

# A High-Sensitivity Analysis Method Of Furanocoumarins In *Radix Angelicae Dahuriae* By Reverse-Phase High-Performance Liquid Chromatography Coupled With Integrated Pulsed Amperometric Detection

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## Research Article

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## Abstract

*Radix Angelicae Dahuricae* is a traditional Chinese medicine. We developed a high-sensitivity method for detection of furanocoumarins in *Radix Angelicae Dahuricae* and *Gumiganghwal-tang* (GMGHT). The six furanocoumarins of *Radix Angelicae Dahuricae* were sonication-extracted from 50% ethanol for 60 min. Six furanocoumarins were separated through a gradient elution system. The limits of detection of the components were 0.002–0.3 ng (0.2–30 ng/mL). The coefficients of determination were 0.9995–1.0000, all inter-day and intra-day precision values were < 4.9%, and the mean recoveries and relative standard deviations were 96.4%–104.5% and 0.5%–4.8% for *Radix Angelicae Dahuricae* extract, respectively. Our method does not require any pretreatment steps and exhibits good reproducibility, selectivity, and sensitivity. Therefore, our method will contribute to a *Radix Angelicae Dahuricae* quality control measure.

## Introduction

*Radix Angelicae Dahuricae*, also known as Baizhi, is a traditional Chinese medicine used for abscess, toothache, headache, acne, and furunculosis (Zhang et al. 2014). The compound contains furanocoumarins such as byakangelicol, imperatorin, oxypeucedanin hydrate, byakangelicin, isoimperatorin, and bergapten (Zheng et al. 2010). *Radix Angelicae Dahuricae* has anti-nociceptive (Lee et al. 2011), anti-oxidative (Cao et al. 2013), anti-inflammatory (Lee et al. 2011), anti-microbial (Lechner et al. 2004), anti-hypertensive (Cao et al. 2013), neuroprotective (Son et al. 2007),  $\beta$ -secretase inhibitory (Marumoto et al. 2010), galactosemic cataract therapeutic (Shin et al. 1994), diabetic complication therapeutic (Shin et al. 1994), and estrogenic (Piao et al. 2006) effects.

*Gumiganghwal-tang* (GMGHT), which includes *Radix Angelicae Dahuricae*, is a representative Chinese medicinal preparation that has multiple effects including anti-inflammatory effects (Kim et al. 2005), analgesic activity (Kim et al. 2005), and treatment of chills (Jin et al. 2014).

Reported analytical methods for furanocoumarins in *Radix Angelicae Dahuricae* are reverse-phase high-performance liquid chromatography (RP-HPLC)-mass spectrometry (MS/MS) (Zheng et al. 2010; Park et al. 2009; Zhang et al. 2011), RP-HPLC-ultraviolet (UV) (Park et al. 2009; Fan et al. 2012; Xie et al. 2007), gas chromatography (GC)-MS (Zhao et al. 2013; Wang et al. 2007), micellar electrokinetic capillary chromatography (MECC) (Ketai et al. 2001), pressurized capillary electrochromatography (pCEC) (Chen et al. 2006), and capillary zone electrophoresis-amperometric detection (CZE-AD) (Wu et al. 2005). High-sensitivity GC-MS and RP-HPLC-MS/MS methods are expensive and not used widely. The RP-HPLC-UV method has low sensitivity, and the pCEC and MECC methods have poor reproducibility. Pulsed amperometric detection (PAD) and the variant integrated PAD (IPAD) (Draisci et al. 1993) are useful for amines and sulfur species (Johnson et al. 1990). With PAD, the current is measured at one potential for a set period of time and integrated to obtain charge. With IPAD, the current is measured over more than one potential during the cycle and integrated. Furanocoumarins have not been analyzed using the IPAD method.

We developed an analysis method of furanocoumarins in *Radix Angelicae Dahuricae* and GMGHT through RP-HPLC-IPAD. GMGHT contains nine medicinal herbs, including *Radix Angelicae Dahuricae*. Determination of furanocoumarins in GMGHT was hindered by the chromatographic peak signals eluted from the other eight herbs. The furanocoumarins in GMGHT have been determined by HPLC-DAD/ESI-MS (Weon et al. 2012) and HPLC-DAD (diode array detection) methods (Yun et al. 2014), but these methods did not overcome the problem of background signals. However, quantification of furanocoumarins in GMGHT was achieved through the selectivity of IPAD. Here, a stable analysis method and an optional IPAD monitoring system are described. This method has allowed remarkable progress in determining furanocoumarins in *Radix Angelicae Dahuricae* and GMGHT.

## Materials And Methods

### Materials

Oxypeucedanin hydrate, byakangelicin, bergapten, byakangelicol, imperatorin, and isoimperatorin were obtained from ChromaDex (Irvine, CA, USA). The 50% sodium hydroxide (NaOH) was obtained from Thermo Fisher Scientific (Fairlawn, NJ, USA). Internal standard (I.S.) vanillin was obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). HPLC-grade acetonitrile (ACN) was bought from Thermo Fisher Scientific. The mobile phase preparation used 18 MΩ-cm purified water. Solvent and samples were filtered using a nylon membrane filter (pore size 0.2 µm) and syringe filters (cellulose acetate, pore size 0.20 µm), respectively. All crude drugs, including *Radix Angelicae Dahuricae*, were purchased from the Kyungdong Market (Seoul, South Korea), in accordance with the standards specified in the Korea Pharmacopoeia (XII). All crude drugs were identified by Professor Chang-Soo Yook (Kyung Hee University, Seoul, South Korea).

### High-performance liquid chromatography

The ICS-3000 series PAD and HPLC equipment were purchased from Thermo Fisher Scientific and Shiseido (Tokyo, Japan), respectively. The following waveforms were used: E1= 0.130 V (0.00–0.04 s), E2= 0.330 V(0.050–0.210 s), E3= 0.550 V(0.220–0.460 s), E4= 0.330 V(0.470–0.560 s), E5= -1.670 V(0.570–0.580 s), E6= 0.930 V(0.590 s). All data of IPAD were obtained from the Chromeleon program (Thermo Fisher Scientific). The column used included a Cadenza C-18 guard column (0.5 × 0.2 cm I.D.; 3.0 µm, Imtakt, Kyoto, Japan) and a Cadenza C-18 column (25 × 0.15 cm I.D.; 3.0 µm) with Hypersil Gold C-18 (25 × 0.46 cm I.D.; 5.0 µm, Thermo Fisher Scientific ). The flow rate was 110 µL /min, the injection volume was 10 µL, and the mobile phase was 50% ACN (Phase A) and water (Phase B). The following gradient condition was used: isocratic elution of [45(A):55(B)] (0–12 min), gradient elution from (45:55) to (70:30) (12–13 min), isocratic elution of (70:30) (13–19 min), gradient elution from (70:30) to (85:15) (19–20 min), isocratic elution of (85:15) (20–35 min), gradient elution from (85:15) to (98:2) (35–36 min), isocratic elution of (98:2) (36–75 min), gradient elution from (98:2) to (45:55) (76–77 min), and maintenance with (45:55) (77–80 min). The post-column mobile phase was composed of 0.10 M NaOH (flow rate; 800 µL /min). The mobile phase was prepared fresh each day. The separation temperature was

25°C, and the UV detector (Shiseido) was set at 254 nm. All UV data were obtained from the dsCHROM program (Donam Instruments, Seoul, South Korea).

### Preparation of standard solutions

The furanocoumarin stock solutions were made by putting 1 mg of each standard to 1 mL 50% ACN and diluting to the appropriate concentration with 50% ACN. The vanillin (I.S.) concentration was 100 µg/mL per sample.

### Preparation of sample solutions

*Radix Angelicae Dahuricae* was powdered with an electric mixer; 1 mg of the powder was put in a round-bottom flask with 40 mL of 50% EtOH and extracted through sonication for 60 min. Filtration of the extract was performed using a Millipore membrane filter with a decompression device. A 0.5-mL aliquot of the filtrate was concentrated using a SpeedVac. The concentrate was dissolved in 50% ACN 0.25 mL. The filtrate was mixed with 0.25 mL of I.S. solution (vanillin, 200 µg/mL) (filtrate:I.S. solution=1:1) and used in the experiment after syringe filtration. The composition of one dose (total 3.2 g) of GMGHT was as follows: *Rhizoma Notopterygii* (0.5 g), *Radix Ledebouriellae* (0.5 g), *Rhizoma Atractylodis* (0.5 g), *Herba Asari* (0.2 g), *Radix Angelicae Dahuricae* (0.3 g), *Radix Rehmanniae* (0.3 g), *Radix Scutellariae* (0.3 g), *Radix Glycyrrhizae* (0.3 g), and *Cnidii Rhizoma* (0.3 g). One dose was ground into powder, placed in a round-bottom flask with 0.1 L of water, and reflux-extracted for 60 min. The extract was pretreated as above.

### Method validation

Standard calibration curves were made for each component. The analysis precision was determined through inter- and intra-day assays. Four injections ([oxypeucedanin hydrate, 0.1 ng; byakangelicin, 0.05 ng; bergapten, 1.0 ng; byakangelicol 0.1 ng; imperatorin, 0.005 ng; isoimperatorin, 1.0 ng], 20.0 ng, 100.0 ng, and 500.0 ng) were conducted five times a day for five days. Recovery tests were performed by placing each standard (100.0, 200.0, and 500.0 ng) in the *Radix Angelicae Dahuricae* and GMGHT samples. Three injections were performed per sample.

## Results And Discussion

### Set up of the RP-HPLC-IPAD system

The RP-HPLC-IPAD system had the following composition for analysis of furanocoumarins in *Radix Angelicae Dahuricae*. The mobile phase, composed of 50% ACN and water, flowed according to the gradient elution program on a C-18 column with a 0.11 mL/min flow rate. The eluent was mixed with NaOH solution before entering the detector. A post-column eluent was used to supply NaOH to the mixer. The post-column eluent concentration and flow rate were set to 100.0 mM NaOH and 0.80 mL/min, respectively. This system delivered a high-sensitivity analysis of furanocoumarins.

The sensitivities of six furanocoumarins were investigated using Hypersil Gold C-18 ( $25 \times 0.46$  cm I.D.;  $5.0 \mu\text{m}$ ) (4.6 mm column) and Cadenza C-18 ( $25 \times 0.15$  cm I.D.;  $3.0 \mu\text{m}$ ) (1.5 mm column) column types (Fig. 1(A)). The flow rates for the two columns were 1.0 and 0.11 mL/min, respectively. The IPAD method was used to measure the current generated by oxidation of a component at more than one potential at a gold electrode in a strongly basic medium ( $\text{pH} > 11$ ). The IPAD sensitivity decreased as ACN volume increased because ACN suppressed detection (Kwon et al. 2008). Our RP-HPLC-IPAD system is a post-column delivery system in which the mobile phase and NaOH are mixed in a mixer. A column of large diameter supplies a large volume of ACN; therefore, IPAD sensitivity decreases with increase in column diameter at a set post-column NaOH flow rate (Kwon et al. 2008). Figure 1(A) shows that the 4.6 mm column had lower baseline stability and sensitivity than the other column. The sharp target peaks of the chromatogram were the basis for selection of the 1.5 mm column for further analyses.

The sensitivities of six furanocoumarins were investigated according to waveforms (triple-, quadruple- or six-potential) (Fig. 1(B)). All target peaks on the chromatogram were adjusted based on an internal standard peak. The peaks with the quadruple-potential waveform had 1.4–2.4-fold better sensitivity than those with the triple-potential waveform. Analysis of glycosides or amino acids usually uses the six-potential waveform of  $E_1 = -0.200$  V,  $E_2 = 0.000$  V,  $E_3 = +0.220$  V,  $E_4 = 0.000$  V,  $E_5 = -2.000$  V, and  $E_6 = +0.600$  V. While glycosides with sugar moieties measured through  $E_3 = +0.220$  V were highly sensitive, furanocoumarins without a sugar moiety were not. Thus, it was necessary to increase the potential of  $E_3$ . Furanocoumarins were detected at high sensitivity by increasing the potential of  $E_3$  to 0.550 V. The peaks with our six-potential waveform had 2.0–8.2-fold better sensitivity than those with the triple-potential waveform. Our IPAD method enabled the first high-sensitivity analysis of furanocoumarins.

### Optimization of extraction efficiency

Water, 50% EtOH, 50% MeOH, and 50% ACN were tested as extraction solvents for furanocoumarins in *Radix Angelicae Dahuricae*, as shown in Figure 2(A). Among them, 50% EtOH with the highest extraction efficiency was suitable as the solvent. Figure 2(B) shows the extraction efficiencies according to extraction method (sonication or reflux). The 50% EtOH was used as an extraction solvent, and the *Radix Angelicae Dahuricae* was extracted under sonication or reflux for 60 min. Oxypeucedanin hydrate and byakangelicin were soluble in water, while the others were soluble in 50% EtOH. The extraction efficiencies of the sonication method were lower than those of the reflux method for oxypeucedanin hydrate and byakangelicin; the same for bergapten; and higher for byakangelicol, imperatorin, and isoimperatorin. For optimal total extraction amount, the sonication method was higher than the reflux method and was selected. Figure 2(C) shows the extraction efficiencies according to extraction time. The *Radix Angelicae Dahuricae* was extracted with 50% EtOH through the sonication method for 30, 60, 90, or 120 min. The extraction efficiency was highest with the 60 min extraction. Based on these results, *Radix Angelicae Dahuricae* was set to be extracted for 60 min with 50% EtOH using the sonication method.

### Method validation

#### Sensitivity and linearity

The linearities of the six target components were confirmed using four standard calibration points (LOQs [oxypeucedanin hydrate, 0.1 ng; byakangelicin, 0.05 ng; bergapten, 1.0 ng; byakangelicol 0.1 ng; imperatorin, 0.005 ng; isoimperatorin, 1.0 ng], 20.0 ng, 100.0 ng, and 500.0 ng). Linear equations and ranges are shown in Table 1. The coefficients of determination were 0.9995 - 1.0000. The IPAD method has 1.05–110-fold better sensitivity than the UV method (Table 1). Figure 3 shows comparative chromatograms of the UV and IPAD methods for *Radix Angelicae Dahuricae* extracts. Our IPAD method produced much better stability and noise reduction of the baseline than did the UV method. While the UV method sensitivity was too low to analyze imperatorin and isoimperatorin, our IPAD method had sufficient sensitivity. Our IPAD chromatogram showed mostly sharp peaks, and byakangelicol and its neighboring unknown peak had good separation. However, the UV chromatogram showed broad peaks, with that of byakangelicol and its neighboring unknown peak overlapping. Therefore, our IPAD method had better sensitivity, peak sharpness, and baseline stability compared to the UV method for analyzing *Radix Angelicae Dahuricae*.

### Accuracy and precision

The inter- and intra-day tests were performed by measuring samples daily for five consecutive days (Table 2). The relative standard deviations (RSDs) were 0.1%–4.2% in the inter-day assay and 0.8%–4.9% in the intra-day assay. The recovery test was conducted for accuracy evaluation of our method. The RSD ranges and mean recoveries were 0.5%–4.8% and 96.4%–104.5% for *Radix Angelicae Dahuricae* (Table 3), showing that this method has excellent precision and accuracy.

### Determination of six furanocoumarins in *Radix Angelicae Dahuricae* and GMGHT

We quantified six furanocoumarins in *Radix Angelicae Dahuricae* and GMGHT through our IPAD method. The samples were used in powdered form. Figure 4 shows the extraction efficiency. *Radix Angelicae Dahuricae* was extracted under reflux for 1 h with 50% EtOH or water and analyzed. Oxypeucedanin hydrate and byakangelicin were soluble in water, and the boiling point in water was higher than that in 50% EtOH. The extraction efficiencies for oxypeucedanin hydrate and byakangelicin in water were 4.0- and 3.6-fold higher, respectively, than those in 50% EtOH owing to the solubility differences. In contrast, bergapten, imperatorin, and isoimperatorin were sparingly soluble in water and soluble in 50% EtOH. The extraction efficiencies for bergapten, imperatorin, and isoimperatorin in 50% EtOH were 3.8-, 21.8-, and 66.4-fold higher, respectively, than those in water. The 50% EtOH had the best total extraction efficiency. However, because GMGHT usually is taken in liquid form by mouth, water instead of 50% EtOH was judged to be more suitable as an extraction solvent for *Radix Angelicae Dahuricae* or GMGHT.

Table 4 shows the results of quantification of the six furanocoumarins in *Radix Angelicae Dahuricae* and GMGHT. In general, the extraction efficiency of target components from a mix of several medicinal herbs, such as that in GMGHT, is lower than that from a single medicinal herb, such as *Radix Angelicae Dahuricae*. In the former case, we used the correction factor for transfer (CFT) (maximum amount of a target component that can be extracted from the Chinese medicinal preparation / measured amount of the target component extracted from the Chinese medicinal preparation). The CFTs for oxypeucedanin

hydrate, byakangelicin, and byakangelicol were in the range of 3.13–11.40. The extraction efficiencies of oxypeucedanin hydrate, byakangelicin, and byakangelicol from GMGHT were lower than those from *Radix Angelicae Dauricae*. It was inferred that the presence of the other medicinal herbs in GMGHT lowered the extraction efficiency. In contrast, the CFTs for bergapten, imperatorin, and isoimperatorin were in the range of 0.07–0.86, beyond the maximum values expected. *Notopterygii Rhizoma* (Kim et al. 1989) and *Ledebouriellae Radix* (Kim et al. 2002) have been reported to contain bergapten and imperatorin, and *Notopterygii Rhizoma* has been reported to contain isoimperatorin (Kim et al. 1989). *Notopterygii Rhizoma* and *Ledebouriellae Radix* are medicinal herbs included in GMGHT. The CFT values of these were low because bergapten, imperatorin, and isoimperatorin were present in large quantities in *Rhizoma Notopterygii* and *Radix Ledebouriellae*. Based on these results, oxypeucedanin hydrate is the most useful marker of *Radix Angelica Dauricae* in GMGHT, for which our method is suitable for quality control.

Figure 5 shows the IPAD chromatograms of GMGHT and *Radix Angelica Dauricae*. Both show good separation of all target components without overlap of unknown peaks. Quantification of the six furanocoumarins from *Radix Angelica Dauricae* or GMGHT was possible without a complex pretreatment.

## Conclusion

We developed a high-sensitivity quantitative method for furanocoumarins in *Radix Angelicae Dauricae* and GMGHT using RP-HPLC-IPAD. This is the first report of such an RP-HPLC-IPAD method for analysis of furanocoumarins. Our method enabled high-sensitivity analysis for furanocoumarins by increasing the potential of E3 to 0.55V. This is a breakthrough approach to quantifying a mixture of furanocoumarins and will contribute greatly to quality control of *Radix Angelicae Dauricae*.

## Declarations

### Conflicts of interest

The authors affirm that there are no conflicting financial interests.

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## Tables

**Table 1.** Investigated linear range, linear equation, coefficient of determination and limit of detection (LOD) and limit of quantitation (LOQ) for six furanocoumarins.

Compounds	Linear range (ng)	Linear equation <sup>a</sup>	R <sup>2</sup>	IPAD		UV <sup>b</sup>	
				LOD(ng)	LOQ(ng)	LOD(ng)	LOQ(ng)
oxypeucedanin hydrate	0.1 - 500	y = 0.05211x + 0.1409	1.0000	0.03	0.1	0.15	0.55
byakangelicin	0.05 - 500	y = 0.03253x + 0.0046	0.9998	0.02	0.05	-	-
bergapten	1.0 - 500	y = 0.01547x + 0.1915	0.9998	0.3	1.0	0.3	1.05
byakangelicol	0.1 - 500	y = 0.01228x + 0.2381	0.9995	0.03	0.1	0.12	0.2
imperatorin	0.005 - 500	y = 0.03688x + 0.2596	0.9996	0.002	0.005	0.2	0.55
isoimperatorin	0.1 - 500	y = 0.03494x + 0.3561	0.9998	0.03	0.1	0.6	1.5

<sup>a</sup>y = ax + b, where y and x are the ratios of the peak areas (analytes/I.S.) and sample mass, respectively.

<sup>b</sup>Data from *Biomedical Chromatography* 23 (2009) 1034-1043 and *Phytochemical Analysis* 23 (2012) 299-307.

**Table 2.** Intra-and inter-day assays for determination of six furanocoumarins.

\*S.D., standard deviation

\*R.S.D., relative standard deviation

**Table 3.** Recovery test for *Radix Angelica Dahuricae* extract (n=3).

\*R.S.D., relative standard deviation

**Table 4.** The contents of six furanocoumarins in *Radix Angelica Dahuricae* and Gumi-ganghwal-tang (n = 3).

Compounds	Added (ng)	Inter-day	Intra-day		*CFT, correction factor for transfer
		Mean±S.D.	R.S.D	Mean±S.D.	
Oxypeucedanin hydrate	0.1	0.1±0.0	4.0	0.1±0.0	3.8
	20.0	20.5±0.2	0.9	20.3±0.8	3.8
	100.0	100.8±2.6	2.6	99.7±4.2	4.3
	500.0	503.9±8.2	1.6	499.8±10.3	2.1
<b>Figures</b>					
byakangelicin	0.05	0.05±0.00	3.1	0.05±0.00	4.1
	20.0	19.9±0.2	1.1	20.2±0.8	3.8
	100.0	102.9±3.3	3.2	102.5±2.3	2.3
	500.0	495.8±5.3	0.5	494.8±9.4	1.9
bergapten	1.0	1.1±0.1	3.3	1.0±0.0	2.0
	20.0	20.3±0.7	3.5	20.6±1.0	4.9
	100.0	104.0±3.0	2.9	103.0±4.3	4.1
	500.0	501.3±8.4	1.7	499.1±4.2	0.8
byakangelicol	0.1	0.1±0.0	0.1	0.10±0.00	3.1
	20.0	20.3±0.2	1.2	20.60±0.50	2.2
	100.0	101.8±4.3	4.2	99.70±2.60	2.6
	500.0	500.6±2.9	0.6	500.60±5.80	1.2
imperatorin	0.005	0.005±0.000	4.1	0.005±0.000	3.9
	20.0	20.4±0.5	2.3	20.70±0.50	2.2
	100.0	98.0±3.8	3.9	99.70±1.90	1.9
	500.0	499.6±6.0	1.2	48.93±15.90	3.3
isoimperatorin	1.0	1.0±0.0	1.9	1.00±0.00	2.6

	20.0	20.4±0.3	1.7	20.20±0.30	1.6
	100.0	101.6±1.9	1.9	100.10±2.70	2.7
	500.0	498.7±5.4	1.1	504.10±5.70	1.1
Compounds	Added (ng)	Recovery (%)	R.S.D.		
oxypeucedanin hydrate	100.0	102.9 ± 4.1	4.0		
	200.0	103.5 ± 1.9	1.9		
	500.0	100.3 ± 0.5	0.5		
byakangelicin	100.0	97.6 ± 3.5	3.5		
	200.0	97.7 ± 3.5	3.6		
	500.0	98.5 ± 0.9	0.9		
bergapten	100.0	103.6 ± 3.3	3.2		
	200.0	99.6 ± 3.5	3.5		
	500.0	102.0 ± 1.0	1.0		
byakangelicol	100.0	97.6 ± 4.5	4.6		
	200.0	98.7 ± 3.8	3.9		
	500.0	100.7 ± 1.1	1.1		
imperatorin	100.0	97.8 ± 3.3	3.3		
	200.0	99.5 ± 4.8	4.8		
	500.0	100.6 ± 1.2	1.2		
isoimperatorin	100.0	104.5 ± 4.4	3.0		
	200.0	96.4 ± 0.0	1.0		
	500.0	100.2 ± 0.0	0.5		

Compounds	Radix Angelicae Dahuricae		Gumiganghwal-tang		*CFT (%)
	Content ( $\mu\text{g/g}$ )	RSD	Content ( $\mu\text{g/g}$ )	RSD	
oxypeucedanin hydrate	295.49 $\pm$ 9.85	3.33	8.86 $\pm$ 0.10	1.17	3.13
byakangelicin	258.39 $\pm$ 4.99	1.93	3.29 $\pm$ 0.68	0.59	7.36
bergapten	4.47 $\pm$ 0.16	3.60	0.49 $\pm$ 0.07	2.44	0.86
byakangelicol	475.74 $\pm$ 12.61	2.65	3.91 $\pm$ 0.81	3.81	11.4
imperatorin	13.31 $\pm$ 0.46	3.47	3.98 $\pm$ 0.02	0.63	0.31
isoimperatorin	3.39 $\pm$ 0.04	1.06	4.65 $\pm$ 0.01	0.28	0.07

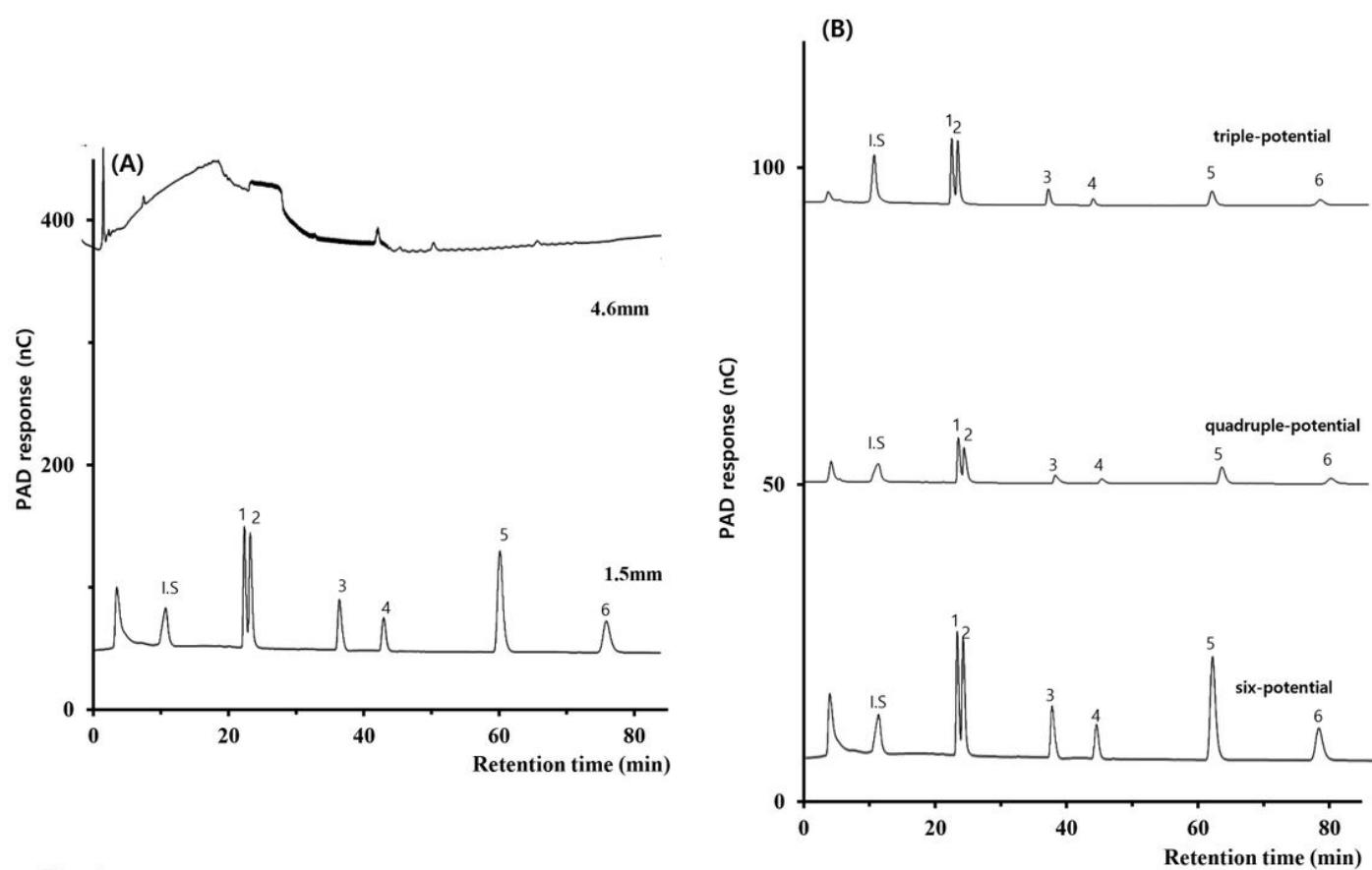
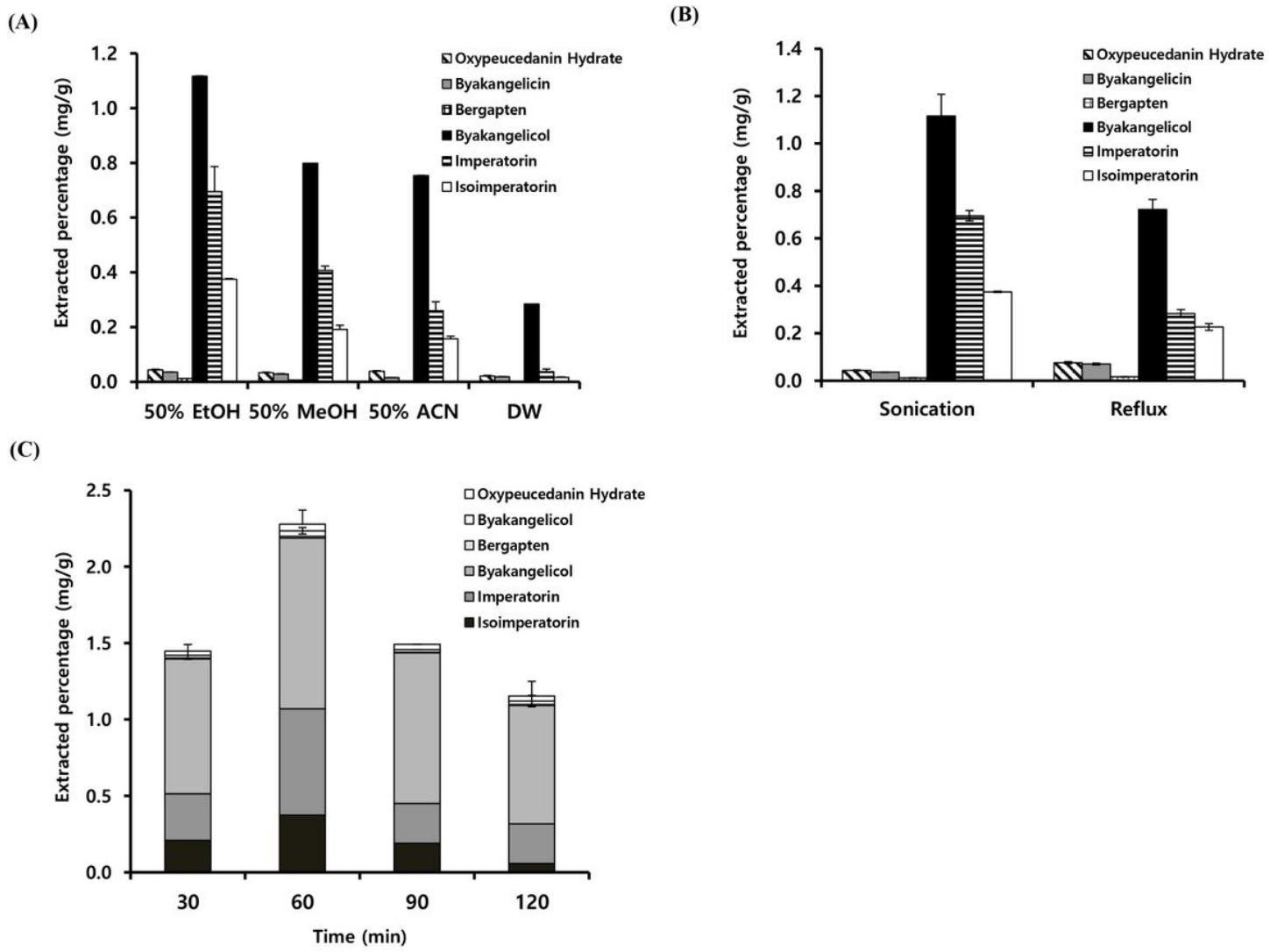


Fig. 1

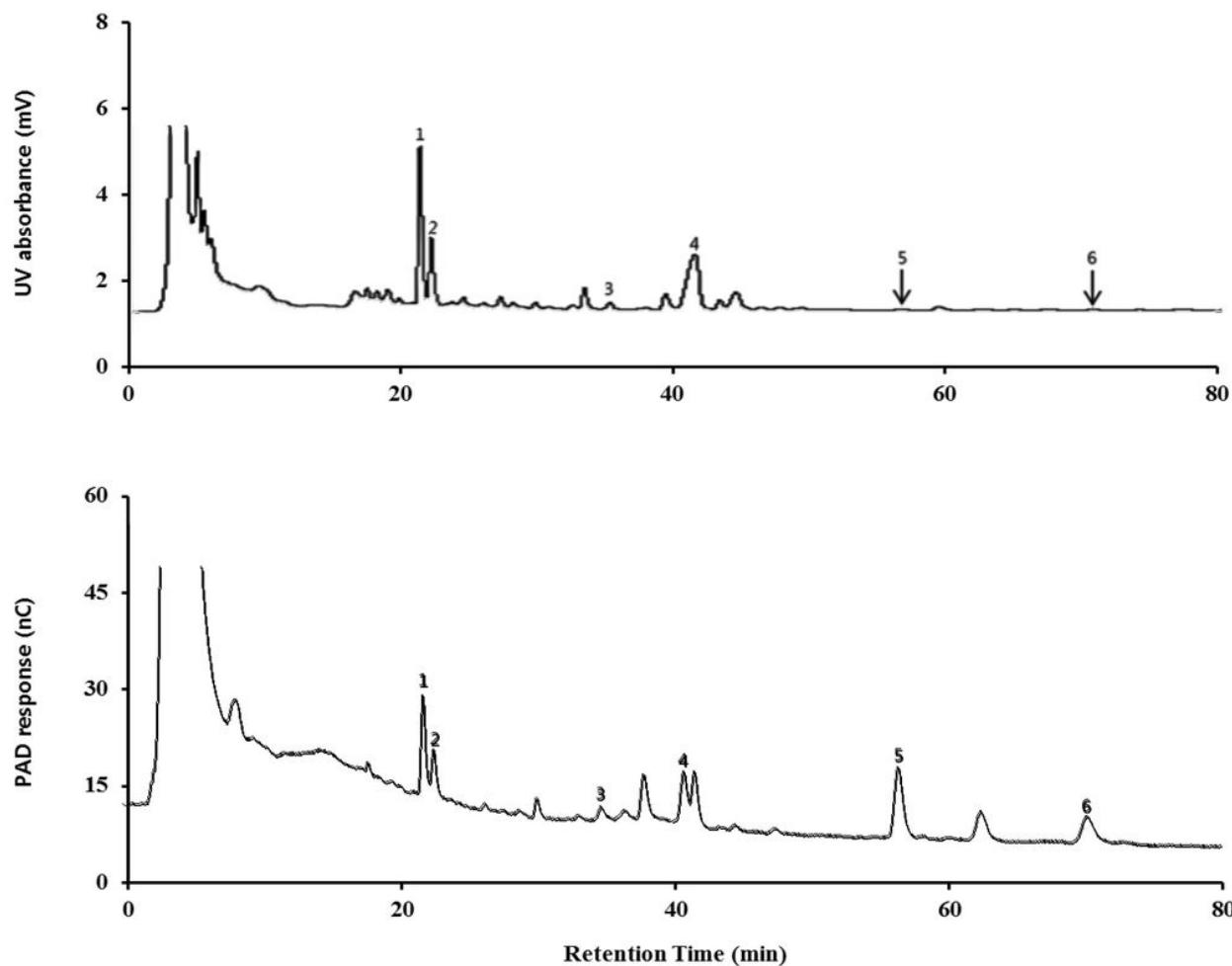
Figure 1

Chromatograms of furanocoumarins according to column type (A) and waveform (B). Peaks: 1, oxypeucedanin hydrate; 2, byakangelicin; 3, bergapten; 4, byakangelicol; 5, imperatorin; 6, isoimperatorin.



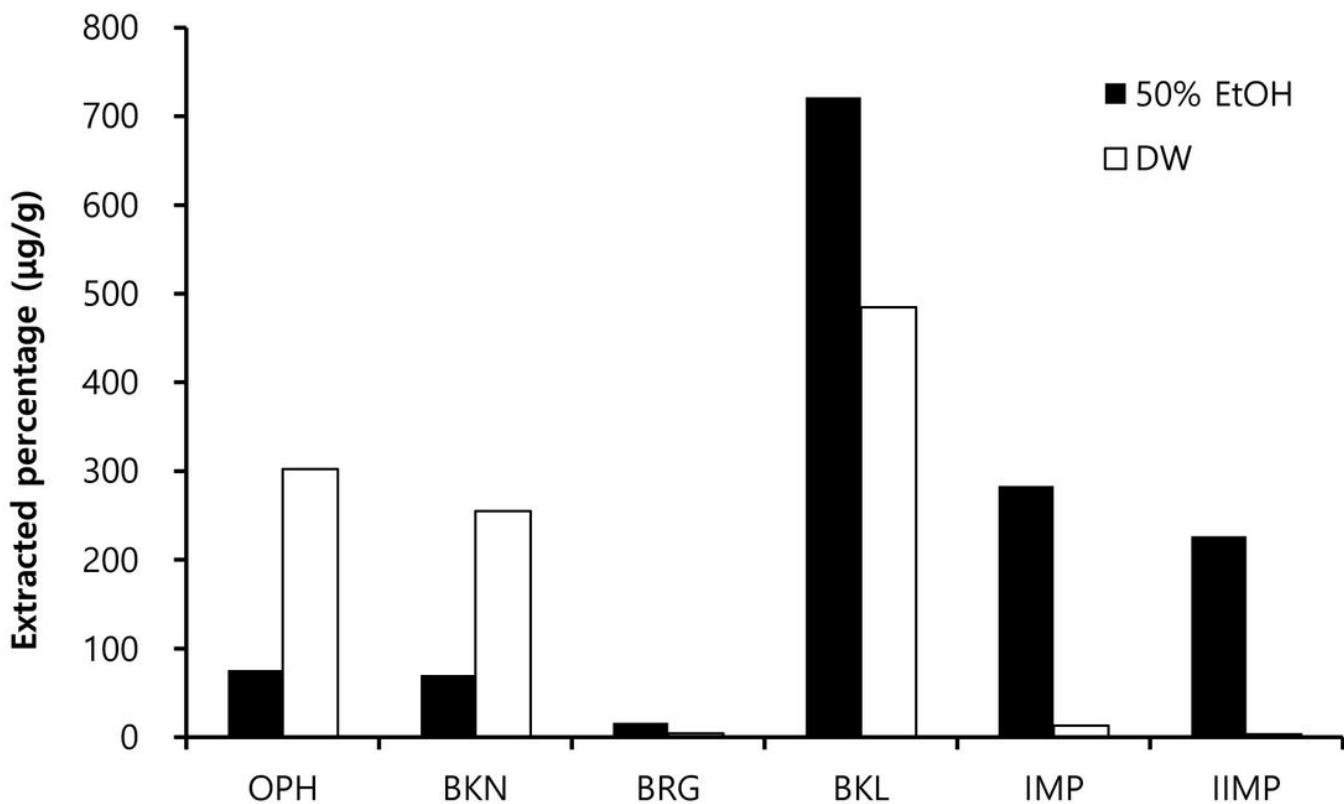
**Figure 2**

Extraction efficiencies for the furanocoumarins in *Radix Angelicae Dahuricae* according to solvent (A), method (B), and time (C).



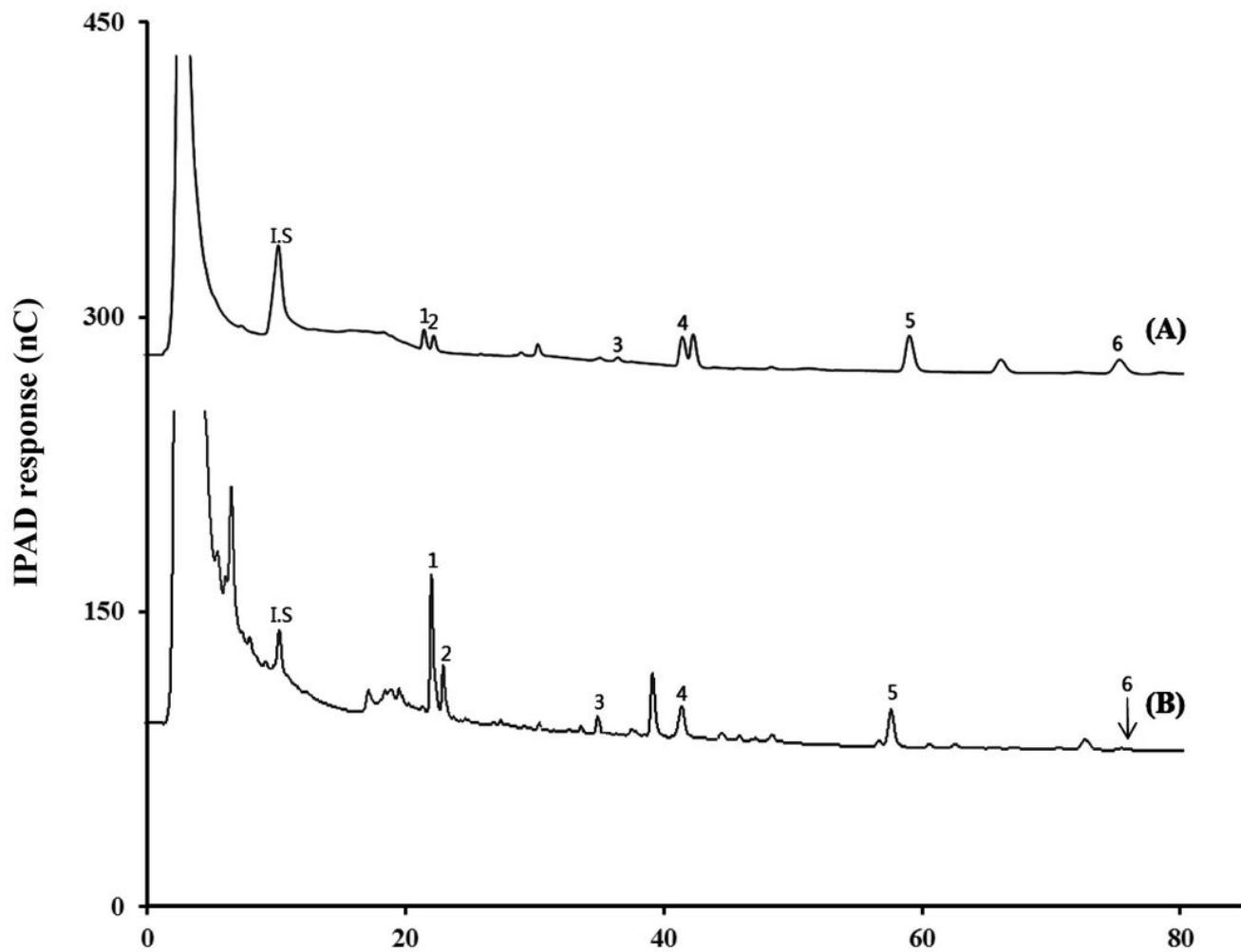
**Figure 3**

Comparative chromatograms of the RP-HPLC-UV (A) and RP-HPLC-IPAD (B) methods for *Radix Angelicae Dahuicae* extract. Peaks: 1, oxypeucedanin hydrate; 2, byakangelicin; 3, bergapten; 4, byakangelicol; 5, imperatorin; 6, isoimperatorin.



**Figure 4**

Extraction efficiency of 50% EtOH or water under reflux for furanocoumarins in *Radix Angelicae Dahuicae* extract. Abbr: OPH, oxypeucedanin hydrate; BKN, byakangelicin; BRG, bergapten; BKL, byakangelicol; IMP, imperatorin; IIMP, isoimperatorin.



**Figure 5**

Chromatograms of *Radix Angelicae Dahuricae* (A) and *Gumiganghwal-tang* (B). Peaks: 1, oxypeucedanin hydrate; 2, byakangelicin; 3, bergapten; 4, byakangelicol; 5, imperatorin; 6, isoimperatorin; I.S., vanillin.