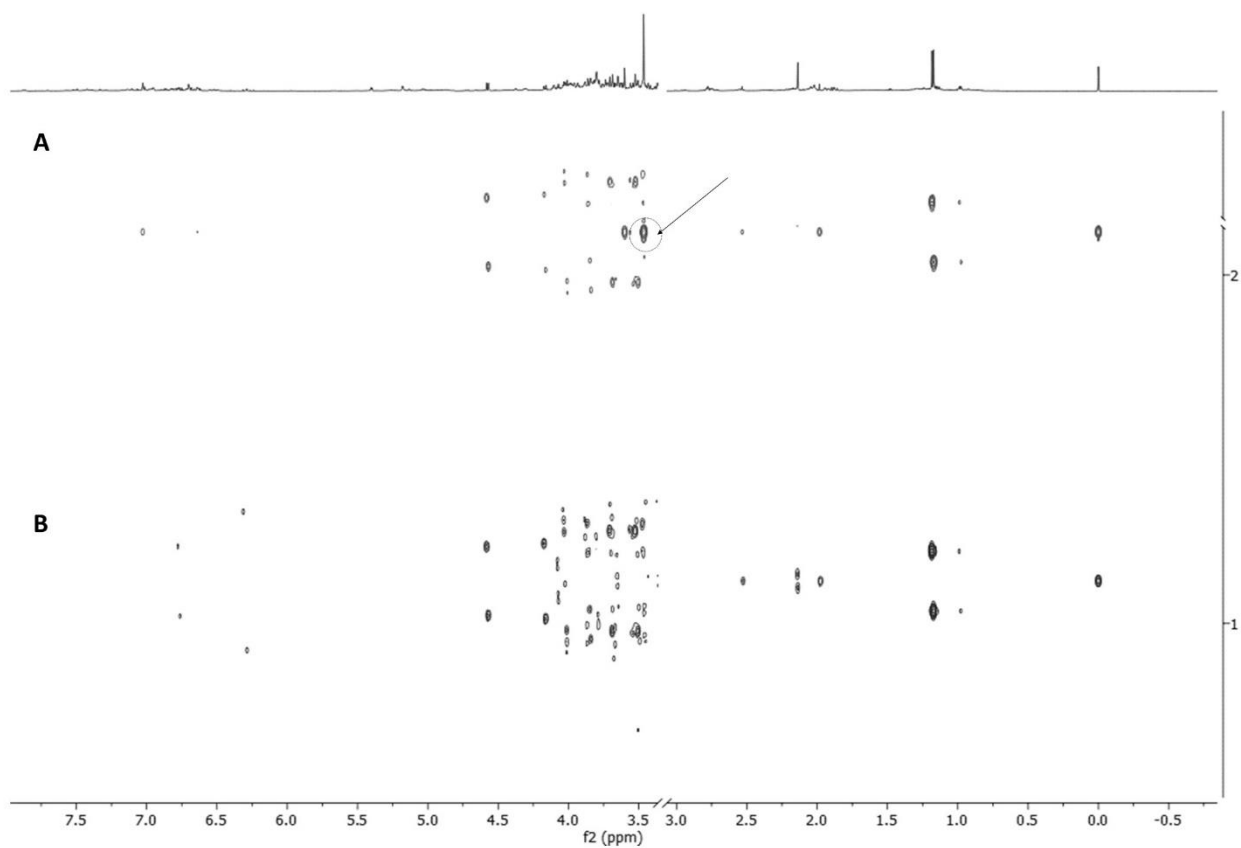
118 **Fig. S13** Graphic obtained by permutation test of the OPLS model built using as y variable the % of
 119 cistus contamination.

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125 **Fig. S14** *J-res* spectra of Oregano contaminated with cistus (A) and pure oregano (B). The
 126 contaminated sample presents a singlet at δ 3.57.

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