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Simultaneous Determination of Five Compounds in *Descurainia sophia* by HPLC-DAD

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Abstract – *Descurainia sophia* was commonly used for treating of cough and asthma in Asian country such as Korea, China and Japan. According to our previous study, it also had anti-influenza activity and anti-influenza compounds were isolated and identified. In this study, simultaneous determination analytical method of isolated five compounds such as daucosterol (**1**), helveticoside (**2**), isorhamnetin (**3**), quercetin (**4**), and sinapic acid (**5**) was established by using HPLC-DAD. The HPLC analysis was carried out using a Dionex C_{18} column (5 μm particle size, 120 Å pore size, 4.6 mm × 150 mm dimensions) at a constant temperature of 25°C. The mobile phase consisted of a blend of 0.1% trifluoroacetic acid (TFA) and acetonitrile and delivered at a flow rate of 1 mL/min. Method validation was conducted to assess its reliability and accuracy. The calibration curve exhibited excellent linearity, with an R^2 value exceeding 0.9994, demonstrating the method's capability to accurately quantify the compounds across a range of concentrations. Limit of Detection (LOD) ranged from 0.93 to 3.19 μg/mL, and limit of quantification (LOQ) spanned from 2.79 to 9.57 μg/mL, respectively. The relative standard deviations (RSD) values of precision test, intra- and inter- day, were less than 1.28 % and 1.14%. The accuracy test results ranged from 100.81% to 105.31% and RSD values were less than 1.26 %. These results showed that the HPLC-DAD method was very reliable and accurate for the quantity analysis of eight compounds in *D. sophia* extract for quality control.

Keywords – *Descurainia Sophia,* Helveticoside, HPLC-DAD, Quality control

Introduction

Swift industrialization has led to a surge in global mobility and concurrent environmental pollution. These environmental shifts have highlighted the pressing issue of escalating virus-induced diseases.[1] Among these ailments, influenza has successfully infiltrated avian and mammalian populations, posing a considerable threat to human health.[2] While pharmaceutical interventions utilizing chemical compounds are available, they frequently come with constraints and adverse effects. Consequently, there has been a notable upswing in research endeavors to pinpoint natural products exhibiting exceptional antiviral properties. Although promising natural compounds have been identified, the path from discovery to effective treatment is intricate, necessitating further research and development efforts.[3-5]

Herbal medicines have played a significant role in preventing and treating various diseases across the world for centuries. It is well-documented that many herbs possess antioxidant properties and act as effective anti-inflammatory agents.[6-7] Furthermore, herbal medicines are generally known for their minimal side effects. In recent years, with a growing focus on health and wellness, research on natural products has continued to expand, leading to active development in the field of health-enhancing functional foods and quasi-drugs derived from natural sources.[8] However, it is important to recognize that the quality of herbal plants can vary considerably depending on factors such as their origin, cultivation methods, timing of harvesting, and processing techniques. This variability underscores the necessity for a more systematic and efficient approach to the management of medicinal herbs. To address this, specific analytical methods using high-performance liquid chromatography (HPLC) have been recently established to individually assess the components of these natural products.[9] Nevertheless, natural

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products often contain a diverse array of compounds, and when utilized as therapeutic agents, their effects tend to be multifaceted and complex. Currently, most herbal medicines are subject to individualized analysis methods, which can result in significant economic and time costs. A more efficient approach to quality control can be achieved by adopting simultaneous analysis of multiple components. This streamlined approach has the potential to enhance the assessment of herbal medicines, making the process more cost-effective and time-efficient.[10]

In a previous study, it was noted that *D. sophia* demonstrated potent antiviral activity against influenza, and we successfully isolated compounds with anti-influenza properties from the *D. sophia* extract.[11] In this study, the simultaneous analysis of *D. sophia* was established by using HPLC-DAD method. We also verified the established method validation and confirmed the availability of this analysis method to *D. sophia* extract for quality control.

Experimental

Plant materials – The dried whole plants of *D. sophia* were procured from the Chunjigayakcho medicinal herbs market in Seoul, Korea. A voucher specimen (CH198M) has been duly deposited in the Natural Product Laboratory at Kangwon National University in Chuncheon, Korea. High-performance liquid chromatography (HPLC) grade solvents, including water, methyl alcohol, and acetonitrile, were sourced from J.T. Baker (U.S.A), while trifluoroacetic acid (TFA) was obtained from DAE JUNG in Seoul, Korea.

HPLC analysis – *D. sophia* solutions were subjected to analysis using HPLC-DAD (High-Performance Liquid Chromatography with Diode Array Detection). The HPLC system utilized in this study was a Dionex instrument comprising an LPG 3X00 pump, an ACC-3000 auto-sampler, a DAD-3000(RS) diode array UV/VIS detector, and a column oven. Each sample was introduced and separated through a Dionex C_{18} column (5 μm, 120 Å, 4.6 mm × 150 mm) maintained at a temperature of 25°C. The mobile phase consisted of a mixture of 0.1% trifluoroacetic acid (TFA) in water and acetonitrile. Samples were injected at a volume of 10 μl. The UV wavelength settings were as follows: 200 nm, 254 nm, 280 nm, and 330 nm, respectively. Chromatograms were acquired at a wavelength of 200 nm to allow for the simultaneous and comprehensive visualization of all peaks.

Preparation of standard solutions for HPLC analysis – Standard stock solutions were prepared by dissolving the five isolated compounds - daucosterol (**1**), helveticoside (**2**), isorhamnetin (**3**), quercetin (**4**), sinapic acid (**5**) - in 80% methanol. The separated compounds exhibited high purity, with daucosterol at 94.2%, helveticoside at 95.6%, isorhamnetin at 92.8%, quercetin at 93.1%, and sinapic acid at 91.2%, respectively. The concentrations of these stock solutions were 512.32 μg/mL, 766.23 μg/mL, 562.11 μg/mL, 663.12 μg/mL, and 879.33 μg/mL, respectively (Fig. 1). Subsequently, working solutions at 1/2, 1/4, 1/8, and 1/16 dilutions were prepared by mixing each standard stock solution with 80% methanol. These diluted samples were then filtered through a 0.45 μm pore-sized filter to ensure purity and consistency. These meticulously prepared working solutions served as the basis for establishing the calibration curve.

Preparation of *D. sophia* extract sample – A total of 100 grams of dried whole plants of *D. sophia* were subjected to extraction using 80% methanol. This extraction process was carried out three times, each time through ultrasonication for a duration of 90 minutes. The resulting extracts were then concentrated under reduced pressure to obtain a powdered form. These powdered extracts were subsequently dissolved in HPLC grade methanol (MeOH) to achieve a concentration of 20 mg/mL Prior to HPLC analysis, the *D. sophia* sample was filtered through a 45 μm membrane filter to ensure the removal of any particulate matter or impurities. Subsequently, the filtered sample was injected into the HPLC system for analysis.

Validation of method

Linearity – The calibration curves were established by plotting the peak area against the concentration of each working solution. To create the working solutions, we dissolved five different compounds, referred to as compound **1-5**, in methanol. The working solutions were prepared at the concentration of compound **1** (32.02, 64.04, 128.08, 256.16 and 512.32 μg/mL), compound **2** (47.89, 95.78, 191.56, 383.12 and 766.23 μg/mL), compound **3** (35.14, 70.27, 140.53, 281.06 and 562.11 μg/mL), compound **4** (41.45, 82.89, 165.78, 331.56 and 663.12 μg/mL) and compound **5** (54.96, 109.92, 219.83, 439.67 and 879.33 μg/mL), respectively. Linear regression equations of the form $y = ax \pm b$ were computed, with 'x' representing concentration and 'y' representing peak areas for each compound. The linearity of these calibration curves was assessed using the coefficient of determination (r^2), which provides insight into the accuracy of our quantification method. To ensure precision and reliability, we conducted triplicate analyses for each working standard concentration.

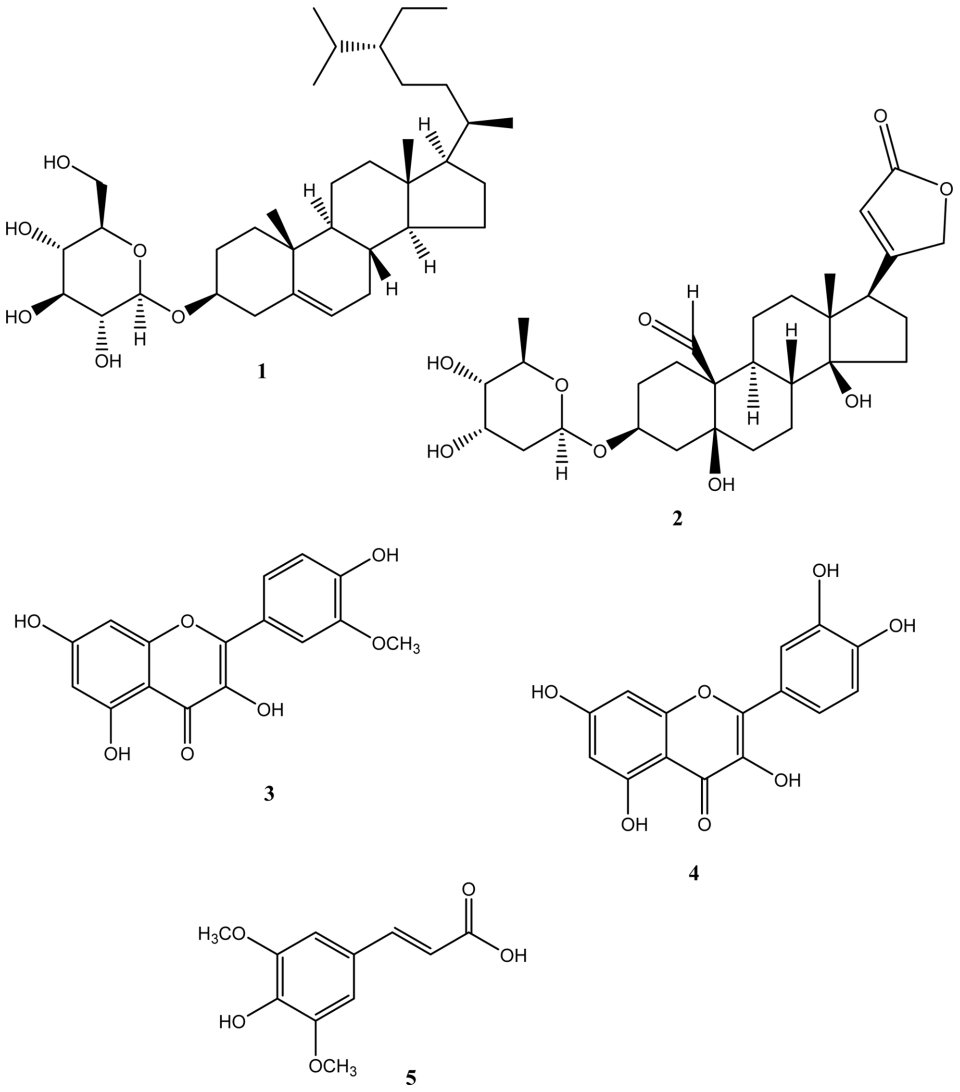

Fig. 1. Chemical structures of compounds **1-5** isolated from *Descurainia sophia* extract.

Limit of detection (LOD) and limit of quantification (LOQ) – The determination of the Limit of detection (LOD) involved identifying the lowest sample concentration that could be reliably detected. Meanwhile, the Limit of quantification (LOQ) was established as the lowest concentration of compounds attainable by injecting a diluted standard solution, ensuring that the signal-to-noise ratio fell within the range of 3.3 to 10.

Repeatability and Precision – The precision of the method was assessed through both intra-day and inter-day injections. The intra-day experiment involved three consecutive injections on the same day, while the inter-day experiment consisted of injections over the course of three days. In both tests, three different concentrations, as confirmed by the calibration curves, were examined. Three distinct concentrations were employed for each compound such as compound **1** (32.02, 64.04 and 128.08 μg/mL), compound **2** (47.89, 95.78 and 191.56 μg/mL), compound **3** (35.14, 70.27 and 140.53 μg/mL), compound **4** (41.45, 82.89 and 165.78 μg/mL) and compound **5** (54.96, 109.92 and 219.83 μg/mL), respectively. Repeatability and precision were quantified using the relative standard deviation (RSD, %).

Accuracy tested by recovery test – To assess the accuracy of the method, a recovery study was conducted by adding precise quantities of compound solutions to *D. sophia* samples. Three distinct concentrations were employed for each compound such as compound **1** (32.02, 64.04 and 128.08 μg/mL), compound **2** (47.89, 95.78 and 191.56 μg/mL), compound **3** (35.14, 70.27 and 140.53 μg/mL), compound **4** (41.45, 82.89 and 165.78 μg/mL) and compound **5** (54.96, 109.92 and 219.83 μg/mL), respectively.

The recovery percentage was determined using the formula: (detected amount – original amount)/spiked amount × 100. This calculation allowed us to evaluate the method's accuracy and its ability to reliably recover known compound amounts when added to the samples.

Sample analysis using established method – The effectiveness of the optimized simultaneous determination method was confirmed through the analysis of a complete *D. sophia* sample. Utilizing the calibration curves for the five compounds, we accurately calculated the quantities of these compounds within the *D. sophia* extract. Notably, all five compounds were successfully detected in the chromatogram without any overlapping peaks, ensuring precise and reliable quantification.

Statistical analysis – All experiments were replicated a minimum of three times to ensure robustness and reliability. The results were presented as the mean ± standard deviation (S.D.), and statistical significance was determined using a one-way analysis of variance (ANOVA) followed by Tukey's test. Significance levels were denoted as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, indicating the degrees of statistical significance. In cell experiments, data were expressed as a relative percentage, with the control group set as the reference at 100%.

Results and discussion

The objective of optimizing the HPLC experiment was to enhance the separation efficiency and peak resolution of the target compounds within a shorter run-time for *D. sophia*. Achieving this goal involved extensive preliminary tests to determine the most effective HPLC-DAD condi-

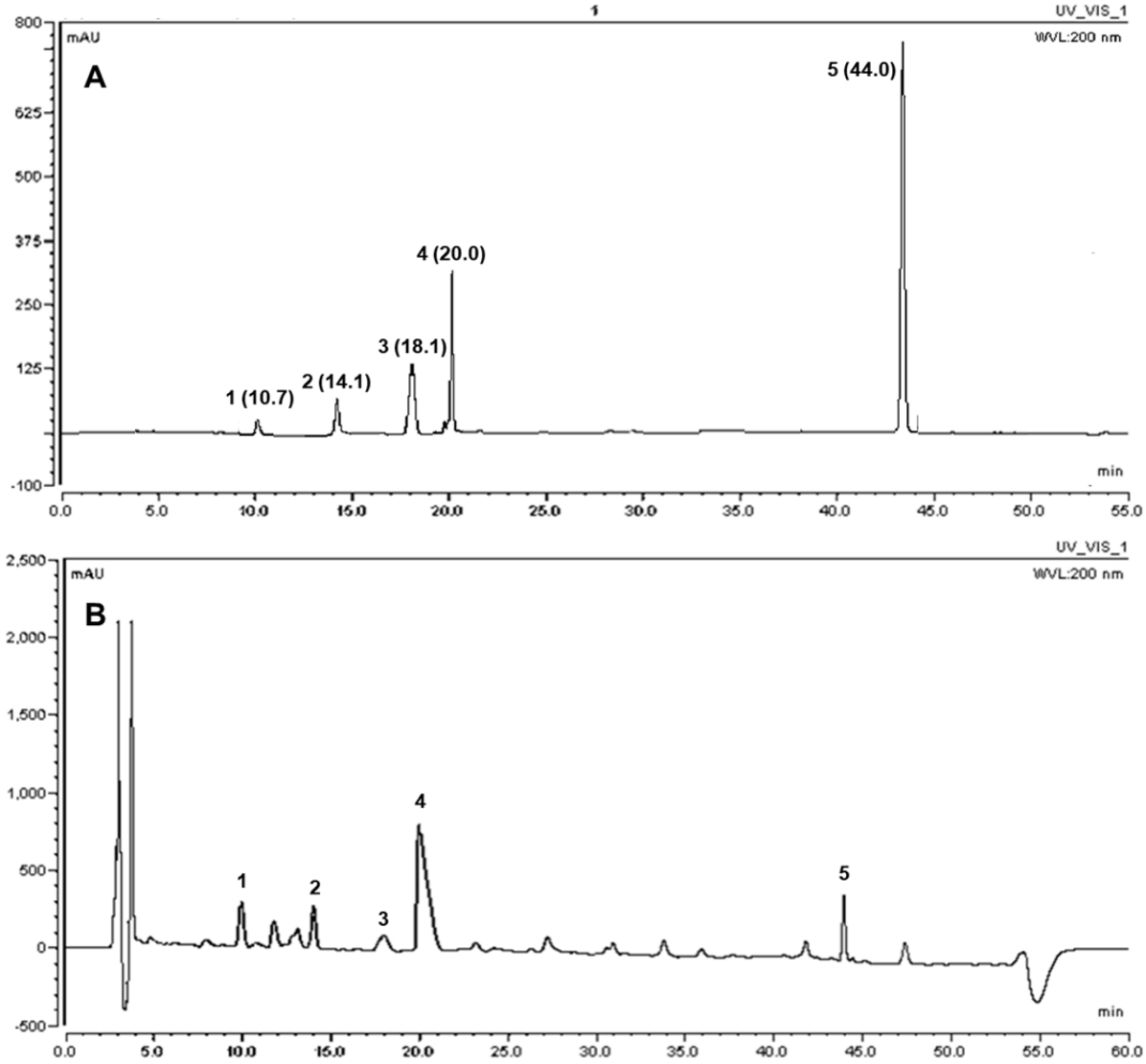

Fig. 2. The HPLC chromatogram of mixture of five standard compounds (A) and *D. sophia* extract (B).

tions, including the selection of an appropriate column, mobile phase, and UV spectrum wavelength. The Dionex C_{18} column, known as a reversed-phase column, was chosen as the optimal column for this purpose. The mobile phase employed a multi-step gradient solution system, comprising 0.1% trifluoroacetic acid (TFA) in water (A) and methanol (B). The most suitable separation conditions for the isolated compounds were as follows: 0-15 minutes, 15% B; 15-25 minutes, 15-25% B; 25-35 minutes, 25-35% B; and 35-45 minutes, 35-40% B; 45-55 minutes, 40%, all at a flow rate of 1.0 mL/min. This gradient method allowed for the separation of five distinct peaks within a 50-minute timeframe. For detection, the diode-array detector (DAD) wavelength was tested at four different settings (200 nm, 254 nm, 280 nm, and 330 nm), and the chromatograms were ultimately selected at 200 nm based on the UV spectrum. The column temperature was found to have no significant impact on the separation, so it was maintained at room temperature. The identification of each compound's peak was confirmed by comparing the retention time and UV spectrum to their respective marker solutions. The separated compounds demonstrated retention times of 10.7 min for daucosterol, 14.1 min for helveticoside, 18.1 min for isorhamnetin, 20.0 min for quercetin, and 44.0 min for sinapic acid on HPLC chromatograms. The chromatograms displaying the entire range of *D. sophia* compounds are illustrated in Fig. 2.

To validate this method, we conducted experiments to assess linearity, determine detection and quantification limits, evaluate precision and accuracy, and measure recovery.

The regression equations were established through five concentration trials of each standard, conducted in triplicate. In the linear regression equation ($y = ax + b$), 'x' represents the concentration of the marker compositions, and 'y' corresponds to the peak area. The slope and intercept of the calibration curve were determined using this equation. The high correlation coefficients ($R^2 > 0.9994$) indicate excellent linearity for all calibration curves within the specified test ranges, as presented in Table 1. Based on this linear regression analysis, the limits of detection (LOD) ranged from 0.93 to 3.19 μg/mL, while the limits of quantification (LOQ) fell within the range of 2.79 to 9.57 μg/mL, respectively. These results demonstrate the method's capability to detect and quantify compounds even at minimal concentrations.

To assess the repeatability and precision of the method, we conducted both within-day tests (intra-day analysis, n = 3) and intermediate-day tests (inter-day analysis, n = 3). The intra-day analysis was carried out three times within a single day, while the inter-day analysis was conducted on three different days (1st, 3rd, and 5th days). The Relative Standard Deviation (RSD) values obtained for the intra-day tests ranged from 0.04% to 1.28%, while for the inter-day tests, they ranged from 0.45% to 1.14%, as detailed in Table 2. These results clearly demonstrate the method's outstanding reproducibility and precision.

The method's accuracy underwent evaluation through a recovery test, wherein each sample underwent triplicate testing with the addition of three different concentrations of solutions to the total *D. sophia* standard solution. The results yielded mean recovery percentages as follows: 102.12% for compound **1**, 101.65% for compound **2**, 101.67% for compound **3**, 102.60% for compound **4** and 102.60% for compound **5** (Table 3). To ascertain the accuracy of the methods, the relative standard deviation (RSD) was employed, resulting in % RSD values of 0.82% for compound **1**, 0.61% for compound **2**, 1.07% for compound **3**, 0.83% for compound **4** and 0.57% for compound **5** (Table 3). Importantly, all analyzed compounds exhibited recovery percentages within the ideal range, and the associated RSDs were consistently low. This dataset unequivocally demonstrates the method's high accuracy and confirms its suitability for the precise quantitative analysis of *D. sophia* samples.

The method outlined in the previous section was effectively applied to investigate the presence of five target compounds within *D. sophia*. As detailed in Table 4, the quantities of these tested compounds exhibited

Table 1. The regression data, LOD and LOQs of five isolated compounds analyzed by HPLC-DAD

Compound	Regression equation[a]	R^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
1	y = 0.0236x + 0.0112	0.9996	12.5-725	1.21	3.63
2	y = 0.0845x + 0.1418	0.9994	6.5-750	0.93	2.79
3	y = 0.0752x + 0.1616	0.9998	10.5-650	1.95	5.85
4	y = 0.1049x + 0.3161	1.0000	5.4-725	3.19	9.57
5	y = 0.2166x + 0.0839	0.9999	5.4-625	2.59	7.77

[a] *y*: peak area, *x*: amount (μg)

Table 2. Intra- and inter- day precision data of five compounds

Compound	Concentration (μg/mL)	Intra-day			Inter-day		
		Mean (μg/mL)	RSD[a] (%)	Accuracy (%)	Mean (μg/mL)	RSD[a] (%)	Accuracy (%)
1	128.08	130.21 ± 0.22	0.04	101.66	131.06 ± 0.42	0.62	102.33
	64.04	66.71 ± 1.12	0.32	104.17	65.12 ± 0.44	0.76	101.69
	32.02	33.12 ± 2.48	0.85	103.44	32.89 ± 0.61	0.45	102.72
2	191.56	191.14 ± 1.25	0.54	99.78	193.22 ± 0.55	0.49	100.87
	95.78	97.12 ± 0.69	0.28	101.40	97.23 ± 0.11	0.65	101.51
	47.89	48.72 ± 0.72	0.68	101.73	49.15 ± 0.83	0.87	102.63
3	140.53	143.22 ± 1.22	0.59	101.91	142.65 ± 0.19	0.55	101.51
	70.27	72.11 ± 0.75	0.89	102.62	72.17 ± 0.94	0.83	102.70
	35.14	35.69 ± 0.65	0.37	101.57	36.78 ± 1.18	1.14	104.67
4	165.78	167.22 ± 0.59	1.28	100.87	168.46 ± 0.59	0.67	101.62
	82.89	84.32 ± 1.16	0.61	101.73	84.34 ± 0.84	0.49	101.75
	41.45	43.21 ± 0.65	1.24	104.25	43.33 ± 0.43	0.86	104.54
5	219.83	224.31 ± 0.91	0.32	102.03	218.59 ± 0.85	0.83	99.44
	109.92	114.89 ± 0.53	0.23	104.52	111.47 ± 1.31	0.85	101.41
	54.96	57.11 ± 0.63	0.77	103.91	57.32 ± 0.65	0.49	104.29

[a] Relative Standard Deviation

Table 3. Recovery test of the five compounds of *D. sophia*

Compound	Spiked (μg/mL)	Found (μg/mL)	RSD (%)	Recovery (%)*
1	128.08	129.12 ± 0.42	0.46	100.81
	64.04	65.28 ± 0.65	0.83	101.94
	32.02	33.18 ± 1.18	0.81	103.62
2	191.56	193.34 ± 0.85	0.38	100.93
	95.78	97.31 ± 0.38	0.84	101.60
	47.89	49.05 ± 0.36	0.61	102.42
3	140.53	142.54 ± 1.59	0.83	101.43
	70.27	71.68 ± 0.19	1.11	102.01
	35.14	35.69 ± 0.65	1.26	101.57
4	165.78	168.32 ± 0.38	0.76	101.53
	82.89	83.69 ± 0.47	0.55	100.97
	41.45	43.65 ± 0.69	1.19	105.31
5	219.83	223.18 ± 0.57	0.76	101.52
	109.92	113.12 ± 0.49	0.38	102.91
	54.96	56.81 ± 0.56	0.56	103.37

[a] Recovery (%) = (amount found – original amount)/spiked amount ×100 %

notable variations. The quantities of the five compounds within the *D. sophia* extract were as follows: daucosterol (**1**) at 10.68 mg/g, helveticoside (**2**) at 12.83 mg/g, isorhamnetin (**3**) at 11.48 mg/g, quercetin (**4**) at 28.07 mg/g, and sinapic acid (**5**) at 20.44 mg/g. respectively (Table 4). The optimized HPLC-DAD conditions allowed for the simultaneous detection of all five compounds.

The developed HPLC-DAD method was employed for the qualitative analysis of compounds within *D. sophia*. It

Table 4. Contents of five compounds in *D. sophia* extract

Compounds	Content (μg/mg)
1	10.68 ± 0.54
2	12.83 ± 0.76
3	11.48 ± 0.32
4	28.07 ± 0.63
5	20.44 ± 0.83

enabled the simultaneous and rapid detection of five compounds within a remarkably short 55-minute timeframe. Furthermore, the optimized HPLC-DAD method demonstrated a high degree of selectivity and accuracy, a fact substantiated through a comprehensive validation process that included assessments of linearity, limits of detection and quantification, repeatability, precision, and accuracy.

In summary, this novel method significantly enhances the separation efficiency of *D. sophia* constituents, resulting in improved peak resolution. The validation tests, covering linearity, detection and quantification limits, precision, accuracy, and recovery, underscore the method's reproducibility and precision. These data collectively support the utility of this simultaneous analysis method for both qualitative and quantitative assessments, rendering it a valuable tool for enhancing the quality control processes associated with *D. sophia* through the simultaneous quantification of its five key compounds.

Conflict of interest

The authors have declared that there are no conflicts of interest.

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