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HPLC-Analysis of Polyphenolic Compounds in *Gardenia jasminoides* and Determination of Antioxidant Activity by Using Free Radical Scavenging Assays

Method Validation

The validation of this developed HPLC method was followed according to the ICH guidelines (ICH, 1995). Linearity ranges, correlation coefficients, detection limits, quantitation limits, and recovery were determined using standard polyphenolics (Table 1).

A series of the standard mixture solutions of nine phenolic compounds were tested to determine the linearity between the standard mixture concentrations and peak areas. Linearity was evaluated on five calibration points (1.25 - 20 µg/ml for all standards except 0.5 - 8.0 µg/ml for CA, and 0.375 - 6.0 µg/ml for QU) with three measurements for each calibration point. All the standard polyphenolics exhibited good linearity over the evaluated range with correlation coefficients (r^2) between 0.995 and 1.000. The limits of detection and quantification were calculated as singnal-to-noise ratios with nominal values of 3:1 and 10:1, respectively. Detection and quantitation limits of these compounds were in the range of 0.11 - 0.35 and 0.36 - 1.15 µg/ml, respectively. In recovery assay, the samples were fortified with all standards at 10, and 20 µg/ml. The recovery tests of all compounds range of 95.0-104%.

Standard mixture solutions of two concentrations (low and high concentrations) were injected five times to determine the reproducibility of the peak areas and retention times under the optimum conditions. Intra-day and inter-day percentage relative standard deviations (%RSD) for retention time and peak areas are considerably low (Table 2) indicating that the precision is good. Stability of crude ethanol extract was tested every 24 h for 7 days, and was found to be stable throughout the 7-day period (RSD < 2.0%).

Table 1. Parameters of calibration graphs for the nine phenolic standards in this study.

Peak no.	Polyphenolic compound	Linearity range (µg/ml)	Correlation coefficients (r^2)	Detection limit (µg/ml)[a]	Quantitation limit (µg/ml)[a]	Recovery (%)[b]
1	GA	1.25 - 20	0.9957	0.25	0.85	98.3± 2.79
2	CH	1.25 - 20	0.9966	0.30	1.12	96.7± 1.65
3	VA	1.25 - 20	0.9958	0.21	1.01	97.9± 2.85
4	CA	0.50 - 8.0	0.9975	0.14	0.47	102.2± 3.19
5	EC	1.25 - 20	0.9955	0.35	1.20	98.3± 2.88
6	PCA	1.25 - 20	0.9992	0.26	1.02	103.1± 2.74
7	RH	1.25 - 20	0.9986	0.28	1.09	102.8± 3.20
8	EA	1.25 - 20	0.9990	0.31	1.17	99.2± 2.02
9	QU	0.375 - 6.0	0.9991	0.11	0.37	100.3± 3.95

[a] Data were expressed as mean of triplicate measurements.
[c] Recovery are expressed as mean ± standard deviation carried out in *Gardenia jasminoides* sample. GA, gallic acid; CH, (+)-catechin; VA, vanillic acid; CA, caffeic acid; EC, (–)-epicatechin; PCA, p-coumaric acid; RH, rutin hydrate; EA, ellagic acid; QU, quercetin

Table 2. Precision study of standard solutions at low and high concentrations (n = 5). GA, gallic acid; CH, (+)-catechin; VA, vanillic acid; CA, caffeic acid; EC, (–)-epicatechin; PCA, p-coumaric acid; RH, rutin hydrate; EA, ellagic acid; QU, quercetin.

Polyphenolic compound	Concentration (µg/ml)	Intra-day precision		Inter-day precision	
		Peak area (% RSD)	Retention time (% RSD)	Peak area (% RSD)	Retention time (% RSD)
GA	1.25	1.03	0.95	1.26	0.68
	20.0	1.28	0.82	1.38	0.92
CH	1.25	1.09	0.47	1.01	0.71
	20.0	1.58	0.67	1.57	0.82
VA	1.25	1.66	0.84	1.32	0.38
	20.0	1.69	0.77	1.89	0.57
CA	0.50	1.34	0.49	1.55	0.82
	8.0	1.72	0.52	1.70	0.68
EC	1.25	1.08	0.79	1.22	0.78
	20.0	1.54	0.91	1.67	0.52
PCA	1.25	1.22	0.75	0.97	0.35
	20.0	1.49	0.96	1.05	0.58
RH	1.25	1.01	0.83	1.27	0.66
	20.0	1.77	0.76	1.58	0.71
EA	1.25	1.34	1.19	1.34	0.39
	20.0	1.94	0.97	1.97	0.48
QU	0.375	1.37	0.57	1.09	0.59
	6.0	1.86	0.91	1.37	0.68

Reference

ICH Q2A. International conference on harmonization: guidance for industry: text on the validation of analytical procedures availability. *Fed Regist* 1995;60(40):11260-2.