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SIMULTANEOUS DETERMINATION OF SELECTED PHENOLIC ACIDS IN TURKISH *Salvia* SPECIES BY HPLC-DAD

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In our previous study, we determined four phenolic acids in 12 *Salvia* species growing naturally in Turkey [1]. In this study we aimed to perform the quantification of phenolic acids in three other *Salvia* species, of which one is endemic.

Reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with UV spectrometry has been used predominantly as an analytical method for quantification of a wide range of phenolic acids in plant materials [2–4].

Good separation was achieved so that successful quantification was done by the applied LC method. For calibration, mixed standard solutions containing gallic acid (0.051–101.4 μg·mL^{-1}), chlorogenic acid (0.207–103.6 μg·mL^{-1}), caffeic acid (0.100–100 μg·mL^{-1}), and rosmarinic acid (0.201–100.5 μg·mL^{-1}) were prepared in the mobile phase. Ten microliters of injections were made in triplicate for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of each drug was plotted against the concentration to obtain the calibration graph. Five concentrations of each compound were subjected to regression analysis to calculate the calibration equation and correlation coefficients.

Table 1 presents the equation of the regression line, relative standard deviation (RSD) values of the slope, and intercept for each compound. Excellent linearity was obtained for compounds between peak areas and concentrations of 0.051–101.4 μg·mL^{-1} with r^2 = 0.9998, 0.207–103.6 μg·mL^{-1} with r^2 = 0.9999, 0.100–100 μg·mL^{-1} with r^2 = 0.9999, and 0.201–100.5 μg·mL^{-1} with r^2 = 0.9999 for gallic, chlorogenic, caffeic, and rosmarinic acids, respectively.

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3. Limits of quantification (LOQ) were established at a signal-to-noise ratio (S/N) of 10. LOD and LOQ were experimentally verified by six injections of gallic, chlorogenic, caffeic, and rosmarinic acids at the LOD and LOQ concentrations. The LOD was calculated as 0.015, 0.062, 0.030, and 0.060 μg·mL^{-1}, and the LOQ was calculated as 0.051, 0.207, 0.100, and 0.201 μg·mL^{-1} for gallic, chlorogenic, caffeic, and rosmarinic acids, respectively (Table 1).

The precision of the method was expressed as the RSD % at the LOQ levels of chlorogenic acid and rosmarinic acid and near the LOQ levels of gallic acid and caffeic acid. RSD % were found to be 2.27, 4.85, 2.15, and 6.15 % for gallic, chlorogenic, caffeic, and rosmarinic acids, respectively.

The results of our study indicate that *Salvia vertisillata* L. subsp. *vertisillata* contains higher amounts of rosmarinic, caffeic, and chlorogenic acids (0.4617, 0.0192, and 0.1165%, respectively), while *Salvia trichoclada* Benth. contains higher amount of gallic acid (0.5463%). The endemic species *Salvia kronenburgii* Rech.f. has lower amounts of gallic, chlorogenic, and caffeic acids compared with the two other *Salvia* species. The assay results of *Salvia* species, and their percent mean and standard deviation values are summarized in Table 2.

Salvia vertisillata L. subsp. *vertisillata* (VANF 14592), *Salvia trichoclada* Benth. (VANF 14594), and *Salvia kronenburgii* Rech.f. (VANF 14593) were collected from the Van-Gurpinar-Catak region in their flowering times. The plants were identified by Mehmet Firat from the Department of Biology, Faculty of Education, University of 100 Yil, Van, Turkey. The voucher specimens have been deposited at the Herbarium of the Department of Biology (VANF), Van, Turkey.

Chemicals and Standards. Gallic acid (Sigma; G-7384), chlorogenic acid (Sigma; C-3878), caffeic acid (Dr. Th. Schuchardt & Co; 822029), and rosmarinic acid (Fluka; 44699) were obtained from their respective manufacturers. Chromatographic grade double distilled water, HPLC grade methanol (Merck-1, 06007), isopropyl alcohol (Merck-101040), and analytical grade *ortho*-phosphoric acid (Merck-563) were used for the HPLC analysis.

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TABLE 1. Linearity Results, Limit of Detection (LOD), and Limit of Quantification (LOQ)

Compound	λ	Equation	Slope, RSD %	Intercept, RSD %	LOQ, μg/mL	LOD, μg/mL
Gallic acid	220	Y = 73.58028 X + 28.57892	0.084	1.982	0.051	0.015
Chlorogenic acid	330	Y = 29.89728 X + 1.629055	0.050	12.807	0.207	0.062
Caffeic acid	330	Y = 50.63197 X + 1.837441	0.061	6.283	0.100	0.030
Rosmarinic acid	330	Y = 27.86734 X – 0.87987	0.039	18.142	0.201	0.060

X = concentration (μg/ mL); Y = Area; $r^2 = 0.999$; RSD % = (Standard Deviation/Mean) × 100.

TABLE 2. Contents of Phenolic Acids (n = 3, mean; Mean ± SD) in *Salvia* Species, %

Salvia species	Rosmarinic acid	Caffeic acid	Chlorogenic acid	Gallic acid
S. kronenburgii	0.2826 ± 0.0011	0.0078 ± 0.0010	0.0257 ± 0.0012	0.2154 ± 0.0001
S. trichoclada	0.2412 ± 0.0002	0.0132 ± 0.0001	0.0327 ± 0.0001	0.5463 ± 0.0002
S. vertisillata subsp. *vertisillata*	0.4617 ± 0.0010	0.0192 ± 0.0002	0.1165 ± 0.0002	0.3352 ± 0.0010

SD: standard deviation.

Extraction. Dried and powdered aerial parts (200 mg) of the *Salvia* species were macerated with 10 mL 0.085% of *o*-phosphoric acid in water, 0.085% *o*-phosphoric acid in methanol, and 0.085% *o*-phosphoric acid in 2-propanol (80:10:10, v/v/v) in a 25 mL erlenmayer flask and sonicated in an ultrasonic bath once for 20 min at room temperature. The extracts were then filtered and made up to the 10 mL mark in a volumetric flask with the mobile phase. Then, the *Salvia* extracts were passed through 0.45 μm filter and injected into the HPLC system.

Apparatus. The analyses were performed with an LC system consisting of an HP Agilent 1100 series quaternary pump, degasser, and photodiode array detector. The samples were injected into an HP Agilent 1100 Autosampler with thermostatted column compartment on a Phenomenex-Hyperclone ODS C_{18} column (5 μm, 250 mm; 4.6 mm) at 30°C. The system was controlled, and data analysis was performed with Agilent ChemStation software. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of the peak areas.

Stock and Standard Solutions. Gallic acid (10.14 mg), chlorogenic acid (10.36 mg), caffeic acid (10.00 mg), and rosmarinic acid (10.05 mg) were accurately weighed into a 10 mL volumetric flask, dissolved in the mobile phase, and filled up to the volume for preparing stock solutions. Standard solutions were prepared in the mobile phase with combination of each phenolic acid at five different concentration levels in 10 mL volumetric flasks for the establishment of calibration curves.

Chromatographic Conditions. HPLC analysis was performed by gradient elution with a flow rate of 1.0 mL·min^{-1}. The mobile phase was delivered from three separate containers with a gradient elution program. The first container was 0.085 % *o*-phosphoric acid in water (solution A), the second container was 0.085 % *o*-phosphoric acid in methanol (solution B), and the third one was 0.085 % *o*-phosphoric acid in 2-propanol (solution C). All solvents were filtered through a 0.45 μm Millipore filter prior to use and degassed in an ultrasonic bath. A gradient system with 0.085% *o*-phosphoric acid in water (A), 0.085% *o*-phosphoric acid in methanol (B), and 0.085% *o*-phosphoric acid in 2-propanol (C) was used as (A–B–C%), 80:10:10 at 0 min, 70:15:15 at 10 min, 60:20:20 at 15 min, and 60:20:20 at 20 min.

Ten microliter volumes of each solution and samples were injected into the column, and the chromatograms were recorded from 200 to 400 nm. Standard solutions were analyzed, and three-dimensional chromatograms (wavelength, time, absorbance) were obtained to select the optimum wavelength for detection of these phenolic acids with maximum sensitivity. Quantification was performed by measuring at 220 nm for gallic acid and 330 nm for rosmarinic, caffeic, and chlorogenic acid using a photodiode array detector. The chromatographic run time was 20 min and the column void volume was 1.60 min.

REFERENCES

1. Y. Kan, A. Gokbulut, M. Kartal, B. Konuklugil, and G. Yilmaz, *Chromatographia*, **66**, S1, 147 (2007).
2. A. Ziakova, and E. Brandsteterova, *J. Liq. Chromatogr. Relat. Technol.,* **26 (3)**, 443 (2003).
3. H. Wang, G. J. Provan, and K. Helliwell, *Food Chem.*, **87**, 307 (2004).
4. G. Zgorka and S. Kawka, *J. Pharm. Biomed. Anal.*, **24**, 1065 (2001).