Separation of Useful Components from SMB, SFA and Garlic by Liquid Chromatography

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액체 크로마토그래피를 이용한 단삼,고삼,마늘로부터 유용성분의 분리

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ABSTRACT

In our work, a reversed-phase high performance liquid chromatographic method has been developed and validated for analysis of three abietane-type diterpenes, tanshinone I, tanshinone IIA and cryptotanshinone, in *Salvia miltiorrhiza bunge*. HPLC separation of the tanshinones was performed on a C18 column and detected by ultraviolet absorbance at 254 nm. A mobile phase composed of methanol-water-acetic acid in the ratio 78:22:0.5 (v/v) was found to be the most suitable for this separation at a flow-rate of 0.5 mL/min and enabled the baseline separation of the three analytes free from interferences with isocratic elution. The analysis time was 30 min per injection. The calibration was linear in the range of 0.1–500.0 μg/mL for tanshinone I, tanshinone IIA and cryptotanshinone. The extracted amounts are 0.0091, 0.15 and 0.23 mg/g and the relative standard deviations were 3.3% for tanshinone I, 2.9% for tanshinone IIA and 3.5% for cryptotanshinone analysis. The method has been successfully applied to the simultaneous determination of tanshinone I, tanshinone IIA and cryptotanshinone in *Salvia miltiorrhiza bunge*.

A reversed-phase high performance liquid chromatographic method (HPLC) has been developed and validated for analysis of two bioactive alkaloids, matrine and oxymatrine, in *Sophora Flavescens Ait.*. HPLC separation of the alkaloids was performed on a C18 column and detected by ultraviolet absorbance at 210 nm. A mobile phase composed of methanol-water-trifluoroacetic in the ratio of 16:84:0.002 (v/v) was found to be the most suitable mobile phase for this separation at a flow-rate of 0.5 mL/min and enabled baseline separation of the two analytes free from interference with isocratic elution. The analysis time was 25 min per injection. The calibration was linear in a range of 3.0–500.0 μg/mL for matrine and 3.0–1000.0 μg/mL for oxymatrine, respectively. The extracted amounts are 0.0091 and 0.15 mg/g and the relative standard deviations were 3.3% for the matrine and 2.9% for the oxymatrine analysis.

The properties of garlic (*Allium sativum* L.) are attributed to organosulfur compounds. Here, a newly developed analytical technique with a rapid and simple sample preparation to determine diallyl disulfide (DADS) in garlic is reported. All garlic samples were simply extracted with different solutions (methanol, benzene or tetrahydrofuran) and prepared for analysis. From the results, with methanol as the extraction solvent was optimized. The mobile phase was composed of methanol and water, and gradient mode was applied. 0.61 mg of DADS per g garlic powder can be extracted with methanol. This work offers some advantages over the currently accepted techniques and would be useful for chemical and biological studies of garlic and its products.
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1. Introduction

Natural products are organic compounds that are formed by living systems. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry. Naturally occurring compounds may be divided into three broad categories. Firstly, there are those compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include the nucleic acids and the common amino acids and sugars. They are known as primary metabolites. Secondly, there are the high-molecular-weight polymeric materials such as cellulose, the lignins and the proteins which form the cellular structures. Finally, there are those compounds that are characteristic of a limited range of species. These are the secondary metabolites. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production. Secondary metabolites, on the other hand, have often attracted interest because of their biological effect on other organisms. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites. It has been estimated that over 40% of medicines have their origins in these natural products. A number of screening programmes for bioactive compounds exist and have led to new drugs, for example taxol, which is used for the treatment of various cancers. Natural products often have an ecological role in regulating the interactions between plants, microorganisms, insects and animals. They can be defensive substances, antifeedants, attractants and pheromones.

1.1 Salvia miltiorrhiza bunge

The root of Salvia miltiorrhiza bunge (SMB) has been widely used in traditional Chinese medicine for promoting blood circulation to remove blood stasis, clearing away heat, relieving vexation, nourishing blood, tranquillizing the mind, cooling the blood to relieve carbuncles, treating hemorrhages, menstrual disorders, and miscarriages [1, 2]. SMB has received much interest due to its ability to accumulate large amounts of active compounds such as tanshinones [3]. Tanshinone I, tanshinone IIA and cryptotanshinone are the main abietane-type diterpenes contained in SMB (molecular structures are shown in Figure 1). It is reported that tanshinones can dilate coronary arteries, increase coronary flow, modulate mutagenic activity, and protect the
myocardium against ischaemia. They also have sedative and tranquilizing effects, and some of them have been used to treat neurasthenic insomnia [4, 5]. In addition, tanshinones have attracted particular attention because they exhibit significant antibacterial [6,7], anti-dermatophytic [7], antioxidant [8,9], anti-inflammatory [7, 10], anti-neoplastic [11], and anti-platelet aggregation [12] activities.

![Chemical structures of three tanshinones](image)

Figure 1. Chemical structures of three tanshinones. a. tanshinone I, b. tanshinone IIA, c. cryptotanshinone

The rapid qualitative and quantitative analyses of these tanshinone compounds have been a central problem in medicinal chemistry and biotechnology. Extraction, using organic solvents, of tanshinones from SMB has been reported by several workers with subsequent analysis by high-performance liquid chromatography [13–15] and spectrophotometry [16]. Conventional extraction methods not only involve the use of large amounts of toxic organic solvents but also need a long time for the pre-concentration of the extracts. In order to eliminate or at least minimize the use of organic solvents and simplify the operating procedure, other extraction methods should be developed. The complexity of tanshinone compounds makes it difficult to separate them in a single chromatographic run. The primary aim of the present study is to develop a reversed-phase HPLC method for the simultaneous separation and determination of these tanshinone compounds in the dried roots.

Following a detailed study, this report describes a simple, sensitive and more reliable HPLC method for simultaneous determination of the abietane-type diterpenes, tanshinone I, tanshinone IIA and cryptotanshinone, in SMB using ultraviolet detection and isocratic elution. The analysis time was 30 min per injection. It has been successfully applied to the determination of tanshinone I, tanshinone IIA and cryptotanshinone in SMB.
1.2 *Sophora flavescens* Ait.

*Sophora flavescens* Ait. (SFA), recorded in the medicine literature Sheng Nong's Herbal Classic as a moderate medicine, is cold by nature with bitter taste, entering three channels of heart, spleen and kidney. According to Herbal Classic of Hundreds of Medicinal Materials, the dried roots of SFA is especially used to treat hotness in the heart with bitter entering heart and cold removing internal hotness. With the development of separating and extracting techniques, intensive investigations and researches have been conducted in China and it has been found that matrine-type alkaloids of the same kind are present in SFA [17]. Matrine and oxymatrine (molecular structures are shown in Figure 2) can obviously inhibit varied clinical gastric mucosa damages. This may result from the direct neutralization of external hydrochloric acid and gastric acid by matrine, and the mitigation of matrine has certain protective effects on gastric mucosa [18]. By means of inhibiting enterocinesia, matrine-type alkaloids have visible effects on antidiarrheal [19]. They have the effects of killing amoebas and giardia lamblia stiles [20]. Matrine can kill trichomonads and slow down the development of subcutaneous abscess caused by the parasites, and also cure infections caused by mouse vaginal trichomoniasis. It is indicated that matrine can be applied as potential medicine for killing parasites, but the mechanism is not quite clear at the present [21]. It has been held that this kind of structure might be the group accounting for the positive myotome effects in matrine-type alkaloids, which may be related to the activation of calcium channel [22].

Figure 2. Chemical structures of two alkaloids. 1, Matrine; 2, Oxymatrine.
Due to the high pharmacological activities of matrine-type alkaloids in SFA, the herb has recently drawn attention in natural medication researches. Several papers have been published concerning the separation and quantification of the alkaloids in SFA, and several methods such as high-performance liquid chromatography (HPLC) [23, 24], high-performance capillary electrophoresis (HPCE) [25, 26], gas chromatography (GC) [27] and thin layer chromatography (TLC) [28, 29] have been applied to the separation and determination of matrine-type alkaloids in SFA root. Undoubtedly, HPLC is the most widely used separation technique for this application for its simplicity and general applicability to matrine-type alkaloids [30, 31]. Moreover, HPCE techniques can also be used when HPLC is not suitable or efficient for the samples of interest. Sample preparation is the most important part in the application of HPLC or HPCE. Some extraction including liquid–liquid extraction (LLE), solid-phase extraction (SPE) or other methods can be selected according to the determination requirement in precision, accuracy and reproducibility [32-33].

Following a detailed study, this report describes a simple, sensitive and more reliable HPLC method for simultaneous determination of the two bioactive alkaloids, matrine and oxymatrine, in SFA using ultraviolet detection and isocratic elution. The analysis time was 25 min per injection. It has been successfully applied to the determination of matrine and oxymatrine in the dried roots.

1.3 Garlic

Garlic (*Allium sativum* L.) has been used universally as a food, spice, and traditional medicine. Numerous studies have previously demonstrated that garlic may be useful for the prevention of carcinogenesis, cardiovascular, and age-related diseases [34]. Especially, it has been strongly suggested that its medicinal and beneficial properties are attributed to specific organosulfur compounds [35, 36]. Many studies on animals showed its protective effects against chemically induced toxicity and against carcinogenesis [35]. The modulation of the metabolism of carcinogens by diallyl disulfide (DADS) was considered one of the possible mechanisms of its protective effect against the occurrence of cancer [36]. However, once garlic is cut, chopped or crushed, the clove’s membrane disrupts and S-allylcysteine sulfoxide is transformed enzymatically into allicin by allinase [37]. The main component of the volatile oil are sulfur compounds especially allicin, diallyl sulfide (DAS), DADS, diallyl trisulfide (DATS). The
chemical structure of DADS was shown in Figure 3. DADS is one of the major volatile degradative compounds of garlic formed from allicin. Therefore, it becomes important to control sample preparation to minimize artificial errors caused by their chemical characters. Moreover, it has been reported that contents of organosulfur compounds in garlic change during cultivation [38, 39] and storage [40]. Therefore, an analytical method for the determination of DADS, in a garlic sample is required for evaluating the quality of garlic. It has been previously reported that organosulfur compounds in garlic were analyzed using reversed-phase high performance liquid chromatography (RP-HPLC) [41-50] gas chromatography [51] thin layer chromatography [52] and biosensors [53]. All of the above methods needed different sample preparation procedures and did not allow simultaneous determination. More recently, the simultaneous analysis has been reported to determine the constituents in garlic preparations [54, 55]. However, these methods were not sufficiently validated with respect to the analytical performance characteristics (e.g., specificity, linearity, limit of detection, accuracy, and precision) despite quantitative analysis.

Figure 3. The chemical structure of DADS.

In the present study, we report a simple and rapid analytical technique using sample preparation procedure and subsequent HPLC techniques to determine DADS in garlic qualitatively and quantitatively.

2. Theoretical Background

M.S. Tswett, a Russian botanist developed a method, utilising a powdered chalk column, to separate and isolate plant pigments. The coloured bands he separated on the column, led to the name chromatography, meaning, “colour writing”. However, in detailed reports published in 1906, he pointed out that the method was not constrained to the separation of coloured substances.

Kuhn and Lederer revived the method in 1930 and within a few years, the technique was used
extensively. Individual variants were developed such as thin-layer chromatography, which was first described by Izmailov and Schraiber in 1938. In 1948, the Nobel Prize in Chemistry was awarded to A. Tiselius “for his work on electrophoresis and adsorption analysis”. Four years later, the prize was given to A.J.P. Martin and R.L.M. Synge for the invention of liquid-liquid partition chromatography. They also applied the concept of theoretical plates as a measurement of column efficiency, setting the stage for gas and paper chromatography. In the same year Martin and James developed gas-liquid partition chromatography, which lead to research on the theory of chromatography. The theory was then generalized for liquid chromatography, resulting in the development of modern liquid chromatography or high performance liquid chromatography (HPLC).

HPLC has become a very important analytical technique as it is characterized by:

• Small diameter columns that are re-usable
• Very small particles for column packings
• High resolution
• Rapid analysis
• Automated instruments
• Detectors capable of detecting small quantities
• Control over the flow of the mobile phase

2.1 Types of Chromatography

All the methods defined as chromatography, have the use of a stationary phase and a mobile phase in common, the mobile phase being either a gas or a liquid. The classification of chromatography into the liquid, gas and supercritical-fluid categories is based upon the type of mobile phase used. Liquid chromatography is performed in columns and on planar surfaces, but gas chromatography is only performed in columns. Figure 4 illustrates the classification of chromatography:
Supercritical-fluid chromatography is a hybrid of gas and liquid chromatography, combining the best features of each. Supercritical fluids’ densities allow them to dissolve large non-volatile molecules, making it a superior technique in certain applications. The method is applied to a group of compounds not amenable to either gas-liquid or liquid chromatography.

Gas chromatography is divided into two types: gas-solid chromatography (GSC) and gas-liquid chromatography (GLC). Gas-liquid chromatography finds more applications in all fields of science and its name is usually shortened to gas chromatography (GC). Gas-solid chromatography uses a solid stationary phase that retains the analytes by physical adsorption. The technique has limited application, and is only used for the separation of low-molecular-mass gases that are not retained by gas-liquid columns. Gas-liquid chromatography uses a liquid that is retained on the surface of an inert solid as a stationary phase. The liquid is retained either by adsorption or chemical bonding. Helium, argon, nitrogen, carbon dioxide and hydrogen are chemically inert gases used for the mobile phase. This method is used to verify the purity of organic compounds, and to identify components in a mixture.

Liquid chromatography is better known as high performance liquid chromatography (HPLC). HPLC includes adsorption, size exclusion, partition and ion chromatography. Partition chromatography is divided into normal phase and reversed phase chromatography. Ion chromatography has been subdivided ion exchange, ion exclusion and ion pair chromatography.

Retention behaviors of solutes and their interactions with the mobile phase and stationary phase has been a very contentious issue in Reversed Phase Liquid Chromatography (RPLC). This area of study is important because an understanding of solute retention, and therefore the retention mechanism will lead to more controlled and selective separations. A greater
understanding of retention requires detailed insight into solute interaction either with the mobile phase or with the stationary phase, but probably a combination of both. Generally, the stationary phase is chosen to maximize strong intermolecular interactions between it and the solutes, because the intermolecular interactions between the solutes and the mobile phase are usually relatively weak. These factors may be optimized to give the required resolution and retention.

2.2 Retention in Reversed Phase Liquid Chromatography

Retention of solutes in RPLC is a complex process, which is affected by a number of different intermolecular interactions taking place that involve the solute molecules, the stationary phase and the mobile phase.

The magnitude of retention in RPLC is measured under isocratic conditions by the retention factor, \( k \), through the following relationship:

\[
k = \frac{(t_r - t_0)}{t_0}
\]  

(1)

where \( t_r \) is the retention time of the solute under investigation, and \( t_0 \) is the retention time of an unretained marker.

The retention factor of a solute is also related to the equilibrium constant, \( K \), for the distribution of the solute between the mobile phase and the stationary phase as below:

\[
k = K \phi
\]  

(2)

where \( \phi \) is the phase ratio of the stationary phase column. The phase ratio is calculated by dividing the volume of the stationary phase by the volume of the mobile phase.

Retention is described in terms of the Gibbs Free Energy change, \( \Delta G^0 \), which is associated with the transfer of a solute from the mobile phase to the non-polar stationary phase, and is related to the equilibrium constant at temperature \( T \) (units of Kelvin) as below:

\[
\Delta G^0 = -RT \ln K
\]  

(3)

where \( R \) is the universal gas constant (8.314 J K\(^{-1}\) mol\(^{-1}\)). By applying the Gibbs-Helmholtz relationship (Equation 4), and combining with Equation 3, Equation 5 defines the dependence of the logarithmic retention factor of the solute as a function of temperature:

\[
\Delta G^0 = \Delta H^0 - T\Delta S^0
\]  

(4)

\[
\ln k = -\Delta H_x^0/RT + \Delta S_x^0 + \ln \phi
\]  

(5)

where \( \Delta H_x^0 \) is a measure of energy exchange in a system, and \( \Delta S_x^0 \) represents the disorder or
chaos of the system, and $x$ denotes the structural variable under investigation. Both the enthalpy and entropy terms are associated with solute transfer from the mobile phase to the stationary phase. The phase ratio, $\varphi$, is a necessary quantity for acquiring thermodynamic information, although obtaining the value of the phase ratio is difficult. The phase ratio of a column is believed to decrease slightly with increasing column temperature and pressure. The assumption that $\varphi = 1$ (phase ratio equal to unity) only affects the value measured for entropy changes, but not the enthalpy changes. A method for the calculation of the stationary phase volume, which differs from previous methods used for the measurement of the phase ratio, in which the packing surface area is not required, which therefore eliminates errors that may be associated with surface area measurement. The carbon load and the total packing weight of the column were measured to calculate the actual volume of the alkyl chains that are bonded to the stationary phase, but this method neglects the effects of intercalated solvent volumes.

When analysing thermodynamic studies on particular solutes, the linearity of the van’t Hoff plots give an indication of the type of retention that is occurring between the solute and the stationary phase. Non-linear van’t Hoff plots are generally assumed to be a result of a change in enthalpy and entropy values due to temperature. For solute molecules, the enthalpy and entropy of transfer from the mobile phase to the stationary phase can be calculated from the retention data through evaluation of van’t Hoff plots (Equation 5), which involve the plot of the natural log of the retention factor, $\ln k$, against the inverse of temperature, $1/T$. For homologues, linear van’t Hoff plots are usually obtained, indicating that $\Delta H^0_x$ and $\Delta S^0_x$ are independent of temperature. From these linear plots, $\Delta H^0_x$ can be derived from the slope and $\Delta S^0_x$ can be determined from the intercept. In general, negative $\Delta H^0_x$ values are found, which demonstrate that the retention of that particular solute molecule is an exothermic process. An exothermic process therefore means that it is energetically more favourable for a solute molecule to remain in the stationary phase as opposed to the mobile phase. $\Delta S^0_x$ gives a measure of the change in the order of the system, which involves the ordering of stationary phase ligands after retention of the solute molecules under investigation and the solvent molecules in the mobile phase.

2.3 Determination of Retention Mechanisms in Reversed Phase Liquid Chromatography

The need for a greater understanding of the underlying retention behavior between solute molecules, the mobile phase and the stationary phase necessitates the determination and
elucidation of the retention mechanisms that are occurring during the chromatographic retention process.

There are a large number of methods that have been employed for the investigation of the retention mechanisms, but the most popular methods include the study of exothermodynamic relationships, the analysis of selectivity, and the measurement of adsorption isotherms.

2.3.1 Exothermodynamic Relationships

There are a number of exothermodynamic relationships that can be employed to investigate and measure the retention mechanism of a particular system in RPLC. Exothermodynamic relationships are empirical correlations of thermodynamic quantities that are popularly utilised to examine the role of structural parameters in chemical equilibria, and therefore assist in the understanding of the underlying retention mechanisms.

2.3.2 Analysis of Selectivity

Chromatographic separation depends on the selective retention of the solute by the stationary phase. The selectivity factor, $\alpha$, for a stationary phase column using two test solutes is calculated using the relative separation of the peaks, and is given by:

$$\alpha = \frac{k_1}{k_2}$$

(6)

where $k_1$ and $k_2$ are the retention factors of the two test solutes 1 and 2 respectively. When $\alpha = 1$ there is no separation. Therefore, in order to gain an increase in the selectivity of a particular separation, manipulation of the selectivity factor is required to improve the resolution.

Factors that may influence the separation selectivity in a particular chromatographic process include solvent selectivity, temperature selectivity, stationary phase selectivity and shape selectivity.

2.3.2.1 Solvent Selectivity

Control and manipulation of a chromatographic separation requires an understanding of the retention process that occurs, and detailed knowledge of the role that is played by the mobile phase composition.
Solvent selectivity is defined as the effect of the solvent on relative retention and band spacing. The mobile phase contributes significantly towards the mechanism of separation in high performance liquid chromatography (HPLC) by intermolecular interactions that occur between the solvent and the solute molecules. The fundamental relationship is between solvent strength and solvent selectivity. One of the most widely adopted methods of solvent classification is the solvent-selectivity triangle. The solvent-selectivity triangle is based on gas-liquid distribution constants, in which solvents are grouped in the triangle according to their ability to either donate or accept protons, and to induce dipole moments. Consequently, solvents that yield similar selectivity are grouped together, resulting in solvents being characterised in eight distinct groups. There are many solvents that have been classified into each group of the solvent-selectivity triangle, but for example, group I includes aliphatic ethers, group II includes alcohols, group III includes amides, group IV includes glycols and acetic acid, group V includes methylene and ethylene chlorides, group VI includes ketones, group VII includes aromatic hydrocarbons and nitro-compounds and group VIII includes water. Snyder was able to classify solvents according to their selectivity, without considering specific intermolecular effects. This solvent triangle is very useful in optimizing separations by substituting the solvent from one particular group with another solvent from a different group.

The solvent-selectivity triangle appears to be inaccurate in measurements of solvent selectivity in RPLC, where changes in the mobile phase concentration will often lead to significant changes in the separation selectivity, and this cannot be predicted by the solvent-selectivity triangle. The solvatochromic solvent-selectivity triangle appears to offer better classifications of solvent selectivity.

Solvent selectivity is an extremely well-documented topic in the literature, which includes an enormous number of examples illustrating how selectivity changes associated with solvents are effective for influencing and altering the selectivity of a separation in a chromatographic process. The effect of mobile phase is composition on the retention and selectivity of aromatic and saturated hydrocarbons. Planar aromatic compounds were preferentially retained on a C\textsubscript{18} stationary phase in methanol, but that a change in mobile phase to acetonitrile resulted in exceptionally different selectivity for the aromatic and saturated hydrocarbons. The C\textsubscript{18} stationary phase seemed to be more ordered in a methanol mobile phase, which has been known to self-associate by hydrogen bonding.

The mobile phase pH can also be adjusted to increase separation selectivity for solutes,
especially ionisable acids or bases. When a change in the mobile phase pH results in the required separation selectivity, but $k$ values do not fall within a specific range, the solvent strength can be adjusted essentially independently of selectivity by varying the components in the mobile phase, such as the salt concentration.

2.3.2.2 Temperature Selectivity

The effectiveness of varying the temperature of a system for increasing the selectivity for a chromatographic separation has been underestimated and under-utilised in HPLC. In HPLC, the temperature of a system is able to influence many physical parameters, such as, the viscosity, diffusivity of solute molecules into the mobile phase and stationary phase, the sample solubility and to decrease the hydrogen-bond acidity of the mobile phase relative to the stationary phase. In general, an increase in temperature leads to a decrease in the dipole-type interactions, and also leads to a decrease in the effectiveness of the mobile phase to compete as a hydrogen-bonding base. These physical parameters determine column efficiency, reduce column back-pressure, and influence the retention and separation time of a separation.

2.3.2.3 Stationary Phase Selectivity

The selectivity of a system can also be adjusted by changing the type of stationary phase that is used in a separation, although a change in the stationary phase is usually not as convenient as a change in the mobile phase composition or temperature. A change in the type of stationary phase can also lead to an increase in resolution for a particular separation, as well as possibly decreasing the retention time of the analysis. There are an enormous number of stationary phases that are available on the market, and these range from the most common stationary phase, being the C18 column, to a number of new stationary phases that have been recently introduced, which incorporate a variety of functionalities. These include pheny phases, octadecyl-based phases, polar-endcapped and polar-embedded phases, electron-donor and electron-acceptor phases, crystalline phases and carbon-type phases just to name a few.

3. Experimental
3.1 SMB

3.1.1 Chemicals

The standard of tanshinone I, tanshinone IIA, cryptotanshinone, matrine, oxymatrine and DADS (analytical grade) were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). SMB, SFA and garlic were purchased from local market. Methanol (HPLC Grade) tetrahydrofuran (THF) and acetic acid were purchased from Duksan Pure Chemical. Co., Ltd. (Korea). Trifluoroacetic acid (TFA) was purchased from Acros Organics (USA). Water was twice distilled and filtered (FH-0.45 μm, Advantec MFS, Inc., Japan) by using a decompressing pump (Division of Millipore, Waters, USA).

3.1.2 HPLC analysis

The chromatography system consisted of a Waters 600s Multi solvent Delivery System and a Waters 616 liquid chromatography (Waters Associates, Milford, MA, U.S.A.), a Rheodyne injector (Cotati, CA, USA) valve with a 20 μl sample loop, and a variable wavelength 2487 UV dual channel detector. Millenium software (Ver. 3.2 Interface Eng., Korea) on a PC was used as a data acquisition system. Experiments were performed with a commercially available RStech Corporation C\textsubscript{18} bonded phase column (4.6×150 mm i.d. 100 Å pore sizes, and 5 μm particles) purchased from Rs-Tech Co. (Daejeon, Korea). The injection volume was 5 μL, the flow-rate was 0.5 mL/min and UV wavelength was set at 254 nm.

3.1.3 Preparation of solutions

The stock standard solution was prepared by transferring about 1 mg of tanshinone I, tanshinone IIA and cryptotanshinone reference standards, respectively, each accurately weighed, to a 5 mL volumetric flask, and added respectively about 1 mL of water and methanol to the flask. These standard stock solutions were stored and further diluted to different working standard solution in a 4 °C refrigerator with light excluded. The SMB roots were oven-dried, sliced and crushed into powder for the extraction experiments.
Selection of a solvent affects the efficiency of extraction. Water, methanol, ethanol, ethyl acetate, and chloroform were selected as extraction solvent.

3.2 SFA

3.2.1 HPLC analysis

The chromatography system consisted of a Waters 600s Multi solvent Delivery System and a Waters 616 liquid chromatography (Waters Associates, Milford, MA, U.S.A.), a Rheodyne injector (Cotati, CA, USA) valve with a 20 μL sample loop, and a variable wavelength 2487 UV dual channel detector. Millennium software (Ver. 3.2 Interface Eng., Korea) on a PC was used as a data acquisition system. Experiments were performed with a commercially available RS Tech Corporation C₁₈ bonded phase column (4.6×150 mm i.d. 100 Å pore sizes, and 5 μm particles) purchased from Rs-Tech Co. (Daejeon, Korea). The injection volume was 5 μL, the flow-rate was 0.5 mL/min and UV wavelength was set at 210 nm.

3.2.2 Preparation of solutions

The stock standard solution was prepared by transferring about 1 mg of matrine and oxymatrine reference standards, respectively, each accurately weighed, to a 5 mL volumetric flask, and added respectively about 1 mL of water and methanol to the flask. These standard stock solutions were stored and further diluted to different working standard solution in a 4 °C refrigerator with light excluded.

The SFA roots were oven-dried, sliced and crushed into powder for the extraction experiments. Selection of a solvent affects the efficiency of extraction. Water, methanol, ethanol, ethyl acetate, and chloroform were selected as extraction solvent. Extraction procedures were shown in Figure 5.
3.3 Garlic

3.3.1 Apparatus

An instrument with an analytical HPLC system was used an M930D solvent delivery module equipped with a M930D solvent delivery pump (Young-In Co, Korea), a UV M720 absorbance detector (Young-In Scientific Co., Korea), and a Reodyne injector (Cotati, CA, USA) valve with a 20 μl sample loop. Experiments were performed with a commercially available column Platinum ESP C_{18} (150 × 4.6 mm i.d. and 5 μm particles) from Alltech Associates Inc. (Waukegan Road, IL, USA). Chromate software (Ver. 3.0 Interface Eng., Korea) on a PC was used as a data acquisition system. BÜCHI heating bath B490 (Flawil, Switzerland), BÜCHI evaporator R200 (Flawil, Switzerland) and aspirator A35 (Tokyo, Japan) were used for sample concentration. Ultrasonic bath was used for homogenization. 1200L single quadrupole gas chromatography (GC) and mass spectrometry (MS) system with 3800GC (Varian, USA) were used for qualitative DADS determination.
3.3.2 Sample preparation

Garlic powder was prepared as follows: fresh garlic was peeled and 500 g of peeled garlic was dried using a dryer at 50 °C for 24 hrs. The resulting was ground into powder with a mortar and pestle and stored at 4 °C until analysis. Preparation of the extract was done according to the scheme described in Figure 6. 2.0 g powder of homogenized with 100 ml of extractant, in an ultrasonic bath at room temperature for 5 min. After mix it round at room temperature for 30 min, and then was enriched to 1 ml solution by concentration system. Distillation was performed with vacuum at 35 °C. As the stability of DADS is very temperature-dependent, it is important to carry out the assay as quickly as possible, so the sample solutions were stored at 4 °C before injection.

Figure 6. Procedures of sample preparation.

3.3.3 HPLC analysis
In this work, flow rate was 1 ml/min, wavelength was UV 210 nm, injection volume was 3 μl and mobile phases were composed of water and methanol. Gradient elution mode was applied. The program used throughout the experiments is described below. The mobile phase composition of the reservoir A was water, while that of the reservoir B was methanol. In first 15 min, the mobile phase compositions of reservoirs A was linearly decreased from 95 to 0 vol. %, and the mobile phase composition of reservoir B was linearly increased from 5 to 100 vol. %, then hold last stage (water 0 vol. %, methanol 100 vol. %) in 3 min, at last return beginning stage (water 95 vol. %, methanol 5 vol. %) in 2 min. The HPLC parameters (retention time and peak area) reported in this study were the averages of at least three determinations. Evaluation of the results of the chromatographic experiment was carried out by mathematical statistic techniques. The relative error of a single measurement did not exceed at 5 %. All chromatographic procedures were performed at an ambient temperature.

3.3.4 GC-MS analysis

Column VF-5MS was utilized. GC oven was programmed: 40 °C 2 min, 10 °C /min to 300 °C, 20 min at 300 °C. The injection temperature was kept on 250 °C (splitless mode). Flow rate of the carrier gas (helium) was 25 cm/s. MS detector was operated at 194 °C, and ionization energy was 70 eV. Scan range was from 0 to 520 m/z at scan rate of 0.9 l/s.

4. Results and discussions

4.1 Results and discussions of SMB

4.1.1 Effect of different extraction solvents

The different extraction solvents used in the experiment for the extraction of tanshinone I, tanshinone IIA and cryptotanshinone from SMB were water, methanol, ethanol, ethyl acetate and chloroform. 50 mL of each solvent was used to extract 0.5 g SMB powder for 12 hr under room temperature respectively. Table 1 show that both of three compounds can be extracted by polar solvents and the highest extracted amount by methanol.
Table 1. Extracted amounts of tanshinone I, tanshinone IIA and cryptotanshinone with different solvents

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Tanshinone I (µg/g)</th>
<th>Tanshinone IIA (µg/g)</th>
<th>Cryptotanshinone (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>0.39</td>
<td>0.52</td>
<td>0.11</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.32</td>
<td>0.49</td>
<td>0.08</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>0.076</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>chloroform</td>
<td>0.0003</td>
<td>0.0006</td>
<td>0.0001</td>
</tr>
<tr>
<td>water</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*: not detected

4.1.2 Effect of different extraction method

The different extraction methods such as dipping extraction and ultrasonic extraction were investigated by 0.5 g SMB powder extracted with 50 mL methanol. In dipping extraction, the powder of SMB was mixed and stirred with solvent for different times. In Figure 7, the extracted amounts of tanshinone I, tanshinone IIA and cryptotanshinone increased as the dipping times was increased from 0.5 hr to 9 hr and no obvious increased after further prolong extraction time. Equivalent samples were then prepared by an ultrasonic method without dipping time. Figure 8 show that the extracted amounts of tanshinone I, tanshinone IIA and cryptotanshinone increased with an increase of ultrasonic time. However, comparing the results of the two methods, it was found that the amounts extracted via the ultrasonic method were lower, while more energy was required in the experiments. Thus, it was determined that the ultrasonic method was not appropriate for this approach.
Figure 7. Effect of different dipping times on extracted amounts of SMB.

Figure 8. Effect of different ultrasonic times on extracted amounts of SMB.
4.1.3 Chromatographic separation

Figure 9 shows typical chromatograms of SMB sample. Water as extract solvent and extraction time 4 hr under 80 °C is the optimum condition to extract tanshinone I, tanshinone IIA and cryptotanshinone from SMB. Under the chromatographic conditions described, tanshinone I, tanshinone IIA and cryptotanshinone had retention times of approximately 16.8, 18.9 and 28.3 min, respectively. The analysis time was 30 min per injection. It can be seen from the figure that good separation and detectability of tanshinone I, tanshinone IIA and cryptotanshinone in SMB sample were obtained with baseline resolved peaks and chromatograms with minimal interference from the herb. Hence, it is relatively easy to estimate the peak area with accuracy.

![Figure 9. Chromatogram of tanshinone I, tanshinone IIA and cryptotanshinone in methanol extract.](image)

4.1.4 Stability of the Solutions
The stability of standard and sample solutions was determined by monitoring the peak area and migration time of standard mixture solutions and sample solutions over a period of 1 week. The results showed that the migration time and peak area of each analyte remained almost unchanged and that no significant degradation is observed within the given period, indicating the solutions are stable for at least 1 week without the results being affected.

4.1.5 Linearity

A series of samples containing of tanshinone I, tanshinone IIA and cryptotanshinone (0.1, 3.0, 10.0, 50.0, 100, 250.0, and 500.0 μg/mL) were prepared to study the relationships between the peak area and the concentrations of the tanshinones under selected conditions. The results of linearity studies are presented in Table 2.

Table 2. Calibration curve for the quantification of tanshinone I, tanshinone IIA and cryptotanshinone

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Standard curve ((r^2))</th>
<th>Test range (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanshinone I</td>
<td>(Y=53735.0X-75327) (0.9998)</td>
<td></td>
</tr>
<tr>
<td>Tanshinone IIA</td>
<td>(Y=37353.0X-88373.0) (0.9997)</td>
<td>0.1-500.0</td>
</tr>
<tr>
<td>Cryptotanshinone</td>
<td>(Y=47839.0X-6967.0) (0.9998)</td>
<td></td>
</tr>
</tbody>
</table>

\(Y\), peak area; \(X\), concentration of analyte (μg/mL).

4.1.6 Precision

In order to determine the accuracy of the method recovery studies were carried out. Known amounts of three compounds were added to an accurately weighted fine power sample of SMB. The mixture of the tanshinones was extracted and analyzed using the proposed method. The control solution was prepared by extracting the same three samples without adding the standards.

In order to determine the accuracy of the method recovery studies were carried out. Known amounts of the three compounds were added to an accurately weighted fine powder sample of SMB. A mixture of the tanshinones was extracted and analyzed using the proposed method.
control solution was prepared by extracting the same three samples without adding the standards. The intra-day and inter-day repeatability of the method evaluated as RSD were performed by injecting 10 μg mL⁻¹ of tanshinone I, tanshinone IIA and cryptotanshinone in quintet in 7-day period.

The mean recoveries of tanshinone I, tanshinone IIA and cryptotanshinone from SMB were evaluated by spiking three different levels (1.0, 10.0, 40.0 μg mL⁻¹) to sample in replicates of three. The measured concentrations were compared with the theoretical concentration to calculate the recovery rates. The experimental results showed that the average recoveries of the three compounds were 89.5% for tanshinone I, 89.6% for tanshinone IIA and 87.9% for cryptotanshinone. The measured concentrations were compared with the theoretical concentration to calculate the recovery rates. The recoveries, RSD, and the LOD are presented in Table 3. Comparison with the real sample analysis, verified that the values noted above were of acceptable precision and accuracy.

Table 3. Recovery studies of tanshinone I, tanshinone IIA and cryptotanshinone (n=3)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RSD (%)</th>
<th>Recovery rate</th>
<th>LOD (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day</td>
<td>Inter-day</td>
<td>Amount added (μg mL⁻¹)</td>
</tr>
<tr>
<td>Tanshinone I</td>
<td>4.6</td>
<td>4.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
<td>4.9</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>4.1</td>
<td>40.0</td>
</tr>
<tr>
<td>Tanshinone IIA</td>
<td>3.6</td>
<td>3.8</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>3.6</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>2.9</td>
<td>40.0</td>
</tr>
<tr>
<td>Cryptotanshinone</td>
<td>4.1</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>4.3</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>4.2</td>
<td>40.0</td>
</tr>
</tbody>
</table>
4.2 SFA

The composition of SFA is very complicated. This herb is known to contain around 10 alkaloids, matrine and oxymatrine being the main bioactive alkaloids. The structures of the two alkaloids are very similar, thus, the results of the separation and analysis were often unsatisfactory when these alkaloids were simultaneously determined.

4.2.1 Effect of different extraction solvents

The different extraction solvents used in the experiment for the extraction of matrine and oxymatrine from SFA were water, methanol, ethanol, ethyl acetate and chloroform. 50 mL of each solvent was used to extract 0.5 g SFA powder for 12 hr under room temperature respectively. Table 4 show that both of two compounds can be extracted by polar solvents and the highest extracted amount by water.

Table 4. Extracted amounts of matrine and oxymatrine with different solvents

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Compounds</th>
<th>Matrine (mg/g)</th>
<th>Oxymatrine (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td></td>
<td>0.0085</td>
<td>0.15</td>
</tr>
<tr>
<td>methanol</td>
<td></td>
<td>0.0066</td>
<td>0.12</td>
</tr>
<tr>
<td>ethanol</td>
<td></td>
<td>0.0009</td>
<td>0.005</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>chloroform</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*: not detected

4.2.2 Effect of different extraction method

The different extraction methods such as dipping extraction and ultrasonic extraction were investigated by 0.5 g SFA powder extracted with 50 mL water. In dipping extraction, the powder of SFA was mixed and stirred with solvent for different times. In Figure 10, the extracted
amounts of matrine and oxymatrine increased as the dipping times was increased from 0.5 hr to 9 hr and no obvious increased after further prolong extraction time. Equivalent samples were then prepared by an ultrasonic method without dipping time. Figure 11 show that the extracted amounts of matrine and oxymatrine increased with an increase of ultrasonic time. However, comparing the results of the two methods, it was found that the amounts extracted via the ultrasonic method were lower, while more energy was required in the experiments. Thus, it was determined that the ultrasonic method was not appropriate for this approach.

Figure 10. Effect of different dipping times on extracted amounts of SFA.
4.2.3 The optimum extraction temperature

Different dipping temperatures ranged from 30 °C to 100 °C were evaluated, the dipping time was 4 hr and the results were shown in Figure 12. The extracted amounts of matrine and oxymatrine increased quickly with the temperature increasing from 30 °C to 80 °C and almost constant when temperature higher than 80 °C. Compared the results with dipping method, the extracted amounts of matrine and oxymatrine from SFA by 4 hr dipping under room temperature were the almost same as 4 hr dipping under 80 °C. The results indicated shorter dipping time with higher temperature and 80 °C was the optimized temperature for SFA extraction in this work.
Figure 12. Effect of different temperatures on extracted amounts of SFA.

4.2.4 Chromatographic separation

Figure 13 shows typical chromatograms of SFA sample. Water as extract solvent and extraction time 4 hr under 80 °C is the optimum condition to extract matrine and oxymatrine from SFA. Under the chromatographic conditions described, matrine and oxymatrine had retention times of approximately 11.2 and 24.8 min, respectively. The analysis time was 25 min per injection. It can be seen from the figure that good separation and detectability of matrine and oxymatrine in SFA sample were obtained with baseline resolved peaks and chromatograms with minimal interference from the herb. Hence, it is relatively easy to estimate the peak area with accuracy.
Figure 13. Chromatogram of matrine and oxymatrine in water extract.

4.2.5 Linearity

A series of samples containing of matrine (3.0, 10.0, 50.0, 100.0, 250.0, and 500.0 μg/mL) and oxymatrine (3.0, 10.0, 50.0, 100.0, 500.0, and 1000.0 μg/mL) were prepared to study the relationships between the peak area and the concentrations of the alkaloids under selected conditions. The results showed that the peak area was linearly related to the matrine for the range of 3.0–500.0 μg/mL. For the oxymatrine, the linear range was 3.0–1000.0 μg/mL. The results of linearity studies are presented in Table 5.

Table 5. Calibration curve for the quantification of matrine and oxymatrine

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calibration curve ((r^2))</th>
<th>Test range (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrine</td>
<td>(Y=17073.0X+4879.9) (0.9999)</td>
<td>3.0-500.0</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>(Y=15333.0X+59432.0) (0.9991)</td>
<td>3.0-1000.0</td>
</tr>
</tbody>
</table>

\(Y\), peak area; \(X\), concentration of analyte (μg/mL).

4.2.6 Precision
In order to determine the accuracy of the method recovery studies were carried out. Known amounts of two alkaloids were added to an accurately weighed fine power sample of SFA. The mixture of the alkaloids was extracted and analyzed using the proposed method. The control solution was prepared by extracting the same two samples without adding the alkaloids.

The mean recoveries of matrine and oxymatrine from SFA were evaluated by spiking three different levels of matrine (3.0, 10.0, 50.0 μg/mL) and oxymatrine (30.0, 100.0, 500.0 μg/mL) to sample in replicates of three. The measured concentrations were compared with the theoretical concentration to calculate the recovery rates. The experimental results showed that the average recoveries of the two alkaloids were 90.8% for matrine and 88.1% for oxymatrine (Table 6). Comparison with the real sample analysis, verified that the values noted above were of acceptable precision and accuracy.

Table 6. Recovery studies of matrine and oxymatrine in SFA (n=3)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Amount added (μg/mL)</th>
<th>Average recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrine</td>
<td>5.0</td>
<td>90.3</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>90.9</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>91.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>50.0</td>
<td>88.5</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>87.6</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>500.0</td>
<td>88.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

4.3 Garlic

The sample preparation procedures used in the work were relatively simple; and extracts were injected. HPLC experiment was conducted to separate DADS using C18 column. Surprisingly, it was found that two peaks were disclosed at injection of the purchased DADS standard solution. Thus, the additional procedures of the identification of the peaks of standard had to be made. The effluents of peaks supposed as DADS from methanol extract were collected and they were injected into GC with MS for detection. The results were shown in Figure 14. Molecular weight of DADS is 146.28. The target molecular ion was finding in the first eluted peak (Figure 14(a)).
The second collected peak has not DADS molecular ion signal (Figure 14(b)). When looked at in more detail, from Figure 14(b) did not find DADS, but we considered that this effluent was most comprised of DAS and DATS. It is more realistic to suppose that the purchased DADS standard was not enough pure. From these experiments it was concluded that the first peak was validated as DADS.

Figure 14. GC-MS of effluent of peaks from methanol extract. ((a): first peak; (b): second peak)

Moreover, in order to calculate the amount of DADS extracted from the garlic, a calibration
curve needs to be drawn. In order to assess the extent of the linear relationship between the known concentrations of DADS (X) and the corresponding absorbance values (Y), the \( r^2 \) was calculated. By plotting of concentration vs. peak area, the regression equation of the calibration curve was \( Y=8367.3X + 19661 \). A value of \( r^2 = 0.998 \) indicates a high positive correlation. This is illustrated in Figure 15.

![Graph showing calibration curve](image)

**Figure 15. The calibration curve of the DADS.**

Figures 16-18 are shown chromatograms of determination of DADS in three differences extracts. In our work, the goal was to generate a convenient technique for rapidly but accurately determining the DADS in garlic. Methanol, benzene or THF were selected as the extracting solvents because they readily dissolve the target DADS but will not easily dissolve other potentially interfering compounds. Without any doubt, the effects of extraction conditions have influence on extraction efficiency.
Figure 16. Chromatogram of DADS in methanol extract.

Figure 17. Chromatogram of DADS in benzene extract.
The extraction amounts of DADS from garlic with three used solvents are shown in Table 7. Contrasted with three results, methanol as the extraction solvent was optimized and 0.61 mg of DADS per g garlic can be extracted. It is worthy of note that presented DADS amounts are the average values, obtained after three experiments. In this work of three different in the polarity of extractant they were used. The best results of the extraction of DADS were achieved with the polar solvent - methanol. Nonpolar benzene extracts practically two times less substance than methyl alcohol. THF, as substance with the middle polarity, it showed the worst results with the extraction of DADS. It was obviously found that the nature of extractant markedly affect on the extraction performance and as a consequence on the amount of extractible DADS.

Table 7. The extraction amounts of DADS from garlic with three different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Amount of DADS mg/g (DADS/garlic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.61</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.33</td>
</tr>
<tr>
<td>THF</td>
<td>0.19</td>
</tr>
</tbody>
</table>
5. Conclusion

The method provides excellent separation and good precision, and is simple and reliable in both chromatographic condition and sample preparation. Methanol as extract solvent and ultrasonic time 20 min is the optimum condition to extract tanshinone I, tanshinone IIA and cryptotanshinone from SMB. The analytical procedure is very suitable for the determination of tanshinone I, tanshinone IIA and cryptotanshinone in SMB.

The analytical procedure is easy to handle and is very suitable for the determination of alkaloids in SFA. Water as extract solvent and extraction time 4 hr under 80 °C is the optimum condition to extract matrine and oxymatrine from SFA. The method has been successfully applied to the simultaneous determination of matrine and oxymatrine in SFA.

A simple rapid analytical technique was used for the separation and determination of DADS in garlic. The experimental results showed that the optimized extraction solvent was methanol and 0.61 mg of DADS per g garlic can be extracted. In addition, this method was validated with respect to reproducibility. Thus, this technique will be useful for chemical and biological studies of garlic and its products.
Summary in English

In this work, the extraction and separation of tanshinone I, tanshinone IIA, cryptotanshinone, matrine, oxymatrine and DADS from natural plant SMB, SFA and garlic were performed with various mechanical and chemical extraction methods.

The method provides excellent separation and good precision, and is simple and reliable in both chromatographic condition and sample preparation. Methanol as extract solvent and extraction time 4 hr is the optimum condition to extract tanshinone I, tanshinone IIA and cryptotanshinone from SMB. The extracted amounts are 0.0091, 0.0085 and 0.15 mg/g, recoveries are 90.8%, 88.1% and 93.5%, and the relative standard deviations are 3.3%, 2.9% and 3.2%, respectively. The analytical procedure is very suitable for the determination of tanshinone I, tanshinone IIA and cryptotanshinone in SMB.

The analytical procedure is easy to handle and is very suitable for the determination of alkaloids in SFA. Water as extract solvent and extraction time 4 hr under 80 °C is the optimum condition to extract matrine and oxymatrine from SFA. The extracted amounts are 0.0091 and 0.15 mg/g, recoveries are 90.8% and 88.1% the relative standard deviations are 3.3% and 2.9%, respectively. The method has been successfully applied to the simultaneous determination of matrine and oxymatrine in SFA.

A simple rapid analytical technique was used for the separation and determination of DADS in garlic. The experimental results showed that the optimized extraction solvent was methanol and 0.61 mg of DADS per g garlic can be extracted. In addition, this method was validated with respect to reproducibility. Thus, this technique will be useful for chemical and biological studies of garlic and its products.
분 연구는 천연물인 단삼, 고삼, 마늘로부터 유용성분인 tanshinone I, tanshinone IIA, cryptotanshinone, matrine, oxymatrine 및 DADS 을 추출하여 분석하였다. 추출량을 비교하였고 표준물질에 의하여 정량 및 정성분석을 하였다. 전처리 단계에서는 추출, 여과, 농축, 원심분리, 막분리로 구성되었으며, 추출액은 역상 고성능 액체 크로마토그래피(RP-HPLC) C18 컬럼을 사용하여 분석하였다.

단삼의 추출물에서 얻어지는 tanshinone I, tanshinone IIA과 cryptotanshinone을 얻기 위하여 분석용 HPLC를 사용하였다. 액체 크로마토그래피는 천연물질의 분리에 많이 사용되고 있다. 전처리한 추출액에 포함된 tanshinone I, tanshinone IIA와 cryptotanshinone을 분석하고 최적의 조업조건을 실험적으로 구하였다. 모든 단삼 시료들은 메탄올, 에틸아세테이트, 에탄올같은 유기용매로 추출하여 분석하였다. 실험결과에 의하면, 메탄올로 추출한 시료가 가장 우수 하였다. 분석조건으로는 C18 컬럼을 사용하였고, 이동상은 물, 메탄올과 acetic acid로 구성되고 분석법에 따른 일정용매조성법과 구배용매조성법을 적용하였다. 메탄올 추출물에서의 메탄올 아세테이트 및 에탄올 추출액에서보다 tanshinone I, tanshinone IIA와 cryptotanshinone의 함량이 매우 높았다.

고삼의 추출물에서 얻어지는 matrine과 oxymatrine을 얻기 위하여 분석용 HPLC를 사용하였다. 전처리한 추출액에 포함된 matrine과 oxymatrine을 분석하고 최적의 조업조건을 실험적으로 구하였다. 모든 고삼 시료들은 메탄올, 에틸아세테이트, 에탄올같은 유기용매로 추출하고 비교 분석하였다. 실험결과에 의하면 메탄올 용매를 사용한 경우, matrine과 oxymatrine의 추출효율이 가장 우수 하였다. 분석조건으로는 C18 컬럼을 사용하였고, 이동상은 물, 메탄올과 trifluoroacetic acid로 구성되고 주로 일정용매조성법을 적용하였다.

마늘의 특성은 organosulfur 화합물이다. 마늘에 포함된 diallyl disulfide (DADS)를 분석하기 위한, 간단하고 신속한 시료준비와 분석방법을 제시하였다. 모든 마늘시료들은 메탄올, 벤젠, 또는 테트라하이드로퓨란과 같은 용매로 추출하여
분석하였다. 실험결과에 의하면, 메탄올로 추출한 시료가 가장 우수하였다. 분석조건으로는 이동상은 메탄올과 물로 구성되고 구배용매조성법을 적용하였다. 분말가루로 된 마늘 1 g에 0.61 mg DADS를 추출하였다. 기존 분석방법에 비해 우수하여 마늘관련 연구에 도움이 될 것이다.
Summary in Chinese

本论文的主要内容是，应用不同种类的提取方法从天然植物的丹参、苦参、大蒜中提取和分离丹参酮、丹参酮 II A、隐丹参酮、苦参碱、氧化苦参碱和二烯丙基二硫。

对于丹参，本论文提供了简单而可靠的色谱条件和样品制备条件。甲醇作为溶剂提取，超声时间 20 分钟是从丹参中提取丹参酮、丹参酮 II A 和隐丹参酮的最佳条件。提取量是 0.0091、0.0085 和 0.15 毫克/克和回收率为 90.8%、88.1% 和 93.5%，相对标准偏差 3.3%、2.9% 和 3.2%。该方法非常适合检测，丹参中丹参酮、丹参酮 II A 和隐丹参酮。

对于苦参，本论文提供了两种生物碱的检测和分析方法。以水为溶剂提取，提取时间 4 小时及温度控制在 80 °C 是从苦参中提取苦参碱和氧化苦参碱的最佳条件。提取量是 0.0091 和 0.15 毫克/克和回收率为 90.8% 和 88.1%，相对标准偏差分别为 3.3% 和 2.9%。该方法已成功地应用于在苦参中同时测定苦参碱和氧化苦参碱。

对于大蒜，本实验建立一个简单的快速分析方法被用于分离和测定的二烯丙基二硫在大蒜。实验结果表明，最优萃取溶剂为甲醇和 0.61 毫克的二烯丙基二硫每克大蒜可以被提取。这一技术是有益于从化学和生物的方向研究大蒜及其产品。
References

Appendix 1. Adsorption Isotherms of Benzene and its Derivatives by RPLC Frontal Analysis

Abstract

The adsorption behavior of solute is one of the most important factors to design a batch and a continuous liquid chromatographic separation process. In liquid chromatography, these behaviors are based on the adsorption equilibrium between a liquid mobile-phase and a solid stationary-phase. However, almost of the retention models were developed under linear adsorption isotherm only few researchers have investigated the relationship between the adsorption parameters and the mobile phase composition, and some empirical models were introduced. In this work, the adsorption isotherms were obtained by the frontal analysis for four low-molecular compounds (benzene, toluene, chlorobenzene and 1,2-dichlorobenzene) on a commercial C18 bonded column. The absorption based on the linear and Lamgmuir models was investigated according to the changes of the composition of methanol high-enriched eluent. The calculations and analysis of the coefficients obtained for both models confirm that the adsorption data for solutes are best modeled with the linear approach. Lamgmuir isotherm model couldn’t satisfactorily describe mechanism and provide the objective information on the physical nature of the absorption in spite of the acceptable accuracy.
Appendix 2. Surface confined ionic liquid as a stationary phase for HPLC

Abstract

Room temperature ionic liquids (RTILs) are salts with melting points at or close to room temperature. Typically, these salts consist of bulky organic cations, such as N-alkylpyridinium and 1-alkyl-3-methylimidazolium combined with an inorganic or organic anion. They represent an interesting class of solvents because they exhibit properties such as negligible vapor pressure, good thermal stability, tunable viscosity, and primarily anion-dependent miscibility with water as well as various organic solvents. In recent years, RTILs have been widely considered as alternatives to classical organic solvents and applied in organic synthesis, electrochemistry, liquidphase extraction, catalysis for clean technology, and separations.

Trimethoxysilane “ionosilane” derivatives of room temperature ionic liquids based onalkylimidazolium bromides were synthesized for attachment to silica support material. The derivatives 1-methyl-3-(trimethoxysilylpropyl)imidazolium bromide and 1-butyl-3-(trimethoxysilylpropyl)imidazolium bromide were used to modify the surface of 3 mm diameter silica particles to act as the stationary phase for HPLC. Columns packed with the modified silica material were tested under HPLC conditions. Preliminary evaluation of the stationary phase for HPLC was performed using aromatic carboxylic acids as model compounds. The separation mechanism appears to involve multiple interactions including ion exchange, hydrophobic interaction, and other electrostatic interactions.
List of publications

SCI/SCIE


Korean Journal


2. **Xiaolong Wan**, Yong An Jung and Kyung Ho Row, “Solvent Extraction of Tanshinone IIA from Salvia Miltiorrhiza Bunge” Korean Chemical Engineering Research, accepted for publication.

International Proceedings
1. **Xiaolong Wan** and Kyung Ho Row, “Solvent Extraction of Tanshinone IIA from Salvia Miltiorrhiza Bunge”

Korean Proceedings


감사의 글

인하대학교 화학공학과 석사졸업을 앞두고 2년 동안 여레모로 가르침과 도움을 주신 교수님과 학우 여러분 들게 감사의 마음을 전하고 싶습니다.

먼저 짧지 않은 석사과정 동안 많은 가르침과 도움을 주신 노경호 교수님께 깊은 감사를 드립니다. 그리고 이 논문이 완성되기까지 세심하게 심사해 주시고 조언을 아끼지 않으신 인하대학교 화공전공 정성택 교수님, 장윤호 교수님께 진심으로 감사를 드립니다. 또한 석사과정 중에 넓은 가르침과 배움의 길을 열어주신 인하대학교 화학공학과 모든 교수님께 감사를 드립니다.

그 동안 같이 연구하며 생활을 같이 한 고순도 분리 연구실의 선두배들에게 감사의 마음을 전합니다. 특히 실험을 같이 진행하고 여러 방면으로 도움을 준 정용안 박사, 이광진 박사, 이주원 박사, 박사과정 염홍원, 김은철, 김춘화 석사, 석사과정 전명래, 왕엽에게 감사의 마음을 전합니다. 그리고 함께 연구하면서 많은 지도를 해 주신 Yulia Polyakova 박사에게도 깊은 감사의 말을 전합니다.

끝으로 긴 석사과정 동안 사랑과 정성으로 보살펴 주신 아버지, 어머니, 묵묵히 제 뒤를 받쳐준 가족들에게 이 논문을 바칩니다.

2008 년 6 월

고순도 분리 연구실에서
만효룡 드림.