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A Study on changes in ingredients according to the manufacturing methods of Samhwangsasim-tang

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Abstract: Objective: We conducted to investigate and compare changes in the content of Samhwangsasim-tang according to different manufacturing methods. **Methods**: The content test of Baicaline, Berberine, and Sennoside A in Samhwangsasim-tang was analyzed using HPLC equipment of LC-2030C 3D. YMC-Pack ODS-A Column was used to separate the surface components. In the HPLC analysis, mobile phase prepared A-0.05% PA in water and B-0.05% PA in ACN, set the flow rate to 1.0 mL/min, and analyzed according to the gradient condition. **Results:** As a result of the analysis experiment with each extract, the content of Berberine was higher in Formulation-1: Samhwangsasim-tang mixture after extracting individual medicinal herbs, and Formulation-3: Samhwangsasim-tang mixture after extracting individual medicinal herbs+excipient. The content of Baicalin and Sennoside A were higher in Formulation-2: Samhwangsasim-tang extracts, and Formulation-4: Samhwangsasim-tang extracts+excipient. In addition, our results show that it was a significant decrease in the average content of berberine, baicalin, and sennoside A in Samhwangsasim-tang when excipients are included, compared to when they are not. **Conclusion**: Our finding suggested that the interaction between components due to mixed extraction at specific ratios might enhance or reduce the extraction of main components. In addition, it might be able to be attributed to the interference caused by the addition of excipients when the analysis of marker compound content. Therefore, this indicates the need for research on various extraction and manufacturing methods to enhance the extraction efficiency of marker compounds when producing herbal formulations.

Keywords: Samhwangsasim-tang, Baicalin, Berberine, Sennoside A, High performance liquid chromatography.

1. Introduction

The traditional herbal formula "Samhwangsasim-tang (三黃瀉心湯)" was first recorded in the ancient Chinese medical text "Geum-Gwe-Yo-Rak (金匱要略)" authored by Zhang Zhongjing during the Han dynasty. This formula consists of three essential herbal components - Scutellariae Radix, Coptidis Rhizoma, and Rhei Rhizoma. Throughout history, it has been employed to mitigate excessive heat within the bloodstream and alleviate symptoms attributed to blood heat, including occurrences like upper body bleeding, skin rashes, abdominal discomfort, insomnia, and dryness. In contemporary medical practice, the formula finds application in treating various conditions such as gastric ulcers, gastritis, hypertension, and gastrointestinal bleeding (1-3). Experimental studies have shown that "Samhwangsasim-tang (三黃瀉心湯)" has demonstrated efficacy in relaxing blood vessel walls and reducing blood pressure, as reported domestically (4,5).

In international research, the direct intravenous administration of water-extracted "Samhwangsasim-tang (三黃瀉心湯)" has been shown to have a therapeutic or protective effect in a hypotensive model induced by lipopolysaccharides (LPS), leading to a reduction in blood pressure. Furthermore, in a hypertension model induced by a Thromboxane A2 analogue, a blood pressure-lowering effect has been demonstrated (6-9).

One of the constituent ingredients of Samhwangsasim-tang (三黃瀉心湯), Huanglian, is derived from the rhizome of *Coptis japonica* Makino (Ranunculaceae). It possesses a bitter taste (味苦) and cold nature (性寒), and is renowned for its efficacy in clearing internal heat (心胃實熱) through heat-clearing and fire-purging actions. It is used to treat symptoms

such as poor appetite (消穀善飢) and dry mouth/thirst (口乾口渴). Huangqin, derived from the root of *Scutellaria baicalensis* Georgi (Lamiaceae), possesses a bitter taste (味苦) and cold nature (性寒), which attributes to its efficacy in clearing heat and dampness (清熱燥濕), as well as purging fire and detoxifying (瀉火解毒). It is also used to clear lung heat (清肺火). Da Huang, derived from *Rheum undulatum* of the Polygonaceae family, is a perennial herbaceous plant known for stimulating the secretion of pancreatic and bile fluids. It exhibits diuretic effects and finds application in traditional medicine as a cathartic, antihypertensive, antipyretic, anti-inflammatory, and analgesic agent (10, 11).

In contemporary society, Samhwangsasim-tang (三黃瀉心湯) is circulated through pharmaceutical companies. According to actual research findings, commercially available general pharmaceuticals based on traditional herbal medicine frequently incorporate blended extracts obtained by combining various herbal components. Nonetheless, in traditional Korean medicine clinics, individual herbal extracts are also employed before creating compounded formulations. In this study, we conducted research to investigate the changes in composition and activity of traditional herbal medicine formulations resulting from modifications in their manufacturing processes. We used Samhwangsasim-tang as the subject of our investigation. Samhwangsasim-tang is composed of Huanglian, Huangqin, and Da Huang. Therefore, we conducted experiments using Huanglian, Huangqin, and Da Huang. Through High-Performance Liquid Chromatography (HPLC) analysis, we compared the changes in constituent components between the extracts of individual herbal ingredients and the mixed formulation of Samhwangsasim-tang.

2. Materials and Methods

2.1. Experimental Materials

For HPLC analysis, we obtained the reference compounds Baicalin and Berberine (both from Chem Faces; Wuhan, Hubei, China), with a purity of over 98%, as well as Sennoside A (sourced from MedChemExpress; NJ, USA) with a purity of 99.44%. The solvents used for the analysis included HPLC-grade Acetonitrile and Methanol, purchased from Fisher Scientific Korea Ltd. (Seoul, South Korea), and Phosphoric Acid sourced from DUKSAN (Ansan, South Korea). Regarding the constituents of Samhwangsasim-tang, we obtained Huangqin, Huanglian, and Da Huang from Hanpung Pharmaceutical Co., Ltd. (Wanju, South Korea), a specialized pharmaceutical company in traditional herbal medicine. These ingredients were chosen based on their suitability for testing according to the Korean Pharmacopoeia (KP).

2.2. HPLC Analysis Conditions

The quantification of the key constituents, Baicalin, Berberine, and Sennoside A, within Samhwangsasim-tang was carried out utilizing the LC-2030C 3D High-Performance Liquid Chromatography (HPLC) system (SHIMADZU; KYOTO, JAPAN), as illustrated in Figure 1. Separation of these major components was accomplished using the YMC-Pack ODS-A Column (250 X 4.6 mm I.D, S-5 μm, 12nm, YMC KOREA Co., Ltd, Seongnam, South Korea). The column temperature was maintained at 25 ℃, and the detection wavelength was set at 230nm. The HPLC analysis employed a mobile phase composition of A-0.05% PA in water and B-0.05% PA in ACN, with a flow rate of 1.0 mL/min. The analysis was conducted following the gradient conditions outlined in Figure 1.

2.3. Preparation of Standard Solutions

Accurate amounts of Baicalin, Berberine, and Sennoside A, the three representative marker components, were carefully weighed and dissolved in Methanol to achieve a concentration of 50mg/L. Ultrasonic extraction was conducted for 30 minutes. The formulated stock solution of standard substances was stored at 4 ℃ and employed for analysis during the study.

2.4. Preparation of Individual Extract Mixtures and Decoction of Samhwangsasim-tang

For the creation of individual extract mixtures of Samhwangsasim-tang, Huanglian, Huangqin, and Da Huang were each precisely weighed at 50g and placed into separate round-bottom flasks containing 500mL of primary distilled water. Utilizing a heating mantle (MS-DM; MISUNG Scientific Instruments Co., Ltd, Seoul, South Korea), reflux extraction was

conducted at 100 ℃ for 2 hours. The resultant extracts were then subjected to filtration, and the filtrates were vacuum-concentrated at temperatures below 60 ℃ to yield concentrated extracts of each ingredient. These individual extracts were subsequently freeze-dried to produce the individual extract mixtures. For the mixture, 30mg of Huanglian extract, 30mg of Huangqin extract, and 40mg of Da Huang extract (in a ratio of 3:3:4) were combined to create a 100mg mixture; Formulation-1 (Samhwangsasim-tang mixture after extracting individual medicinal herbs). Following the preparation of the individual extract mixtures, the formulation of commercially available Samhwangsasim-tang from Hanpung Pharmaceutical Co., Ltd. (Wanju, South Korea) was executed by combining 48mg of the individual extract mixture, 82mg of lactose monohydrate, and 192mg of cornstarch, resulting in a 320mg mixture of Samhwangsasim-tang (including excipients); Formulation-3 (Samhwangsasim-tang mixture after extracting individual medicinal herbs with excipient).

To prepare the decoction of Samhwangsasim-tang, Huanglian, Huangqin, and Da Huang were each weighed at 30g, 30g, and 40g (in a ratio of 3:3:4), totaling 100g of raw materials. An additional 1000mL of primary distilled water was added, and reflux extraction was carried out at 100 ℃ for 2 hours using a heating mantle (MS-DM; MISUNG Scientific Instruments Co., Ltd, Seoul, South Korea). The resulting extracts were filtered, and the filtrates were vacuum-concentrated at temperatures below 60 ℃ to obtain condensed extracts. These extracts were then freeze-dried to generate the final Samhwangsasim-tang decoction; Formulation-2 (Samhwangsasim-tang extracts). For the preparation of commercially available Samhwangsasim-tang from Hanpung Pharmaceutical Co., Ltd. (Wanju, South Korea), 48mg of the decoction, 82mg of lactose monohydrate, and 192mg of cornstarch were combined to yield a 320mg mixture of Samhwangsasim-tang (including excipients); Formulation-4 (Samhwangsasim-tang extracts with excipient). In this manner, four formulations were produced and analyzed, with 20mg of each sample collected and placed into 10mL volumetric flasks. Following mixing with 50% MeOH, the samples were subsequently filtered through a 0.45 μm syringe filter (BioFact, Daejeon, South Korea) for further analysis.

3. Results

3.1. Establishment of Analytical Condition

To analyze the primary constituents, namely Baicalin, Berberine, and Sennoside A, which are characteristic compounds of *Scutellaria baicalensis* Georgi, we fine-tuned our column selection, mobile phase gradient conditions, solvent options, and UV detection wavelength for the analytical process (Figure 1).

Operating condition				
UV Absorbance	240nm			
Column temp.	25℃			
Injection vol.	10㎕			
Mobile phase A	0.05% PA in Water			
Mobile phase B	0.05% PA in Acetonitrile			
Gradient profile	**Time (min)**	**%A**	**%B**	**Flow(ml/min)**
	0	86	14	1.0
	15	75	25	1.0
	30	50	50	1.0
	35	30	70	1.0
	40	86	14	1.0
	45	86	14	1.0

Figure 1. HPLC conditions for the analysis of various extracts for each formulation of Samhwangsasim-tang

We compared two columns, namely YMC Pack ODS-A 5µm 250 x 4.6mm 12nm and CAPCELL PAC ODS-250 x 4.6mm. Our mobile phase employed 0.05% phosphoric acid (PA) in water for solvent A, while solvent B consisted of 0.05% PA in acetonitrile. Through meticulous exploration of various analytical conditions, we opted for the YMC Pack ODS-A 5µm 250 x 4.6mm 12nm column to enhance separation efficiency. Our optimized analytical conditions were established by incorporating phosphoric acid into the mobile phase to enhance peak symmetry.

3.2. Chromatograms and UV Spectra for Different Preparation Methods

First, a comparison was carried out between the UV spectra and HPLC chromatograms of Berberine, known as the primary component of Huanglian, and Baicalin, the principal component of Huangqin. Berberine, an alkaloid found in Huanglian (*Coptis chinensis*) and Huangbai (*Phellodendron amurense*), is recognized for its diverse pharmacological effects, including anti-inflammatory activity (12). The comparison of Berberine's UV spectrum and HPLC chromatogram showed a retention time (R.T) of 26.854 for standard analysis. For the Samhwangsasim-tang individual extract mixture (Formulation-1) post extraction of individual medicinal herbs, the R.T was 27.638; for Samhwangsasim-tang extracts (Formulation-2), the R.T was 27.692; for Samhwangsasim-tang individual extract mixture + excipient (Formulation-3), the R.T was 27.735; for Samhwangsasim-tang extracts + excipient (Formulation-4), the R.T was 27.737; and for Huanglian extract, the R.T was 26.845. All retention times fell within a 0.5 range, confirming consistent components within the extracts (Figures 2-8).

Baicalin, also known as 5,6-Dihydroxy-4-oxo-2-phenyl-4H-1-benzopyran-7-yl-ß-D-glucopyranosiduronic acid, is the predominant component found in Huangqin (13). A comparison of the UV spectrum and HPLC chromatogram of baicalin revealed a retention time (R.T) of 26.854 for standard analysis. For the Samhwangsasim-tang individual extract mixture (Formulation-1) after extracting individual medicinal herbs, the R.T was 26.857. For Samhwangsasim-tang extracts (Formulation-2), the R.T was 26.851, for Samhwangsasim-tang individual extract mixture + excipient (Formulation-3), the R.T was 26.899, for Samhwangsasim-tang extracts + excipient (Formulation-4), the R.T was 26.860, and for Huangqin extract, the R.T was 26.845. All retention times fell within a 0.5 range, and the UV spectra were consistent, confirming the presence of identical components within the extracts (Figures 2-8).

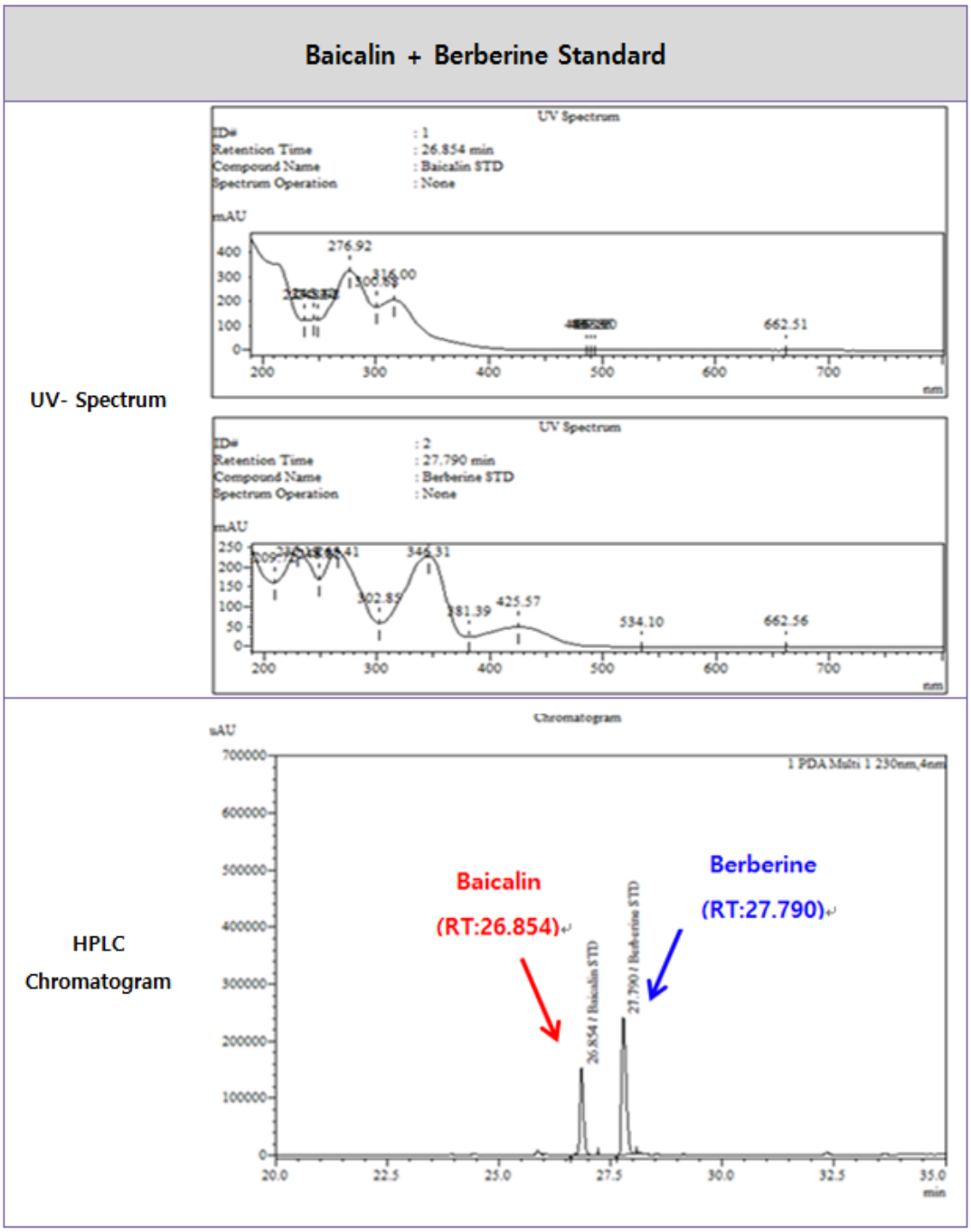

Figure 2. HPLC chromatogram and UV spectrum of berberine and baicalin as standards.

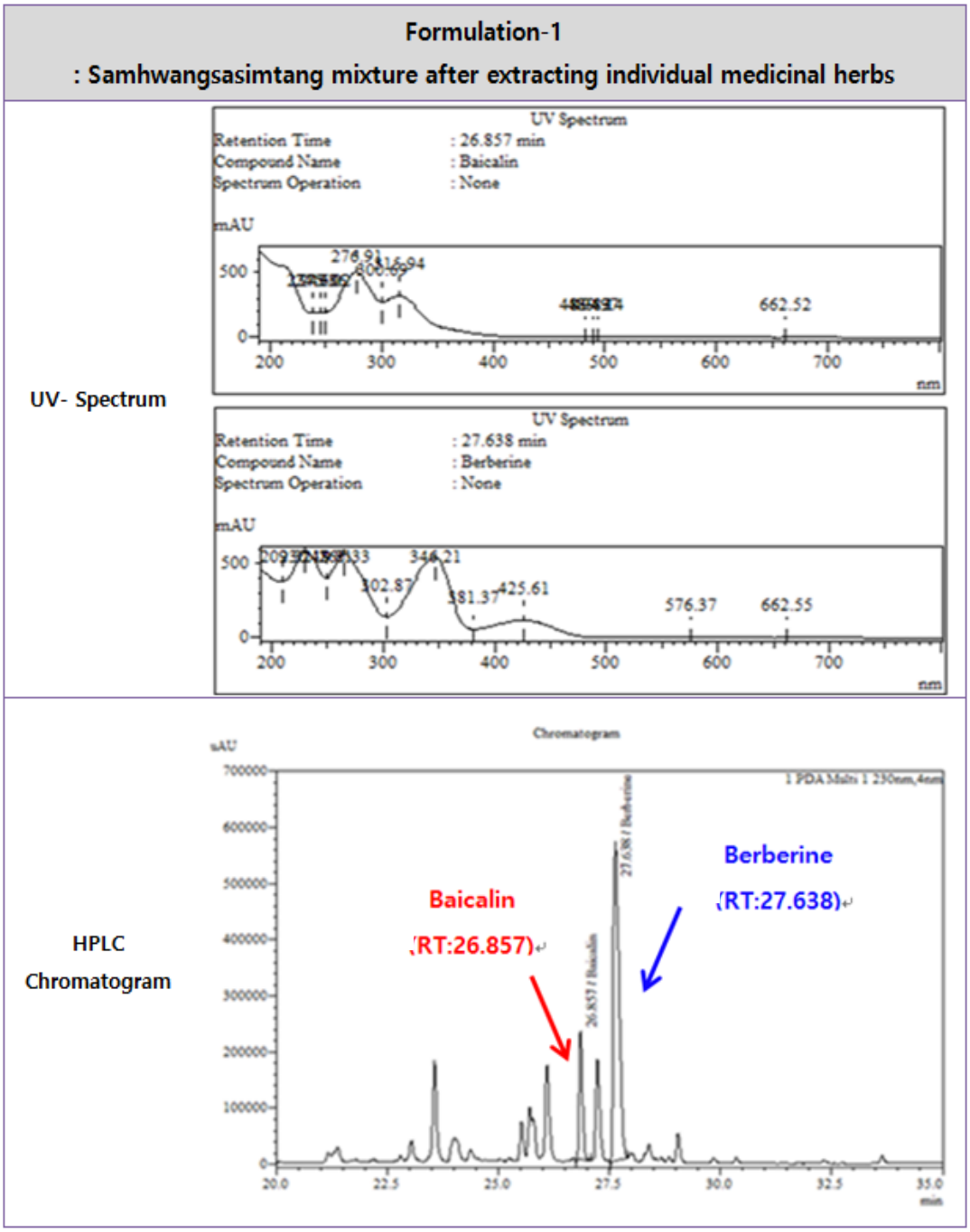

Figure 3. HPLC chromatogram and UV spectrum of berberine and baicalin in Formulation-1: Samhwangsasim-tang mixture after extracting individual medicinal herbs

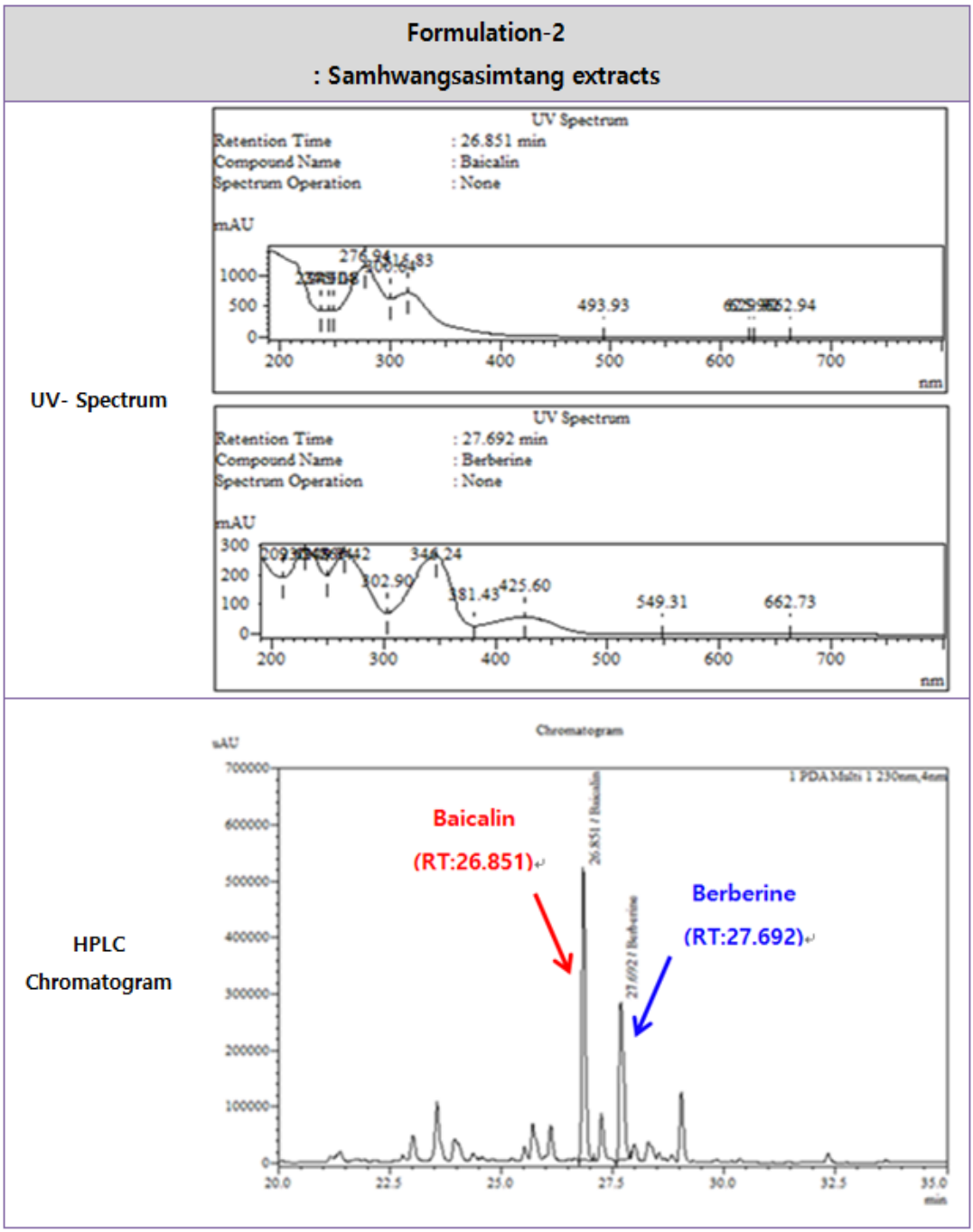

Figure 4. HPLC chromatogram and UV spectrum of berberine and baicalin in Formulation-2: Samhwangsasim-tang extracts

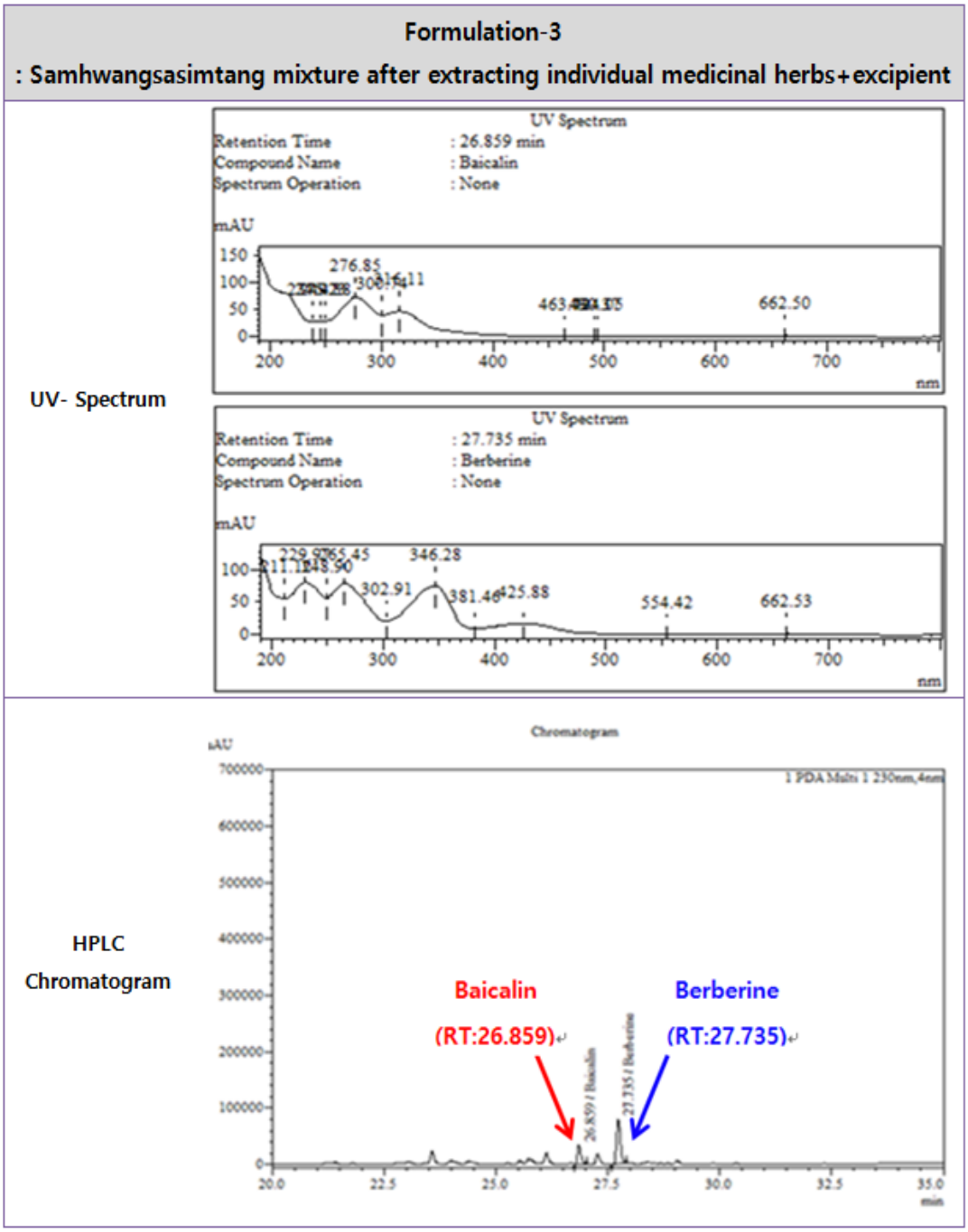

Figure 5. HPLC chromatogram and UV spectrum of berberine and baicalin in Formulation-3: Samhwangsasim-tang mixture after extracting individual medicinal herbs+excipient

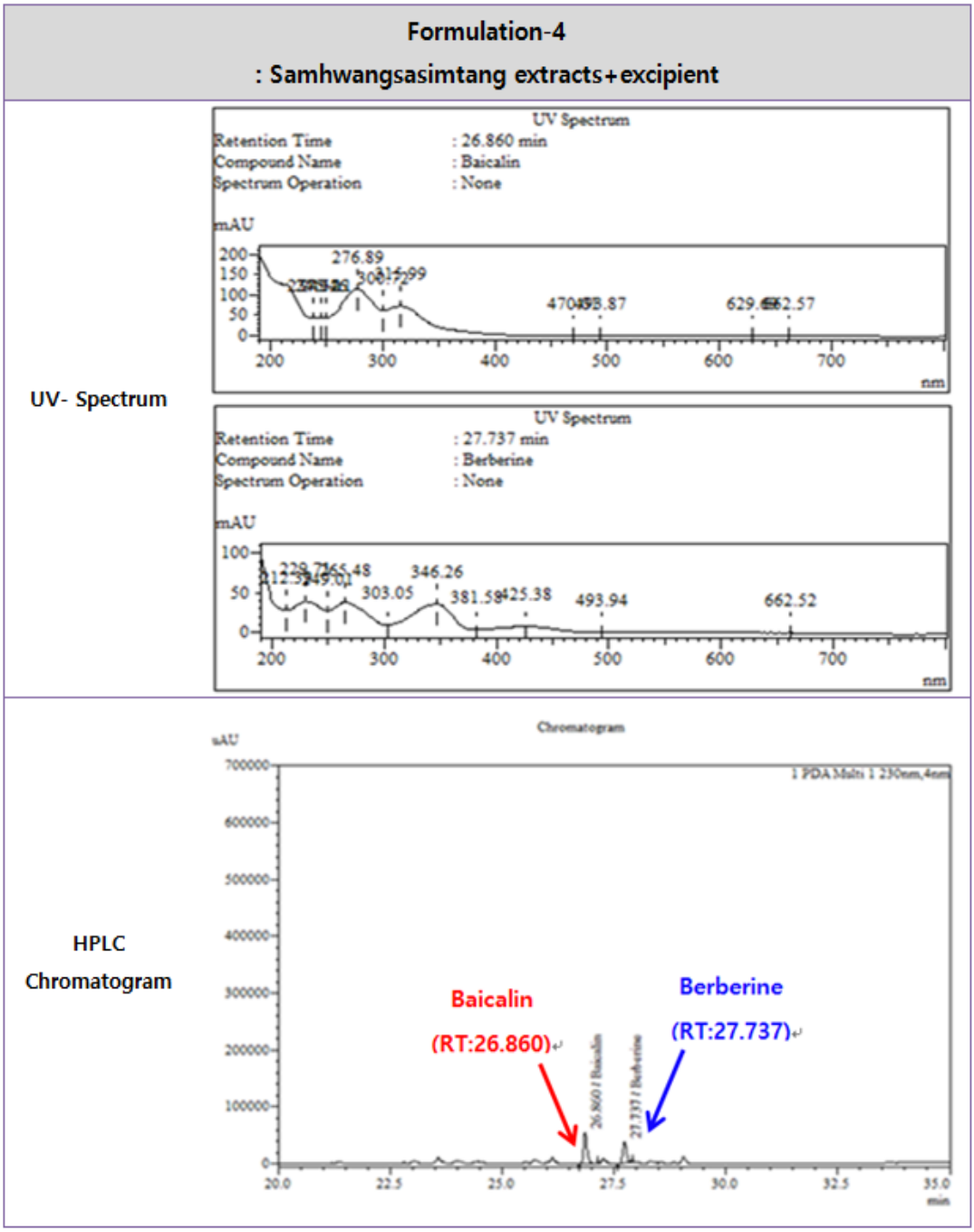

Figure 6. HPLC chromatogram and UV spectrum of berberine and baicalin in Formulation-4: Samhwangsasim-tang extracts+excipient

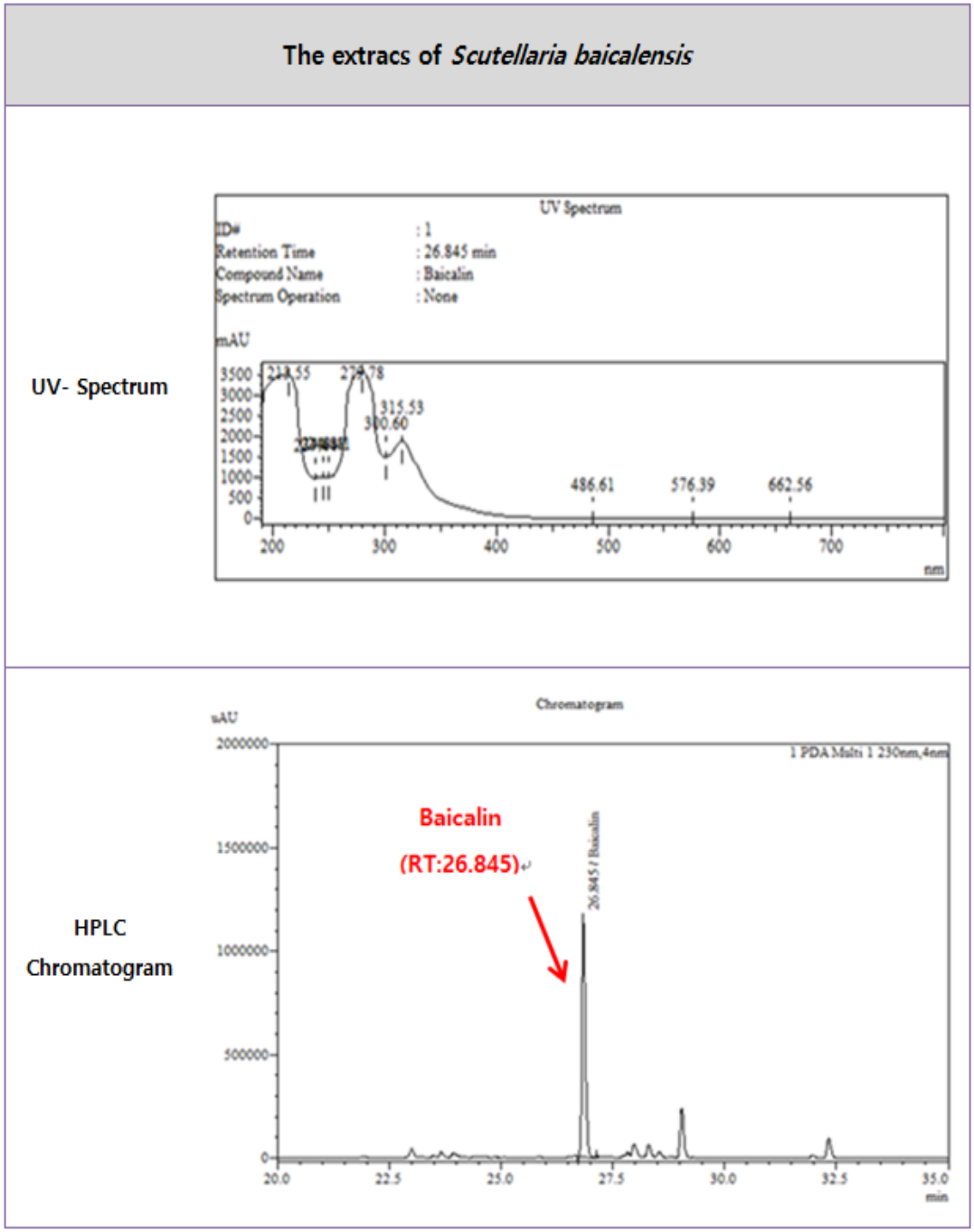

Figure 7. HPLC chromatogram and UV spectrum of berberine and baicalin in the extracs of Scutellaria baicalensis

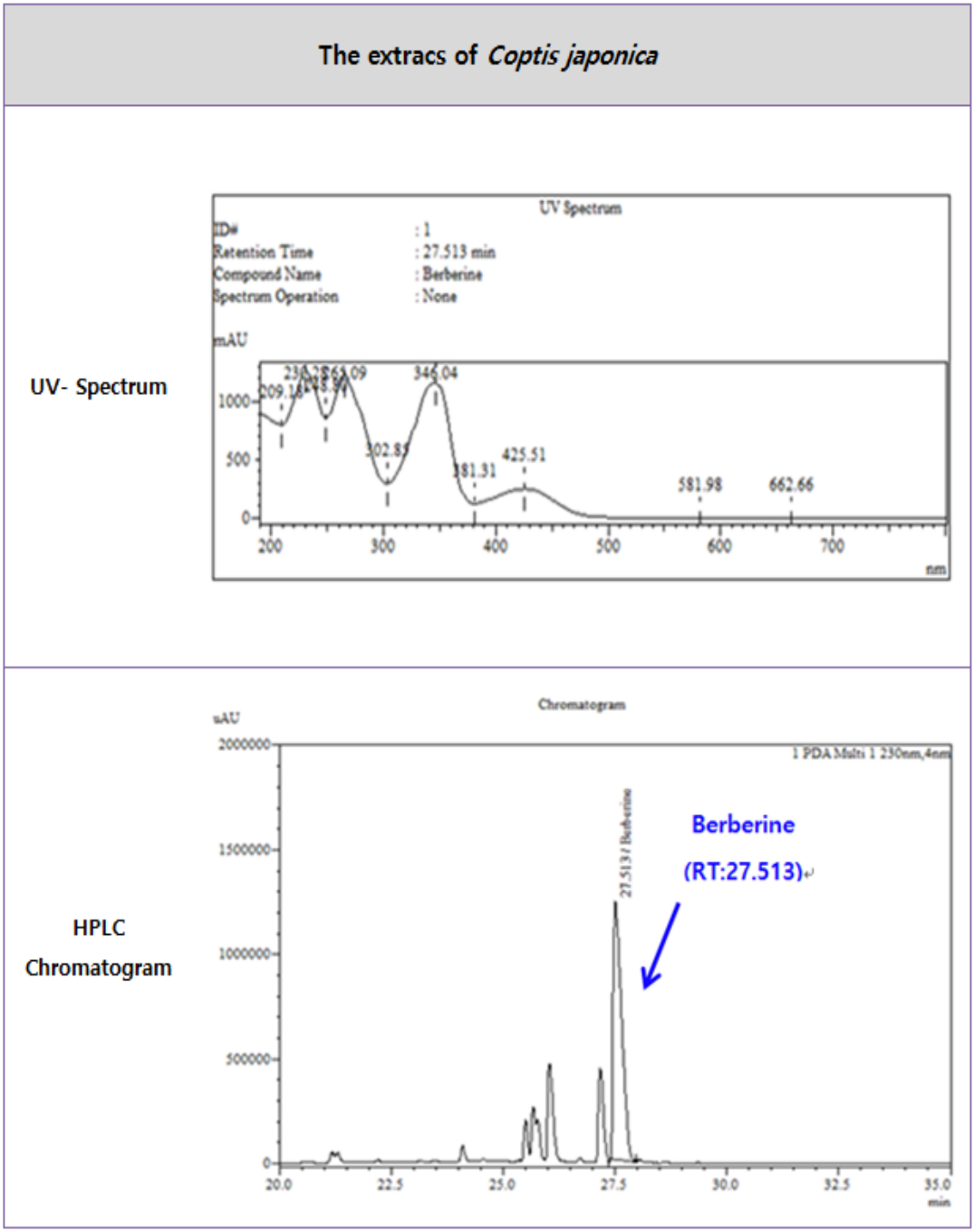

Figure 8. HPLC chromatogram and UV spectrum of berberine and baicalin in the extracs of Coptis japonica

Subsequently, a comparison was made between the UV spectrum and HPLC chromatogram of Sennoside A, a prominent constituent of Da Huang (Rhubarb). Sennoside A (14), recognized as a major component of Da Huang, was initially isolated by Miyamoto et al. (15), and subsequent studies by Oshio et al. reported the isolation of Sennoside D from Shinjudaehwang and Sennoside E from Jangyeopdaehwang (16,17). The current quality control standard primarily focuses on Sennoside A. Upon comparing the UV spectrum and HPLC chromatogram of Sennoside A, the retention time (R.T) for standard analysis was 35.292. For the Samhwangsasim-tang individual extract mixture (Formulation-1) after extracting individual medicinal herbs, the R.T was 35.481. For Samhwangsasim-tang extracts (Formulation-2), the R.T was 35.481. For Samhwangsasim-tang individual extract mixture + excipient (Formulation-3), the R.T was 35.592. For Samhwangsasim-tang extracts + excipient (Formulation-4), the R.T was 35.642. The R.T for the Huangqin extract was 35.435. All retention times were within a 0.5 range, and the UV spectrum exhibited consistency, confirming the presence of Sennoside A within the extracts. This underscores the presence of the same component in each extract (Figures 9-14).

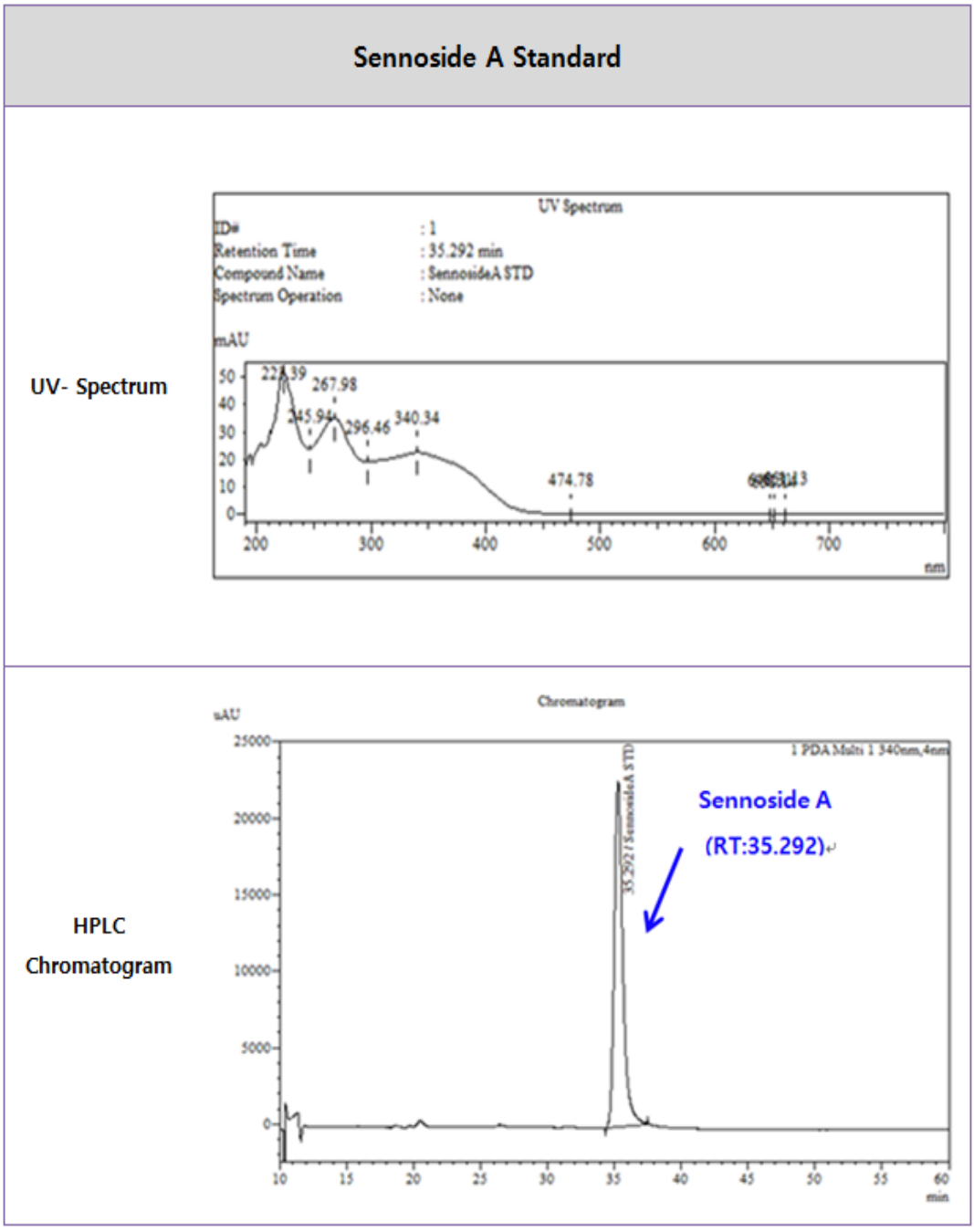

Figure 9. HPLC chromatogram and UV spectrum of Sennoside A as a standard

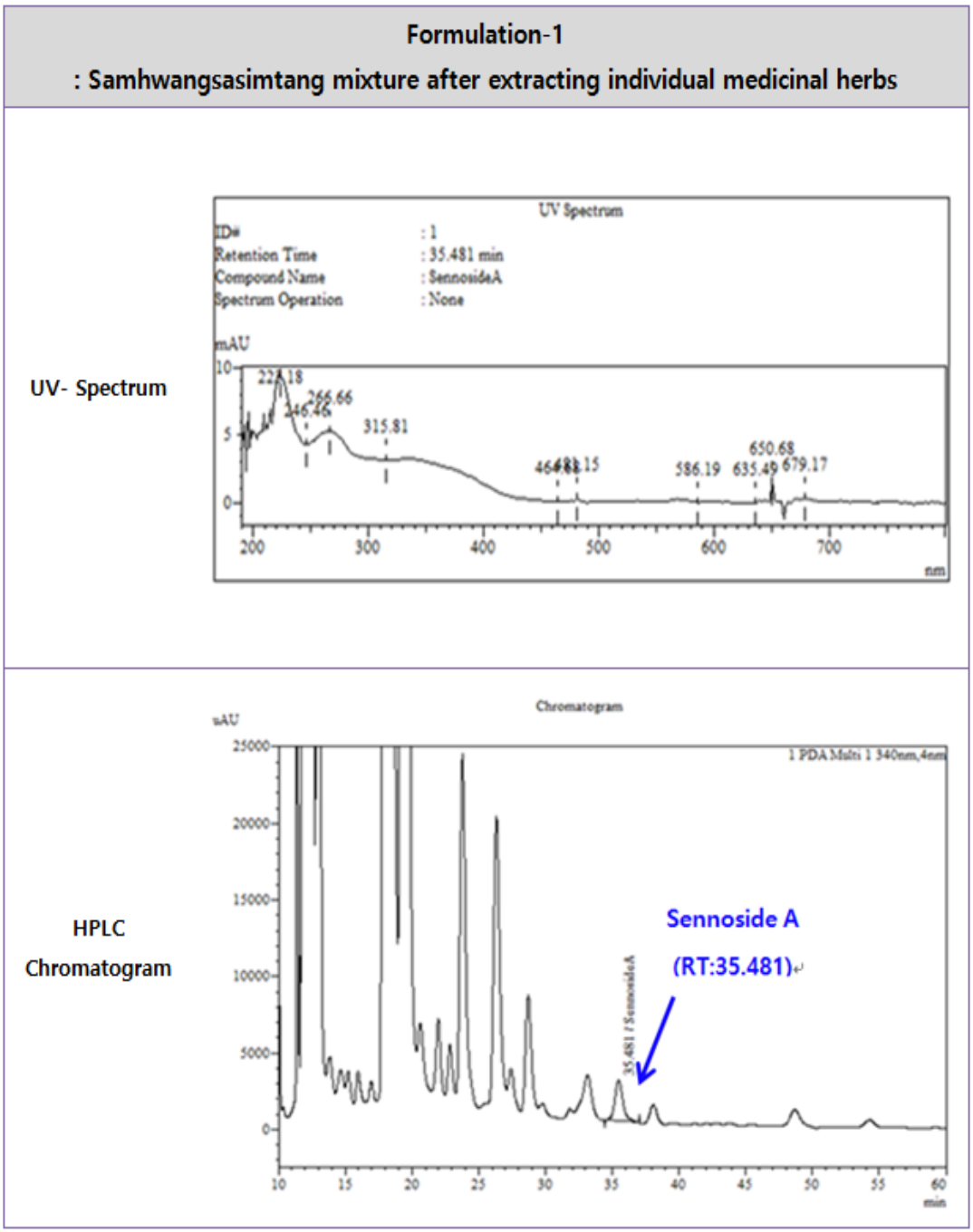

Figure 10. HPLC chromatogram and UV spectrum of Sennoside A in Formulation-1: Samhwangsasim-tang mixture after extracting individual medicinal herbs

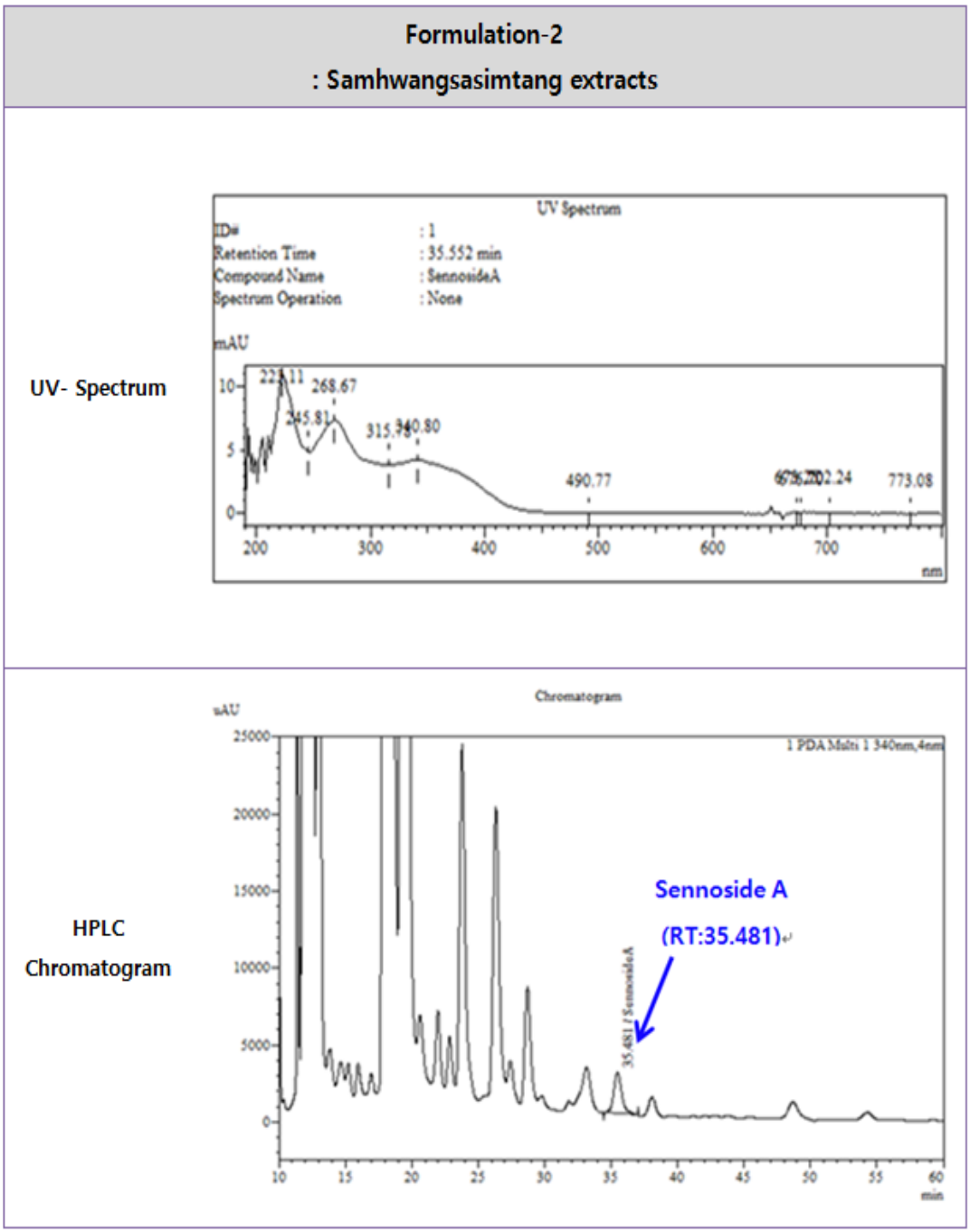

Figure 11. HPLC chromatogram and UV spectrum of Sennoside A in Formulation-2: Samhwangsasim-tang extracts

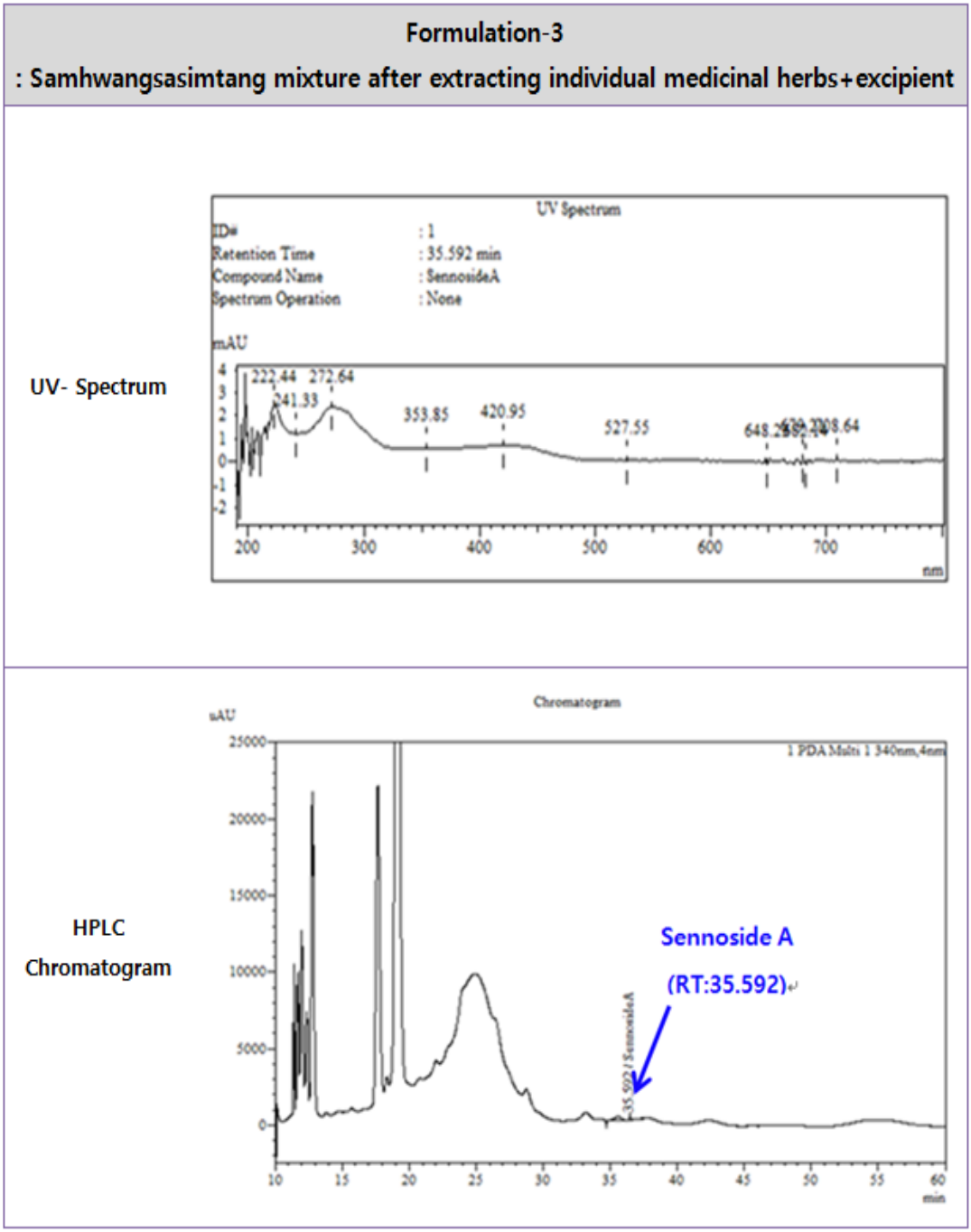

Figure 12. HPLC chromatogram and UV spectrum of Sennoside A in Formulation-3: Samhwangsasim-tang mixture after extracting individual medicinal herbs+excipient

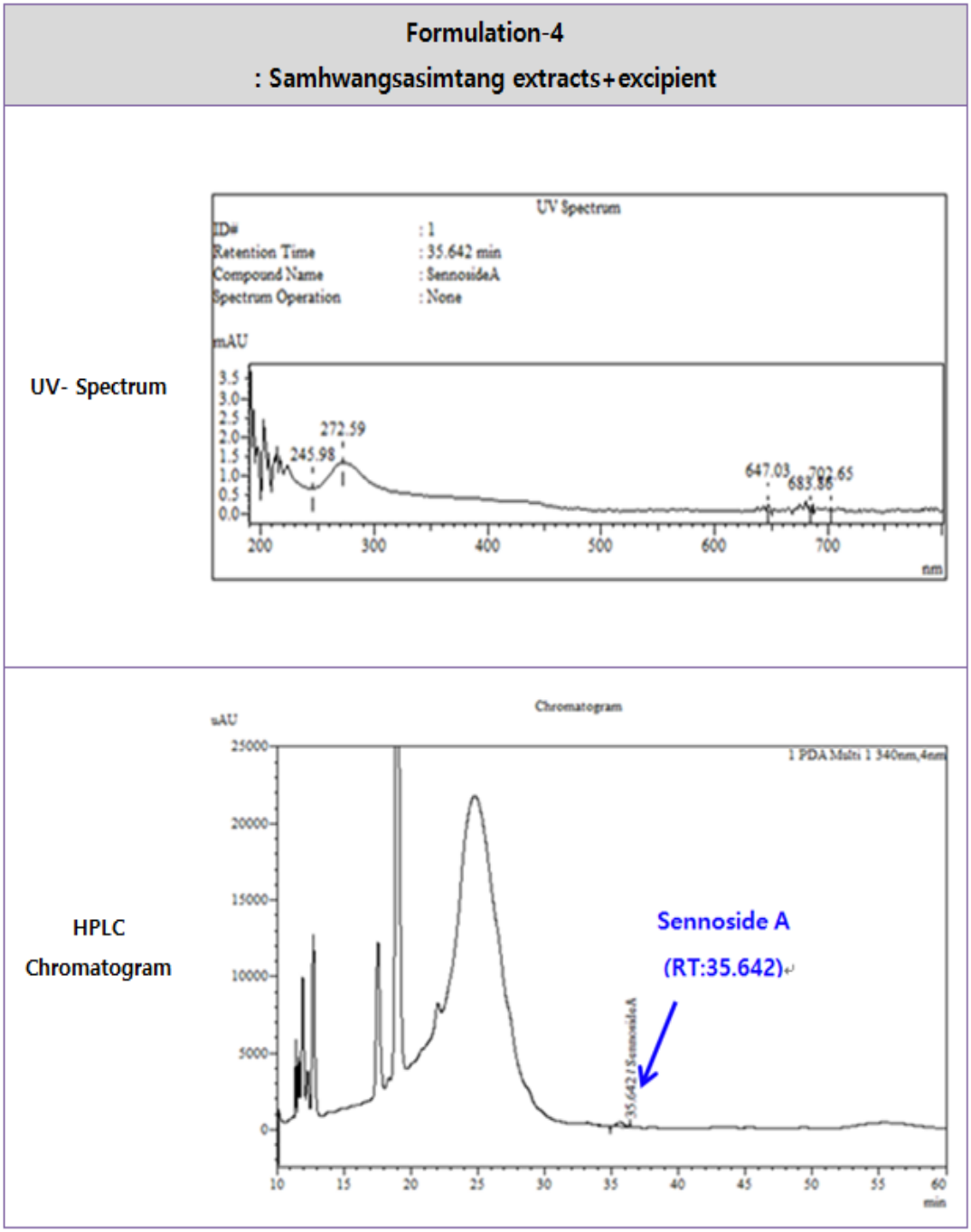

Figure 13. HPLC chromatogram and UV spectrum of Sennoside A in Formulation-4: Samhwangsasim-tang extracts+excipient

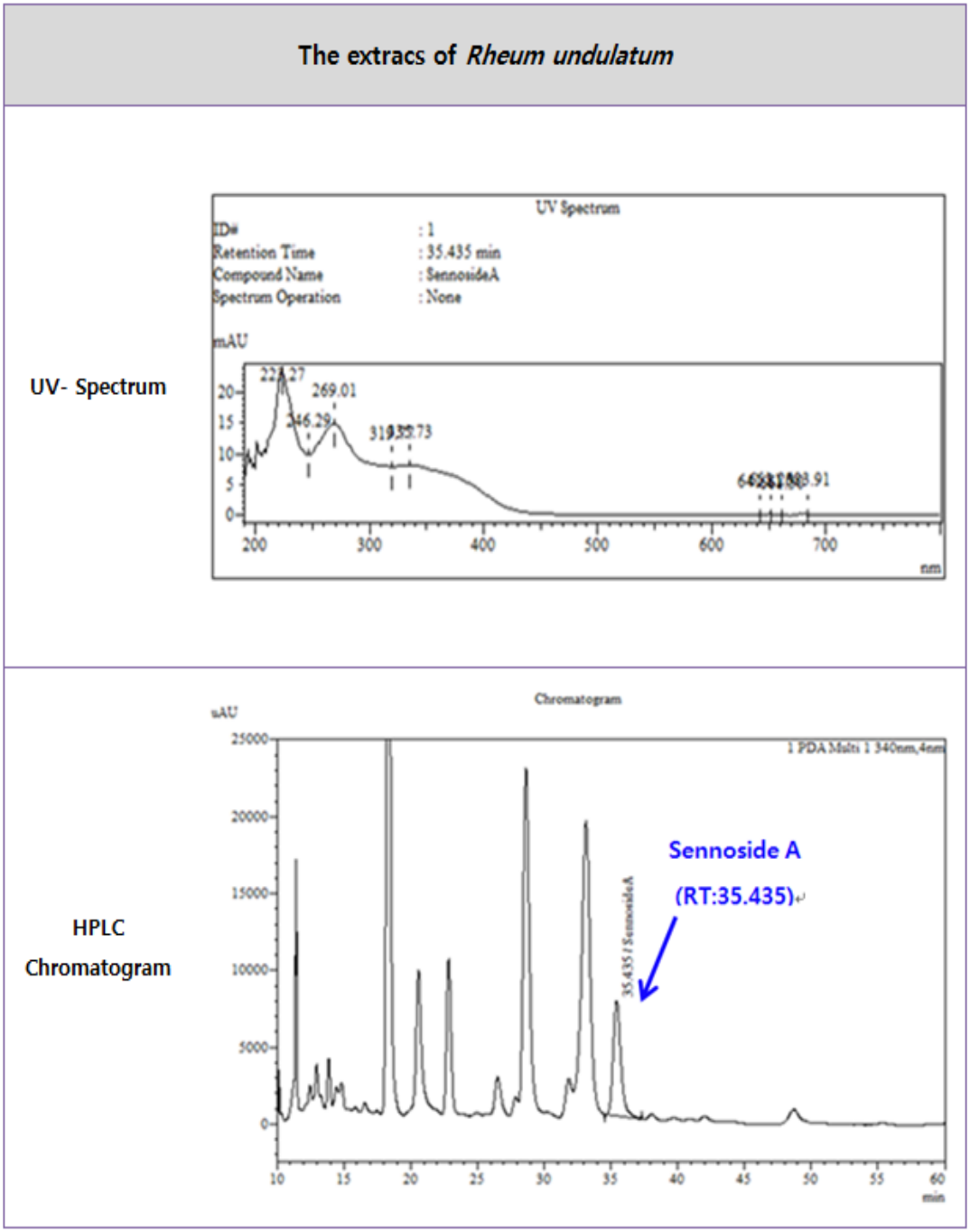

Figure 14. HPLC chromatogram and UV spectrum of Sennoside A in the extracts of Rheum undulatum

3.3. Content Analysis According to Manufacturing Methods

To conduct a comparative analysis of the samples, we examined the Peak Area and calculated content values for each sample. In the case of Berberine, for the Samhwangsasim-tang individual extract mixture (Formulation-1), the Peak Area was 5053248, with a corresponding content of 75.53 mg/g. For Samhwangsasim-tang extracts (Formulation-2), the Peak Area measured 1947338, and the content was determined as 28.19 mg/g. Moving on to Samhwangsasim-tang individual extract mixture + excipient (Formulation-3), the Peak Area was found to be 462012, while the content was 6.40 mg/g. Similarly, for Samhwangsasim-tang extracts + excipient (Formulation-4), the Peak Area recorded was 215061, and the content calculated was 3.13 mg/g. As for the Huanglian extract, its Peak Area stood at 16228454, reflecting a content of 224.86 mg/g (Table 1). Notably, the content of Berberine was observed to be higher in the Samhwangsasim-tang individual extract mixture and Samhwangsasim-tang individual extract mixture + excipient, as compared to Samhwangsasim-tang extracts and Samhwangsasim-tang extracts + excipient, which were measured separately from the formulation.

Table 1. The average content of the Berberine in the Samhwangsasim-tang extract

Compound	Sample Name	Peak Area	Content
Berberine	Samhwangsasim-tang mixture after extracting individual medicinal herbs	5053248	73.53 mg/g
	Samhwangsasim-tang extracts	1947338	28.19mg/g
	Samhwangsasim-tang mixture after extracting individual medicinal herbs+excipient	462012	6.40 mg/g
	Samhwangsasim-tang extracts+excipient	215061	3.13 mg/g
	Coptis japonica extracts	16228454	224.86 mg/g

In the case of Baicalin, the Peak Area for the Samhwangsasim-tang individual extract mixture (Formulation-1) was 1202770, with a content of 37.68 mg/g. For Samhwangsasim-tang extracts (Formulation-2), the Peak Area measured 2775132, with a content of 86.50 mg/g. Moving on to Samhwangsasim-tang individual extract mixture + excipient (Formulation-3), the Peak Area was 174767, and the content was 5.21 mg/g. Similarly, for Samhwangsasim-tang extracts + excipient (Formulation-4), the Peak Area recorded was 282850, and the content calculated was 8.44 mg/g. As for the Huanglian extract, its Peak Area stood at 6267748, reflecting a content of 171.48 mg/g (Table 2). Notably, the content of Baicalin was observed to be higher in the Samhwangsasim-tang extracts and Samhwangsasim-tang extracts + excipient, compared to Samhwangsasim-tang individual extract mixture and Samhwangsasim-tang individual extract mixture + excipient, which were measured separately from the formulation.

Table 2. The average content of the Baicalin in the Samhwangsasim-tang extract

Compound	Sample Name	Peak Area	Content
Baicalin	Samhwangsasim-tang mixture after extracting individual medicinal herbs	1202770	37.68 mg/g
	Samhwangsasim-tang extracts	2775132	86.50 mg/g
	Samhwangsasim-tang mixture after extracting individual medicinal herbs+excipient	174767	5.21 mg/g
	Samhwangsasim-tang extracts+excipient	282850	8.44 mg/g
	Scutellaria baicalensis extracts	6267748	171.48 mg/g

Furthermore, in the case of Sennoside A within the Samhwangsasim-tang individual extract mixture (Formulation-1), the Peak Area measured 113227, corresponding to a content of 2.94 mg/g. For Samhwangsasim-tang extracts (Formulation-2), the Peak Area measured 147846, and the content was determined to be 3.82 mg/g. In the case of Samhwangsasim-tang individual extract mixture + excipient (Formulation-3), the Peak Area was 7208, while the content was 0.18 mg/g. Similarly, for Samhwangsasim-tang extracts + excipient (Formulation-4), the Peak Area recorded was 9951, and the content calculated was 0.26 mg/g. As for the Da Huang extract, its Peak Area stood at 301565, reflecting a content of 7.10 mg/g (Table 3). Similar to Baicalin, the content of Sennoside A was found to be higher in the Samhwangsasim-tang extracts and Samhwangsasim-tang extracts + excipient, as compared to Samhwangsasim-tang individual extract mixture and Samhwangsasim-tang individual extract mixture + excipient, which were measured separately from the formulation.

Table 3. The average content of the Sennoside A in the Samhwangsasim-tang extract

Compound	Sample Name	Peak Area	Content
Sennosied A	Samhwangsasim-tang mixture after extracting individual medicinal herbs	113227	2.94 mg/g
	Samhwangsasim-tang extracts	147846	3.82 mg/g
	Samhwangsasim-tang mixture after extracting individual medicinal herbs+excipient	7208	0.18 mg/g
	Samhwangsasim-tang extracts+excipient	9951	0.26 mg/g
	Rheum undulatum extracts	301565	7.10 mg/g

4. Discussion

This study aimed to compare the levels of marker compounds in Samhwangsasim-tang, specifically Huanglian, Huangqin, and Da Huang, based on different extraction methods. The objective was to investigate the influence of extraction methods on the content of these marker compounds in the extracts. We focused on four formulations: Formulation-1 (Samhwangsasim-tang mixture after extracting individual medicinal herbs), Formulation-2 (Samhwangsasim-tang extracts), Formulation-3 (Samhwangsasim-tang mixture after extracting individual medicinal herbs with excipient), and Formulation-4 (Samhwangsasim-tang extracts with excipient), aiming to elucidate their respective effects on the content of marker compounds.

Berberine particularly exhibits robust anti-inflammatory activity by inhibiting intracellular reactive oxygen species production and MAPK signal transduction, consequently curtailing pro-inflammatory reactions. Moreover, berberine promotes apoptosis in various tumor cells, induces tumor cell differentiation, restrains cancer cell proliferation, impedes tumor cell metastasis, regulates the expression of Bcl-2 family proteins, and modulates mitochondrial membrane potential, thereby influencing several cell signaling pathways (12). Significantly, berberine's inhibition of the binding of transcription factors NF-κB and activator protein-1, pivotal in the interplay between cancer and inflammation, has been reported, leading to the suppression of inflammatory responses (12,18). Recent studies have also unveiled berberine's efficacy in anti-aging and antioxidant roles through reactive oxygen species inhibition and reduced MMP9 and MMP-2 expression, mediated by the regulation of TIMP-2 and TIMP-1 (19). Baicalin, also known as 5,6-Dihydroxy-4-oxo-2-phenyl-4H-1-benzopyran-7-yl-ß-D-glucopyranosiduronic acid, is the predominant component found in Huangqin (13). Active research on the constituents of Huangqin has predominantly focused on flavonoid compounds, such as baicalin, baicalein, wogonin, and oroxylin A. Among these, baicalin, a major flavone glycoside compound, has exhibited a wide range of physiological effects. These effects encompass anticancer properties (20-23), anti-inflammatory and anti-allergic actions (24-28), antioxidative effects (29-33), and the inhibition of human immunodeficiency virus type 1 (HIV-

1) (26-28). Additionally, studies have reported its anti-obesity effects (34-36). Baicalin's antioxidative action induces directional movement and chemotaxis of leukocytes, leading to the release of various immune mediators and playing a role in angiogenesis regulation. This biological function is achieved through baicalin binding to various chemokines, restraining their biological activities and inhibiting the formation of reactive oxygen species (20). Belonging to the flavonoid group, baicalin demonstrates potent antioxidant effects by inhibiting oxidative DNA damage and cell apoptosis caused by reactive oxygen species (ROS) and hydroxyl radicals (31). Baicalin exhibits inhibitory effects against aging, characterized by oxidative damage and the continuous accumulation of radicals in cells. These antioxidant effects are also associated with anti-inflammatory effects (24) and the suppression of histamine release from adipocytes in cases of obesity, contributing to its anti-allergic effects (27). Furthermore, baicalin suppresses adipogenesis and obesity by regulating adipocyte differentiation factors and pathways. Notably, baicalin has been shown to inhibit the proliferation of bladder cancer cells (21) and induce apoptosis in some prostate cancer cell lines (22), suggesting its potential in inhibiting tumor growth and metastasis (24). In addition, sennosides are known to remain unabsorbed in the stomach and small intestine upon oral administration. They subsequently reach the colon, where they undergo metabolism by colonic bacteria to form rhein anthrone. This process stimulates colonic motility, promotes mucus secretion, and inhibits water and Na absorption, thereby inducing laxative effects (37).

Following the guidelines established in the Korean Pharmacopoeia, Samhwangsasim-tang was formulated in a ratio of Huanglian : Huangqin : Da Huang (3 : 3 : 4) using the methods of decoction and dried extraction. Additionally, for comparative purposes, excipients commonly used in commercially available products from Hanpung Pharmaceutical Co., Ltd. (Wanju, South Korea) were used. In this study, the analysis of each extract revealed that Formulation-1 and Formulation-3 resulted in higher berberine content, while Formulation-2 and Formulation-4 exhibited higher levels of baicalin and sennoside A. The analysis of Berberine content revealed variations based on the extraction techniques employed. Specifically, the content of Berberine was observed to be lower in Samhwangsasim-tang extracts (Formulation-2: Samhwangsasim-tang extracts) and Samhwangsasim-tang extracts with excipient (Formulation-4: Samhwangsasim-tang extracts+excipient) in comparison to Samhwangsasim-tang mixture after individually extracting medicinal herbs (Formulation-1) and Samhwangsasim-tang mixture after individually extracting medicinal herbs with excipient (Formulation-3). This trend can be elucidated by the characteristics associated with mixed extractions, as expounded upon in prior studies (38, 39). In a previous study, it was reported that the combination ratio of *Atractylodes japonica* and *A. macrocephala* during the process of heat extraction influences the extraction rate of components in licorice (*Glycyrrhiza uralensis*) (38). Additionally, it has been noted that the extraction yield of main constituents is affected by the mixing ratios of *Paeonia lactiflora*, Ramulus Cinnamomi Cassiae, and *G. uralensis* (39). Furthermore, in this study, the berberine content demonstrated a significant decrease in extracts of Samhwangsasim-tang compared to the mixture of individual medicinal herbs after separate extraction. Therefore, our results suggested that the results of the extraction yield of main constituents are influenced by the method of mixed extraction.

In the cases of baicalin and sennoside A, the extracts of Samhwangsasim-tang (Formulation-2: Samhwangsasim-tang extracts) and Samhwangsasim-tang extracts with an excipient (Formulation-4: Samhwangsasim-tang extracts+excipient) were measured to have higher levels compared to Samhwangsasim-tang mixture after individually extracting medicinal herbs (Formulation-1) and Samhwangsasim-tang mixture after individually extracting medicinal herbs with an excipient (Formulation-3). This finding suggests that the interaction between components due to mixed extraction at specific ratios, as indicated by studies on changes in major chemical constituents before and after the blending of Huanglian and Huangqin (40), might enhance the extraction of Baicalin and Sennoside A.

In summary, our results presented intriguing findings, demonstrating a decrease in berberine and an increase in baicalin and sennoside A in Samhwangsasim-tang extracts compared to the Samhwangsasim-tang mixture after extracting individual medicinal herbs. This finding suggests that the interaction between components due to mixed extraction at specific ratios might enhance or reduce the extraction of main components. In addition, our results show that it was a significant decrease in the average content of berberine, baicalin, and sennoside A in Samhwangsasim-tang when excipients are included, compared to when they are not. These results might be able to be attributed to the interference caused by the addition of excipients in the analysis of marker compound content. This indicates the need for research on various extraction and manufacturing methods to enhance the extraction efficiency of marker compounds when producing herbal formulations. Therefore, for pharmaceutical companies dealing with herbal products, it is advantageous to tailor production based on various factors and the specific circumstances of the company. Furthermore, the efficacy of traditional herbal medicines prescribed as a mixture of multiple herbs, rather than single herbs, may not solely rely on contents of each herb, and it may also be influenced by the proportions of the constituents (41). And,

further research is necessary to explore these aspects in products for Samhwangsasim-tang and other traditional herbal prescriptions.

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