Supporting Information

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Quantitative determination of some phenolics in *Origanum laevigatum* Boiss. extracts via validated LC-MS/MS method

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S1. LC-MS conditions

Experiments were implemented by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometry equipped with a Synergy Max C18 column (250 x 2 mm i.d., 5μm particle size). The mobile phase was made up of water (A, 0.1% formic acid) in methanol (B, 0.1% formic acid), the gradient programs of which were 0-1.00 minute 55% A and 4 % B, 1.01-20.00 minutes 100% B and finally 20.01-23.00 55% A and 45% B. The flow rate of the mobile phase was 0.25 mL/min, and the column temperature was set to 30 ^{o}C. The injection volume was 10 μL.

Ionization technique and collision energies of the experiments are the most important parameters in quantitative mass spectrometry analysis. The three-part quad-pole mass spectrometry system was chosen to use triple quadrupole mass spectrometry because it is widely used for fragmented ion stability. The optimum ESI parameters were determined as 2.40 mTorr CID gas pressure, 5000 V ESI needle voltage, 600 V ESI shield voltage, 300.00 °C drying gas temperature, 50 °C API housing temperature, 55 psi Nebulizer gas pressure and 40 psi drying gas pressure.

S2. Preparation of test solution

50 mg of the each extract (M1, M2, C and Ac) was dissolved in 5 mL of ethanol-water (50:50 v/v) in a volumetric flask, refluxed in ethanol-water (50:50 v/v) for 1h, from which 1 mL was transferred into a 5 mL of volumetric flask. Then, 50 μL of curcumin was added and diluted to the volume with methanol. From the final solution, 1 mL was transferred into capped auto-sampler vial and 10 μL of sample was injected to LC. The samples in auto-sampler were kept at 15 ^{0}C during the experiment.

Stock solutions were prepared as 10 mg/L in methanol, which were prepared as 0,1 and 5 mg/L, respectively. HPLC grade methanol was purchased from Merck. Calibration solutions were prepared in methanol in a linear range (Table 1). Dilutions were performed using automatic pipettes and glass volumetric flasks (A class), which were stored at -20 ^{o}C in glass containers. 100 mg/L curcumin solution was freshly prepared, from which 50 μL was used as an Internal Standard (IS) in all experiments.

S3. Method validation

LOD (limit of detection) and LOQ (limit of quantification) of the LC-MS/MS methods for the above compounds were calculated to be 0.5-50 mg/L. The LODs were determined to be three times bigger than standart deviation while LOQs were determined to be ten times bigger than standard deviation.

The concentration of each analyte within the linear range and concentration of the reported method was obtained from the calibration curve. The linearity for each compound for the reported method was determined by the analysis of the corresponding standard solutions. Peak areas versus the analyte concentrations in mg/kg was plotted to obtain the calibration curves for phenolic acids. Linearity was evaluated using linear regression analysis of a six-point linear plot. The plot was consisted of three replicates per point and squared correlation coefficients, r2 was estimated for each analyte. The correlation coefficients (r2) for all analytes were found to be ≥0.98.

Finally, the calculated concentrations were converted to mg/kg of crude sample with the below equation.

$$Amount\ \ (mg/kg) = \frac{C_a \text{ x } V_{final}}{m \text{ x } V_{initial}} x1000$$

C_a is the analyte concentration obtained by calibration curve (mg/L), m is the amount of extract in gram and V_{final} and $V_{initial}$ are for the final diluted volume, respectively before the analysis and the initial sample volume. The EURACHEM/CITAC guide was used for evaluation of sources and quantification of uncertainty of LC-MS/MS method. The maximum contribution comes from the calibration curve.

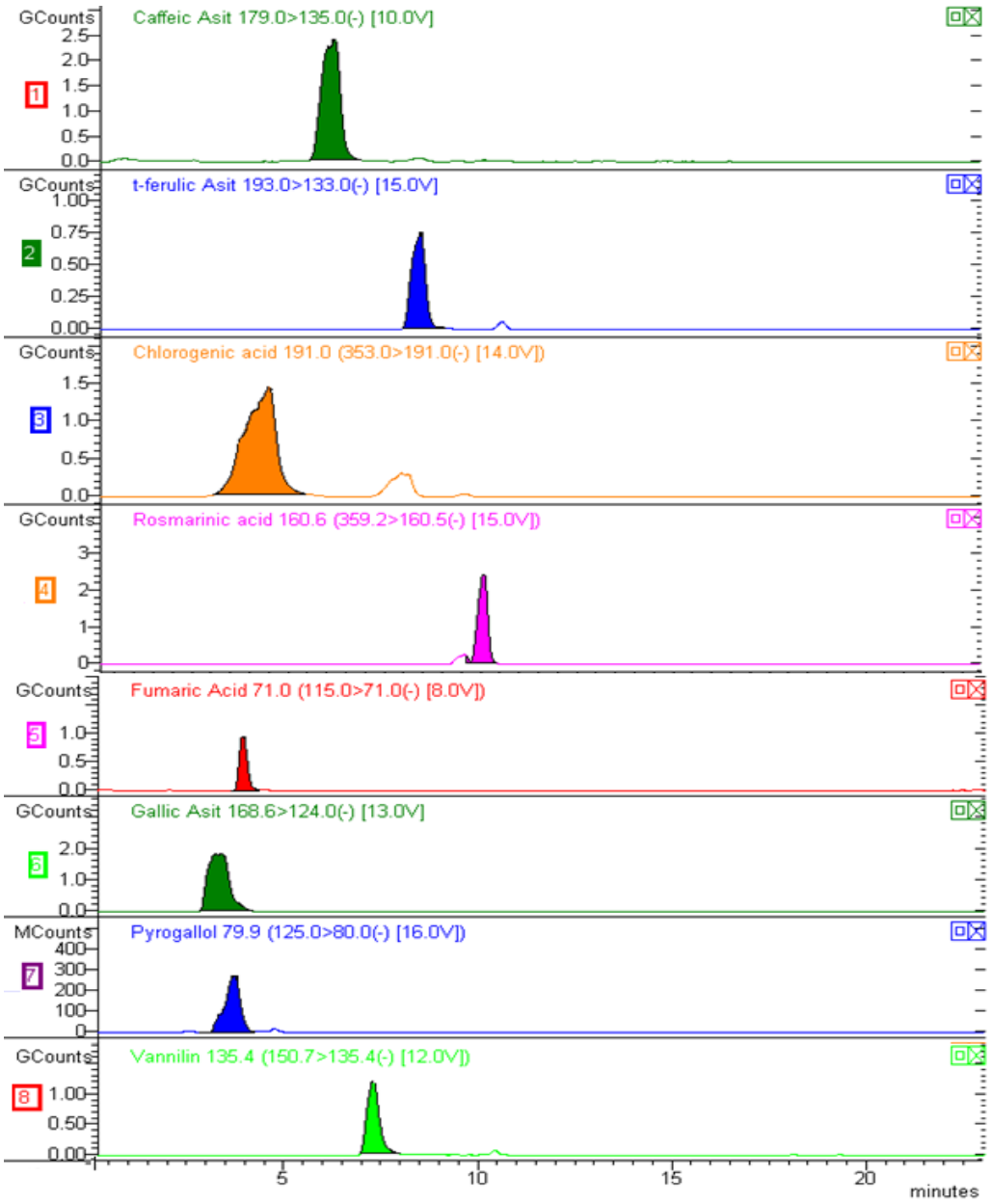
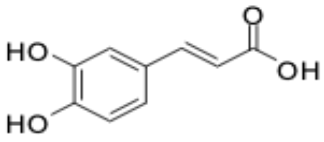
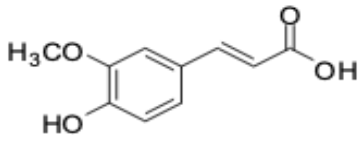


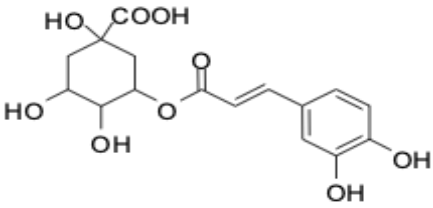
Figure 1. Chromatogram of secondary metabolites (phenolics) by LC-MS/MS



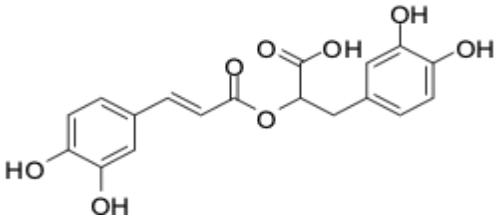
Caffeic acid (1)



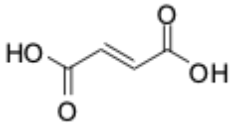
(*E*)-ferulic acid (2)



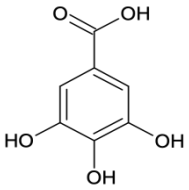
Chlorogenic acid (3)



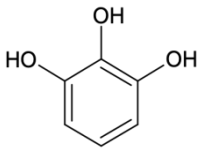
Rosmarinic acid (4)



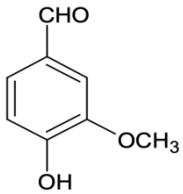
Fumaric acid (5)



Gallic acid (6)



Pyrogallol (7)



Vanillin (8)

Figure 2. Chemical structure of the determined phenolics