Quality Assurance of Chinese Herbal Medicines: Procedure for Multiple-Herb Extraction

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A quality assurance (QA) procedure for multiple-herb extraction, which takes into account the existence of common chemical markers and multiple-herb-extraction effects, has been developed for producing Chinese herbal medicines (CHMs) of consistent quality. The experimental method for determining related correlation function of the multiple-herb-extraction effect was designed. A systematic solution strategy was also developed to appropriately decompose the multiple-herb extraction system into several subsystems for obtaining solution(s) and determining the overall behavior of the system. An example of QA of Danshen–Gegen (DG) decoction was used to demonstrate the QA procedure. An H9c2 cell assay was used to test the efficacy of consistent quality DG decoctions prepared by different herb combinations with different material costs of herbs. It was observed that a multiple-herb-extraction effect was present in the aqueous extraction of Danshensu and this effect was depended on the extraction solvent. The possible mechanism of this multiple-herb-extraction effect in the aqueous DG extraction was speculated to be the change of initial pH value of the aqueous extraction solvent by an unknown component from Gegen. The experimental chemical marker concentrations fell within ±10% of the specified chemical marker compositions by using the amount of herb from each herb class as predicted by the QA model. Furthermore, an H9c2 cell assay was used to test the efficacy of three consistent quality DG extracts, which were produced by different herb combinations with different material costs of herbs. The results showed that the three DG extracts provided consistent biological efficacy against menadione-induced toxicity. This study extended a recently developed QA procedure of single-herb extraction to multiple-herb extraction. It provides a solution of QA in extraction, which is one of the most important unresolved problems in the modernization of traditional Chinese medicines. With this modified model and the companion experiments, the amount of herbs needed from different quality classes to produce a multiple-herb formula CHM product decoction with consistent quality can be exactly determined.
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Keywords: Chinese herbal medicine, product quality, quality assurance, multiple-herb extraction, process design

Introduction

Chinese herbal medicinal (CHM) products on the market often exhibit different product qualities (as quantified by the proportions of the bioactive markers or simply referred to as chemical markers). The root cause of the problem is due to the fact that the chemical composition of raw herbs can vary significantly depending on the place and conditions of growth, and so forth. These raw herbs are classified into superior, medium, or inferior; the amount of a specific chemical marker in the raw herb does not necessarily correlate with its classification. Despite the herb variations, the same recipe, in terms of the relative amount of each herb, is fixed in extraction, resulting in a CHM product with the bioactive markers not in the desired proportions (i.e., the desired quality). Recently, a systematic quality assurance (QA) procedure has been developed by Lau et al.,[1] which assures that a consistent quality CHM product is produced in the extraction of a single herb. The procedure has two major components. One is a model which calculates the amount of each herb

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class that should be used in the extraction so that the same proportion of chemical markers is obtained despite the variations in the composition of raw herbs. Basically, this is a blending approach commonly used in chemical engineering, where consistent quality CHM product is produced by combining herbs from different classes. Whether a solution exists or not depends on the number of chemical markers and the number of available herb classes. Another is an experimental procedure that allows the determination of the relevant model parameters.

For a multiple-herb product, the QA procedure has to deal with the added complexity of common chemical markers and multiple-herb-extraction effects. A common chemical marker is a marker that exists in more than one type of herb. Thus, the concentration of a given chemical marker in the extraction solvent is the sum of the individual amounts of the same marker extracted from herbs of different quality classes and different herbs. Multiple-herb-extraction effects are caused by the interactions among chemical components being extracted from different herbs. For example, a chemical marker from one herb can decompose in the presence of another herb in the mixture.

The objective of this study was to extend the QA procedure developed for single-herb to multiple-herb extraction with proper modifications to the model and experimental procedure. A systematic strategy was developed to appropriately decompose the multiple-herb extraction system into several subsystems for obtaining solution(s) to the model. Because of the complexity, an empirical approach in the form of a correlation function was used to quantify the multiple-herb-extraction effects.

The extraction of Danshen (D) and Gegen (G) to form a decoction was used to illustrate the QA procedure. Also demonstrated was the experimental method for determining the correlation function for multiple-herb-extraction effects. The possible mechanisms causing the multiple-herb-extraction effects were identified. In addition, an H9c2 cell assay was used to check the efficacy of three DG decoctions, prepared by different herb combinations as predicted by model, that were supposedly of the same quality.

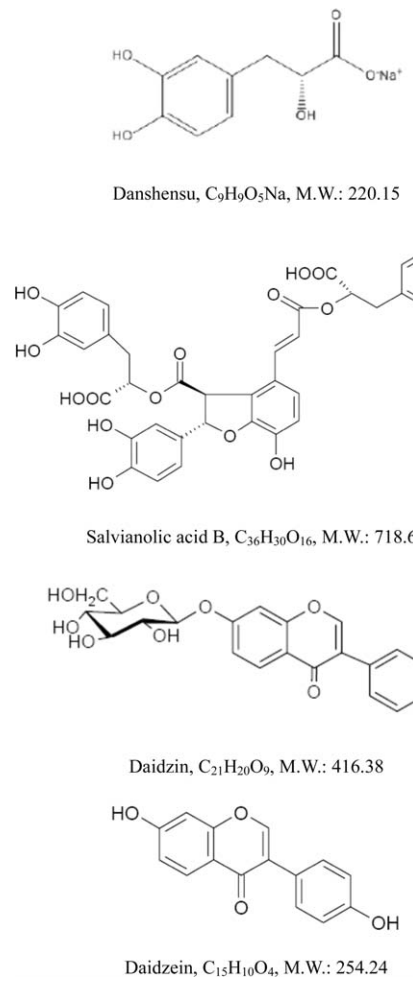

Figure 1. Molecular structure of DS, SAB, DN, and daidzein.

Materials and Experimental Methods Used

Herbs and chemical markers

Danshen (*Radix Salviae Miltiorrhiza*) and Gegen (*Radix Puerariae Lobatae*) are medicinal herbs widely used in China and other Asian countries. Danshen–Gegen (DG) decoction has been shown to produce beneficial effects on cardiovascular functions in animals, humans, and cultured human endothelial cells.[2–4] For each herb, three different quality classes (categorized as superior, medium, or inferior class) were used. Based on the market price in Hong Kong in the spring of 2010, the cost of Danshen was HK$ 100/kg for superior class (1S), HK$ 100/kg for medium class (1M), and HK$ 40/kg for inferior class (1I), and the cost of Gegen was HK$ 30/kg for superior class (2S), HK$ 60/kg for medium class (2M), and HK$ 20/kg for inferior class (2I). Note that the herb class was assigned by a herbalist; it does not necessarily reflect the herb composition or price. Raw herb was ground into powder form and sieved such that all the herb powders used in this study had a particle size of 1.0 mm or smaller. All the herb powders were stored in a 4°C refrigerator before use. Water used in the experiments was double deionized with a resistivity of 18.2 Megaohm-cm at room temperature. Except for the chemicals specifically mentioned, all chemicals were of analytical grade.

Danshensu (DS) and salvianolic acid B (SAB) were selected as chemical markers of Danshen, and daidzin (DN) and daidzein (DeN) were selected as chemical markers of Gegen (Figure 1). All standards of the chemical markers were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), with purities higher than 98%. The product quality of DG decoction was specified as the ratio of DS:SAB:DN:DeN = 2:49:1.77:1 (or equivalently the concentrations in the extract $C_{DS}^{QA} = 0.140$ mg/mL, $C_{SAB}^{QA} = 3.430$ mg/mL, $C_{DN}^{QA} = 0.124$ mg/mL, and $C_{DeN}^{QA} = 0.070$ mg/mL). This ratio was determined by the medical researchers in a 10-year long project supported by the University Grants Council of Hong Kong.

Chemicals

All chemicals were of analytical grade. Pyruvate and reduced nicotinamide adenine dinucleotide (NADH) were purchased from Sigma Chemical Co. (St. Louis, MO). H9c2 cell line was purchased from ATTC (Rockville, MD). Cell culture medium and fetal bovine serum (FBS) were obtained from GIBCO BRL Life Technologies (Grand Island, NY). The H9c2 cells, which are cultured as monolayer in Dulbecco's modified Eagle's medium (GIBCO BRL) supplemented with 10% (v/v) FBS, are permanent cell line derived from cardiac myoblast.

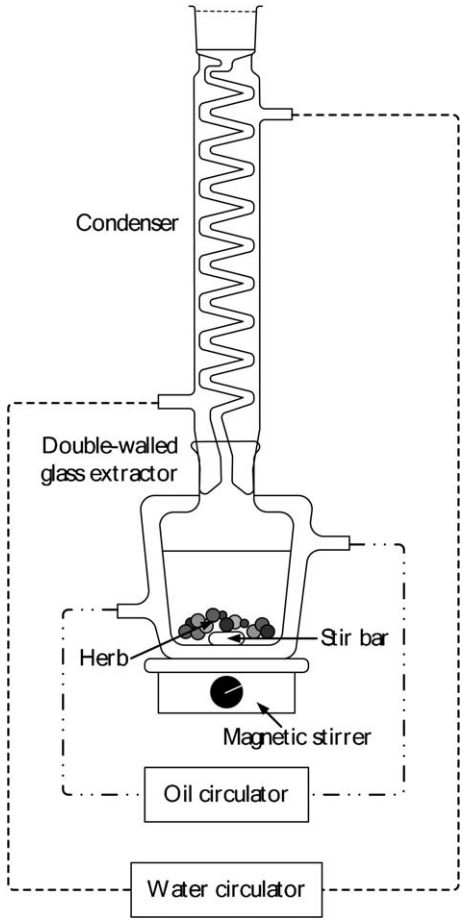

Figure 2. Schematic diagram of setup for heat-and-reflux extraction.

Herbal extraction

The heat-and-reflux extraction method was used in this project. The schematic diagram of the experimental setup is shown in Figure 2. Herbs were extracted in a double-walled glass extractor with magnetic stirring and temperature control by oil circulator. Water circulated condenser for reflux of extraction solvent was installed on the top of the extractor. For each extraction, a certain amount of Danshen and/or Gegen powders was macerated in 50 g of water for 60 min at room temperature. After maceration, an additional 50 g of water was added. The system was then heated under reflux at 100°C to start the extraction process under gentle magnetic stirring. Samples of 0.5 mL in volume were taken at certain time points during extraction. The chemical contents in the samples were determined using a Waters Alliance high performance liquid chromatography (HPLC) system which comprises the Waters 2956 Separation Module and the Waters 2996 Photodiode Array Detector. An ODS-2 Hypersil 5 μm (4.6 $\times$ 250 mm^2) column was used. The mobile phase was water and acetonitrile (with 0.1% acetic acid). A sample of 40 μL was injected and the elution was monitored by UV absorbance at 270 nm.

H9c2 cell assay

The medium contained glucose (4.5 g/L) and glutamine (4.5 mM), supplemented with $NaHCO_3$ (17 mM), penicillin (100 IU/mL), and streptomycin (100 μg/mL). The H9c2 cells were grown under an atmosphere of 5% (v/v) CO_2 in air at 37°C. The medium was replaced by fresh medium every 2 or 3 days. A stock of cells was grown in a 75 cm^2 culture flask and split before confluence at a subcultivation ratio of 1:10. Cells used for experiments were seeded at a density of 3.75 $\times$ 10^4 cells/well in a 12-well culture plate, and cells in each well were allowed to grow to achieve 60–80% confluence within 24 h prior to drug pretreatment.

The efficacy of three DG extracts supposedly with consistent quality produced in this project (DG-1, DG-2, and DG-3) on oxidant-induced cell injury was investigated. Cells were exposed to DG-1, DG-2, or DG-3 (100, 200, or 300 μg/mL) for 24 h. Vehicle, which was phosphate-buffered saline (PBS), was added for the control cells.

Cells were seeded at 3.75 $\times$ 10^4 cells/well in a 12-well culture plate and they were allowed to grow overnight. Immediately following the drug pretreatment, the cells were challenged with menadione (dissolved in ethanol) at 12.5 μM (0.2% (v/v) ethanol, final concentration) for 4 h. Unchallenged (i.e., control) cells were treated with the medium containing 0.2% ethanol only.

After the menadione challenge, the microtiter plate containing cells and medium was centrifuged at 300*g* (Himac CF 9RX, Hitachi Koki Co., Japan) for 15 min at 4°C, and the medium samples were retained in respective microcentrifuge tubes. The cells were then washed once with PBS. Aliquots of 200 μL 0.1% (v/v) Triton-X were added to the cells. After shaking for 10 min at 4°C, the cell lysates were transferred to microcentrifuge tubes. The medium and lysate samples were then centrifuged at 540*g* for 15 min at 4°C. The resultant supernatants were used for the measurement of lactate dehydrogenase (LDH) activity.

For the measurement of LDH activity, an aliquot of 60 μL medium or 20 μL lysate (made up to 60 μL with phosphate buffer [0.1 M; pH 8.0]) was added to a well of a 96-well microtiter plate. 140 μL of prewarmed reaction mixture (containing 10 μL of 20 mM pyruvate, 10 μL of 3 mM NADH, and 120 μL of phosphate buffer) were added to initiate the reaction. Absorbance changes at 340 nm were monitored spectrophotometrically by Victor V^3 Multilabel Counter (Victor V^3, Perkin-Elmer) every minute for 5 min at 37°C. The enzyme activity was estimated by using an extinction coefficient for reduced form of NADH at 340 nm of 6.22 $\times$ 10^3 M^{-1} cm^{-1} and expressed as U/L of medium/lysate. One unit (U) represents the activity of enzyme that can catalyze the oxidation of 1 μmol NADH per min. The percentage of LDH release (% LDH release) was estimated as an index of cellular injury, which was calculated as follows

$$\%\ \text{LDH release} = \frac{\text{LDH in medium}}{(\text{Cellular LDH} + \text{LDH in medium})} \times 100\% \quad (1)$$

where LDH in medium means the amount of LDH in the medium of unchallenged or challenged cells and cellular LDH means the amount of cellular LDH measured in cell lysate prepared from control cells, with or without drug pretreatment.

QA Procedure of CHM Product in Multiple-Herb Formula

The herbal extraction model

A schematic diagram of herbal extraction by heat and reflux in a well-mixed stirred tank with an extraction solvent

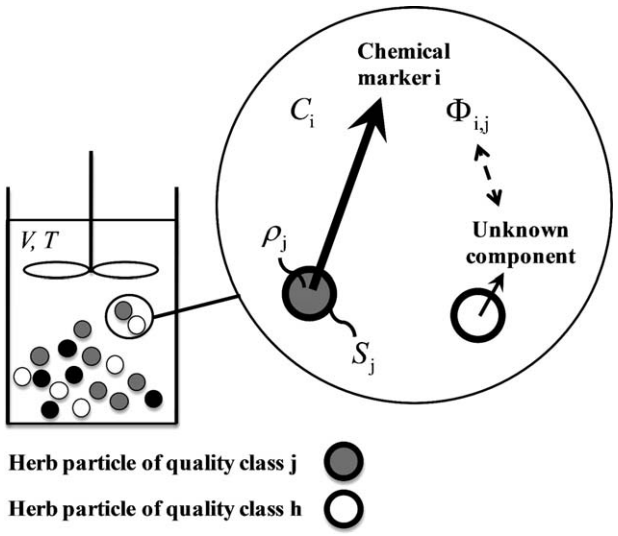

Figure 3. Illustration of multiple-herb extraction.

The herb particles are shaded differently to represent different quality classes. Chemical marker i is being extracted from herb of quality class j. A multiple-herb-extraction effect exists during the extraction including herb of quality class h of another herb. An unknown chemical component from quality class h of another herb changes the maximum available amount of chemical marker i than that in single-herb extraction.

of volume V (mL) and at temperature T (°C) is shown in Figure 3. Several herb types containing one or more chemical markers are present and each herb type can include one or more quality classes. The herb particles are shaded differently to represent different quality classes. Several chemical markers are simultaneously extracted from these herb particles. Chemical marker i is being extracted from herb particle of quality class j. The maximum extractable amount of chemical marker i for herb quality class j is $\Phi_{i,j}$ (mg i/g herb of quality class j). C_i (mg i/mL) is the concentration of chemical marker i in the extraction solvent at any given time t (min). As demonstrated elsewhere,[1] the internal mass transfer rate is fast compared with that external to the small herb particles. Also, as shown in Appendix, the absorption of chemical marker extracted from one herb by other herbs is insignificant. Since the multiple-herb extraction model, except for the correlation function accounting for multiple-herb-extraction effect, is similar to that in single-herb extraction discussed in Lau et al.,[1] only the governing equations are summarized below. The rate of extraction of chemical marker i from an herb particle of quality class j, $\dot{C}_{i,j}$ (mg/mL•min) can be expressed as

$$\dot{C}_{i,j}=\frac{k_{i,j}M_jS_j}{V}\left[\left(K_{i,j}\rho_j\Phi_{i,j}-\frac{K_{i,j}\rho_jV}{M_j}C_{i,j}\right)-C_i\right] \tag{2}$$

where $k_{i,j}$ (cm/min) is the mass-transfer coefficient, $K_{i,j}$ (dimensionless) is the partition coefficient of chemical marker i which is the ratio of the concentration of chemical marker i inside the herb particle of quality class j under consideration and that at the interface, M_j (g herb of quality class j) is the mass of the herb of quality class j under consideration, S_j (cm^2/g) is the specific surface area and ρ_j (g/cm^3) is the density of herb of quality class j, which are assumed to be constant and $C_{i,j}$ (mg i/mL) is the concentration of chemical marker i in extraction solvent extracted from the herb of quality class j. Note that the index j in $C_{i,j}$ has a special meaning. Here, j represents a quality class of a specific herb type in the extraction tank. For example, if there are two herb types with one herb type having two quality classes and the other having three quality classes, the total number of j is 5. In this way, the need for an index for herb type and another for quality class is avoided. If chemical marker i does not exist in the herb of quality class j or a herb type simply does not have quality class j, $C_{i,j}=0$.

The mass balance of chemical marker i in the extraction solvent is

$$C_i=\sum_{j=1}^{n}C_{i,j} \tag{3}$$

Here, n is the total number of quality classes of all types of herb. If there are x chemical markers in the product specifications, their concentrations in an extract that satisfy the product specifications can be expressed as

$$\boldsymbol{c}=\boldsymbol{c}^{QA} \tag{4}$$

where

$$\boldsymbol{c}=[C_1 \quad C_2 \quad \cdots \quad C_x]^T \tag{5}$$

$$\boldsymbol{c}^{QA}=[C_1^{QA} \quad C_2^{QA} \quad \cdots \quad C_x^{QA}]^T \tag{6}$$

At a specified set of extraction conditions, if the quality constraint is satisfied, $C_i=C_i^{QA}$ is a constant and $\dot{C}_{i,j}=\dot{C}_{i,j}^{QA}$ is of a given value. Equation 2 of the x chemical markers can be rearranged as

$$\boldsymbol{D}\boldsymbol{m}=\boldsymbol{b} \tag{7}$$

where

$$\boldsymbol{D}=\begin{bmatrix} \frac{\Phi_{1,1}}{V}-\frac{C_1^{QA}}{K_{1,1}\rho_1V} & \frac{\Phi_{1,2}}{V}-\frac{C_1^{QA}}{K_{1,2}\rho_2V} & \cdots & \frac{\Phi_{1,n}}{V}-\frac{C_1^{QA}}{K_{1,n}\rho_nV} \\ \frac{\Phi_{2,1}}{V}-\frac{C_2^{QA}}{K_{2,1}\rho_1V} & \frac{\Phi_{2,2}}{V}-\frac{C_2^{QA}}{K_{2,2}\rho_2V} & \cdots & \frac{\Phi_{2,n}}{V}-\frac{C_2^{QA}}{K_{2,n}\rho_nV} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\Phi_{x,1}}{V}-\frac{C_x^{QA}}{K_{x,1}\rho_1V} & \frac{\Phi_{x,2}}{V}-\frac{C_x^{QA}}{K_{x,2}\rho_2V} & \cdots & \frac{\Phi_{x,n}}{V}-\frac{C_x^{QA}}{K_{x,n}\rho_nV} \end{bmatrix} \tag{8}$$

$$\boldsymbol{m}=[M_1 \quad M_2 \quad \cdots \quad M_n]^T \tag{9}$$

$$\boldsymbol{b}=\begin{bmatrix} C_1^{QA}+\sum_{j=1}^{n}\frac{\dot{C}_{1,j}^{QA}}{(k_{1,j}S_j)(K_{1,j}\rho_j)} \\ C_2^{QA}+\sum_{j=1}^{n}\frac{\dot{C}_{2,j}^{QA}}{(k_{2,j}S_j)(K_{2,j}\rho_j)} \\ \vdots \\ C_x^{QA}+\sum_{j=1}^{n}\frac{\dot{C}_{x,j}^{QA}}{(k_{x,j}S_j)(K_{x,j}\rho_j)} \end{bmatrix} \tag{10}$$

If a herb of quality class j does not have chemical marker i or quality class j simply does not exist, the corresponding element of $\boldsymbol{D}$ and the corresponding term in the summation part of each element of $\boldsymbol{b}$ are set to be equal to zero. The solution(s) for $\boldsymbol{m}$ can be determined numerically.

Multiple-herb-extraction effect

It is well known that the proportions of chemical marker concentrations in a multiple-herb CHM extract prepared by extraction of the herbs together in the same pot can be different from the extract obtained by carrying out the extraction of each of the herbs in separate pots and then mixing them together.[5,6] Such multiple-herb-extraction effects are caused by the interactions among chemical components being extracted from different herbs.

Figure 3 depicts the presence of a multiple-herb-extraction effect where an unknown chemical component from quality class h leads to a $\Phi_{i,j}$ that is different than that in single-herb extraction. Evidence shows that the presence of an unknown component(s) from another herb can alter the structure of the herb under consideration, cause a chemical reaction or change the pH of the extract solution, which in turn changes its concentration in the extract.[5,6] However, it is difficult to find out what the unknown component is and the interaction mechanisms are always complicated. Therefore, an empirical approach is proposed to quantify the effect. If there is an unknown chemical component from quality class h of another herb being extracted together with the herb of quality class j under consideration, the maximum extractable amount of chemical marker i from herb of quality class j can be represented by

$$\Phi_{i,j} = \Phi^0_{i,j} r_{i,j,h} \tag{11}$$

$$r_{i,j,h} = f(M_h) \tag{12}$$

where $\Phi^0_{i,j}$ (mg i/mL) is a constant representing the maximum extractable amount of chemical marker i from herb of quality class j for the case without multiple-herb-extraction effect and decomposition of chemical marker i during extraction. Here, f is a case-specific correlation function and M_h (g herb of quality class h) refers to the amount of the other herb types other than the herb type under consideration. Since $r_{i,j,h}$ (dimensionless) depends only on M_h, Eq. 12 has assumed that the multiple-herb-extraction effect is the same for different quality classes of the same herb type.

Depending on the extraction situations of chemical marker i, there are three possibilities of $\Phi_{i,j}$ as

$$\Phi_{i,j} = \begin{cases} \Phi^0_{i,j} \\ \Phi^0_{i,j} e^{-\beta_{i,j} t} \\ \Phi^0_{i,j} r_{i,j,h} \end{cases} \tag{13}$$

where $\beta_{i,j}$ (min^{-1}) is the decomposition rate constant of chemical marker i extracted from herb of quality class j.

Existence and uniqueness of solution

As discussed in our previous article,[1] a single-herb extraction system behaves as a linear system with all the elements of matrix $\boldsymbol{D}$ (Eq. 8) being constants. The number of chemical markers (x) represents the number of quality constraints and the number of quality classes (n) represents the number of variables. The behavior of a given system is determined by comparing x and n. In general, as long as $\det(\boldsymbol{D}) \neq 0$, there are three possibilities. When $x = n$, the system is exactly specified and a unique solution of $\boldsymbol{m}$ exists which can be used to produce an extract with the desired quality. When $x < n$, the system is underspecified and has infinitely many solutions of $\boldsymbol{m}$. When $x > n$, the system is overspecified and there is no exact solution.

A multiple-herb extraction system, without multiple-herb-extraction effect, even with the presence of common chemical markers also behaves as a linear system, as no variable has been introduced into $\boldsymbol{D}$ (Eq. 8) and $\boldsymbol{b}$ (Eq. 10). Therefore, the uniqueness of solution in the case of exactly specified system is still valid. In contrast, if multiple-herb-extraction effect exists, the system is a nonlinear system, since a variable $r_{i,j,h}$ (which is a function of M_h) is introduced into $\boldsymbol{D}$ (Eq. 8). This means that even if the system is exactly specified, a unique solution may not exist.

Table 1. Three Possible Scenarios of Overall Behavior of Multiple-Herb Extraction System

Scenario 1	If all the subsystems are exactly specified, all the variables are specified to satisfy every quality constraint. The system is exactly specified and a unique solution (for linear system) or a finite number of solution(s) (for non-linear system) exist.
Scenario 2	If at least one of the subsystems is underspecified and the remaining subsystems are exactly specified, there exist degree(s) of freedom after satisfying all the quality constraints. The system is underspecified and infinitely many solutions exist.
Scenario 3	If at least one of the subsystems is overspecified, there are quality constraint(s) that cannot be satisfied by specifying the variables of this system. The system is overspecified and has no solution.

Solution strategy of multiple-herb extraction system

For a multiple-herb extraction system, the number of chemical markers (x) represents the total number of quality constraints and the number of quality classes (n) represents the total number of variables of the given system. The behavior, or to be more specific as the overall behavior, of a multiple-herb extraction system cannot be determined by just comparing x and n as in the single-herb extraction system. Instead, a given multiple-herb extraction system is decomposed into several subsystems and the overall behavior of the given system is determined by checking the behaviors of all the subsystems. Here, subsystem is a system that contains several chemical markers and several quality classes of one type or more types of herb. Each subsystem can be considered independently that the quality constraints of chemical markers in each subsystem can be satisfied only by specifying the herb masses of quality classes in that subsystem. The behavior of each subsystem can be determined by exactly the same method used in single-herb extraction, which compares the number of chemical markers and the number of quality classes in that subsystem. There are three possible scenarios of the overall behavior of a given multiple-herb extraction system (Table 1).

Chemical markers in multiple-herb extraction system are classified into two types. If a chemical marker is neither a common chemical marker nor affected by multiple-herb-extraction effect, it is referred to as an independent chemical marker, and its extracted concentration depends on only one type of herb; otherwise it is a nonindependent chemical marker, as its extracted concentration depends on more than one type of herb. Obviously, if all the chemical markers are independent in a given multiple-herb extraction system, each herb forms a subsystem. For instance, if there are two different herb types in a multiple-herb formula where Herb 1 has two quality classes and involves two chemical markers and Herb 2 has four quality classes and four chemical markers.

Table 2. Rules of Solution Strategy

Rule 1	For any subsystem, if the number of quality classes is equal to the number of chemical markers, this subsystem is exactly specified.
Rule 2	For any subsystem, if the number of quality classes is larger than the number of chemical markers, this subsystem is underspecified.
Rule 3	For any subsystem, if the number of quality classes is smaller than the number of chemical markers, this subsystem is overspecified.

If all six chemical markers are independent, both Herbs 1 and 2 form a subsystem that is exactly specified. The overall system is exactly specified as well.

Once the given multiple-herb extraction system involves nonindependent chemical marker, the way of satisfying the quality constraints is no longer trivial. The following systematic solution strategy with three rules summarized in Table 2 has been developed to appropriately decompose the given multiple-herb extraction system into several subsystems.

Step 1: For a given multiple-herb extraction system, identify independent and nonindependent chemical markers.

Step 2: Count the number of independent chemical markers associated with each quality class. Indentify Nu quality classes containing the same Ne independent chemical markers. If $Nu \leq Ne$, form a subsystem with the same.

Step 3: Count the number of chemical markers associated with each quality class. Indentify Nu quality classes containing the same Ne chemical markers. If $Nu \leq Ne$, form a subsystem with the same.

Step 4: For each subsystem, determine its behavior based on Rules 1-3.

Step 5: Determine the overall behavior of the given multiple-herb extraction system based on the behaviors of all the subsystems (Table 1)

Next, three examples are given to illustrate the solution strategy. It is easier to appreciate the utilization of solution strategy using these examples.

Example 1. *Consider a multiple-herb extraction system with four different herbs in the formula and each herb has a different number of quality classes available to be used and different chemical markers desired in the product specifications (Figure 4). In this system, there are four nonindependent chemical markers, which are N1, N2, N3, and N4. Common chemical markers N1 and N2 exist in Herbs 1, 2, and 3 and N3 exists in Herbs 3 and 4 (rows 1–3). Herb of quality class 4i has multiple-herb-extraction effect on chemical markers N2 and N4, which are extracted from all quality classes of all herbs that have these two chemical markers. The solution strategy proceeds as follows*

Step 1:

The chemical markers in the multiple-herb extraction system are classified as independent chemical markers (I1, I2, I3, I4, and I5) and nonindependent chemical markers (N1, N2, N3, and N4).

Step 2:

We deal with the independent chemical markers in each herb first, as their quality constraints can only be satisfied by specifying the herb masses of quality classes of that herb. We want to separate the exactly specified and overspecified parts out from the overall system by checking the number of quality classes and the number of independent chemical markers of each herb. To do this, the number of independent chemical markers associated with each quality class is calculated. If there are Nu quality classes containing the same Ne independent chemical markers and $Nu \leq Ne$, these Nu quality classes and Ne independent chemical markers form a subsystem.

After calculated the number of independent chemical markers associated with each quality class, it can be seen that independent chemical marker I1 only associates with quality class 2i. Therefore, quality class 2i and chemical marker I1 formed a subsystem.

Step 3:

After step 2, all the remaining quality classes in different herbs are considered together for all the remaining chemical markers. Again, we intend to separate the exactly specified and overspecified parts out from the overall system by the method similar to the previous step. The number of chemical markers associated with each quality class is calculated. If there are Nu quality classes containing the same Ne chemical markers and $Nu \leq Ne$, these Nu quality classes and Ne chemical markers form a subsystem.

After calculated the number chemical markers associated with each quality class, it can be seen that both chemical markers N3 and I5 associate with quality classes 4ii and 4iii.

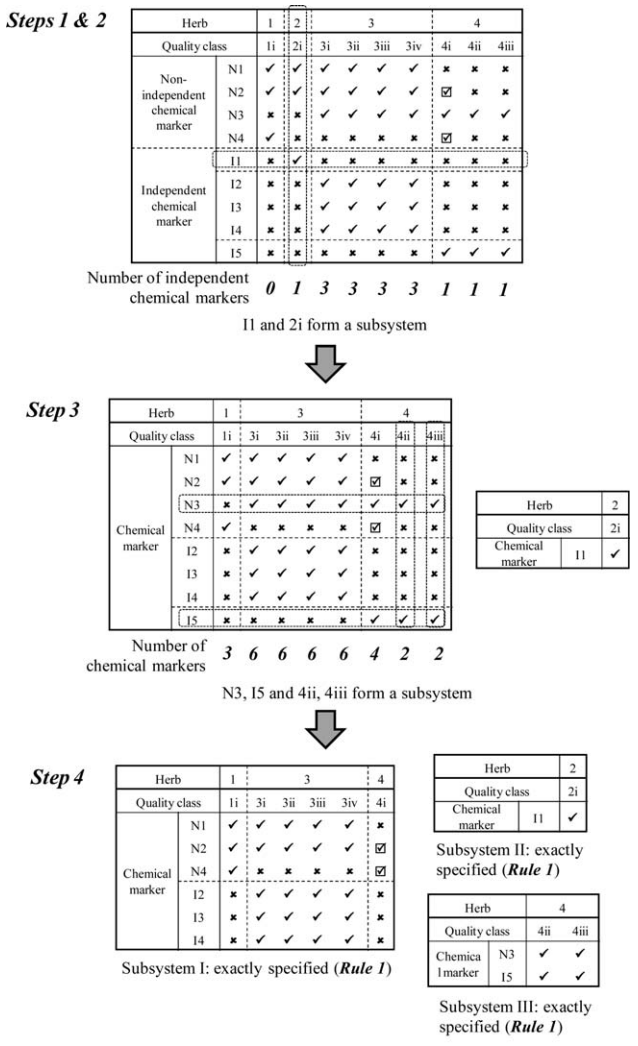

Figure 4. Solution strategy of Example 1.

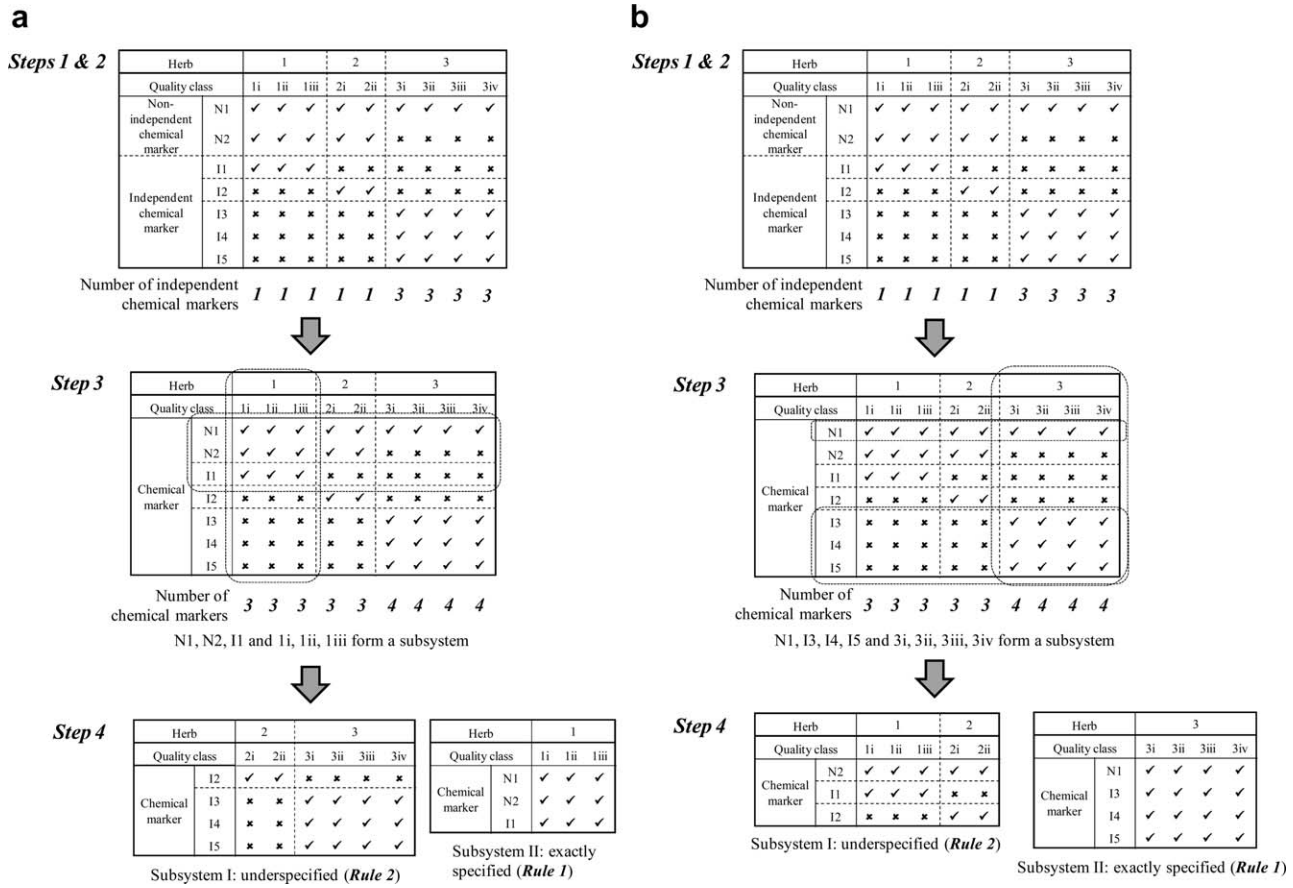

Figure 5. a: First alternative of solution strategy of Example 2 (symbols have same meaning as in Figure 4). b: Second alternative of solution strategy of Example 2 (symbols have same meaning as in Figure 4).

Therefore, quality classes 4ii and 4iii and chemical markers N3 and I5 formed a subsystem.

Step 4:

After step 3, all the remaining quality classes and chemical markers formed a subsystem. The behavior of each subsystem is determined based on the Rules 1-3.

There are six quality classes for six chemical markers in subsystem I. According to Rule 1, this subsystem is exactly specified. Similarly, subsystem II, which has one quality class for one chemical marker, and subsystem III, which has two quality classes for two chemical markers, are also exactly specified (Rule 1).

Step 5:

Finally, the overall behavior of the given multiple-herb extraction system is determined based on the behaviors of all the subsystems as mentioned in Table 1.

Since all the subsystems are exactly specified, this system is exactly specified and a finite number of solution(s) exist.

Example 2. *Consider a multiple-herb extraction system with three different herbs in the formula and each herb has a different number of quality classes available to be used and different chemical markers desired in the product specifications (Figure 5). There are two nonindependent chemical markers, which are N1 and N2. Common chemical markers N1 exists in all herbs and N2 exists in Herbs 1 and 2 (rows 1 and 2). Based on the steps, two alternatives of solution strategy can be used. Both of them determine that this multiple-herb extraction system is underspecified with two degrees of freedom and infinitely many solutions exist as mentioned in Table 1. The first alternative of solution strategy is shown in Figure 5a. By following the steps, the given system is decomposed into one underspecified subsystem with two degrees of freedom and one exactly specified subsystem. The second alternative of solution strategy is shown in Figure 5b. Similarly, the given system is decomposed into one underspecified subsystem with two degrees of freedom and one exactly specified subsystem.*

Conceptually, how we define the two degrees of freedom in the two alternatives is different. For the first alternative, the two degrees of freedom come from the subsystem of Herbs 2 and 3 (i.e., the subsystem I in Figure 5a), but for the second alternative, two degrees of freedom come from the subsystem of Herbs 1 and 2 (i.e., the subsystem I in Figure 5b). Although the two degrees of freedom in the two alternatives are different, they do not cause any difference when they are utilized for optimization. This is because the optimized solution (i.e., the herb masses of all the quality classes) is determined by optimizing the objective function (such as the material cost of herbs) subjected to the quality constraints of all the chemical markers in the product specifications. No matter which two decision variables (i.e., two herb masses of quality classes) are defined as the degrees of freedom, the two alternatives would lead to the same optimized solution based on the objective function.

Example 3. *Consider a multiple-herb extraction system with three different herbs in the formula and each herb has a different number of quality classes available to be used and different chemical markers desired in the product specifications (Figure 6). There are three nonindependent chemical markers, which are N1, N2, and N3. Common chemical*

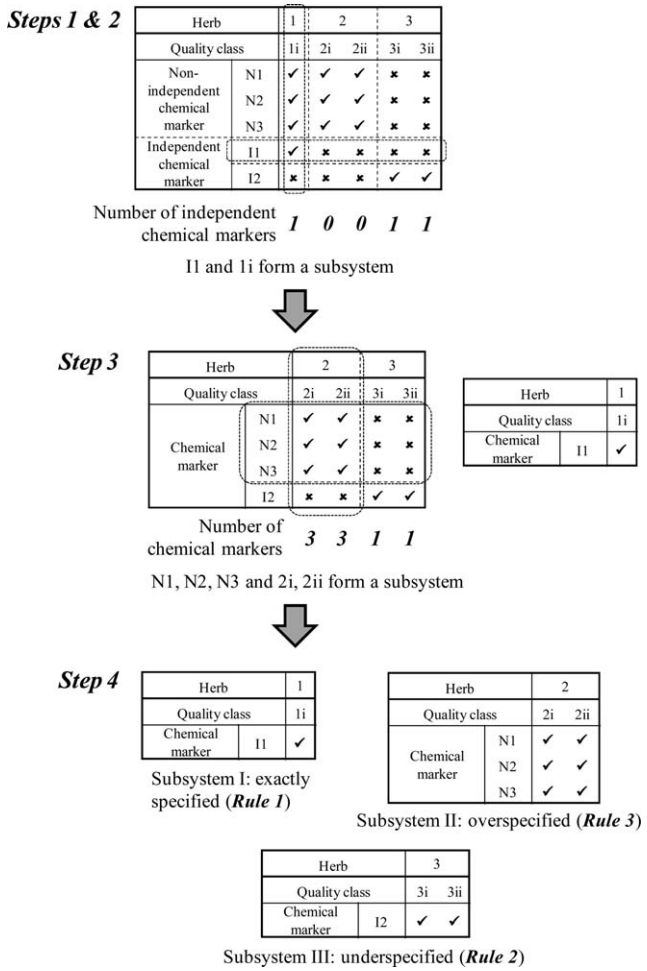

Figure 6. Solution strategy of Example 3 (symbols have same meaning as in Figure 4).

markers N1, N2, and N3 exist in Herbs 1 and 2 (rows 1–3). Based on the steps, this multiple-herb extraction system is decomposed into three subsystems, which include one exactly specified subsystem (subsystem I), one overspecified subsystem with one unsatisfied quality constraint (subsystem II), and one underspecified subsystem with one degree of freedom (subsystem III). Therefore, this multiple-herb extraction system is overspecified and there is no solution as mentioned in Table 1.

This example illustrates that the overall solution behavior cannot be determined simply from the total number of quality constraints and the total number of variables. It can be seen that the overall system is overspecified because of the unsatisfied quality constraint in the subsystem II that involves chemical markers N1, N2, and N3 and quality classes 2i and 2ii. Although there is one degree of freedom in the underspecified subsystem III, it cannot be used to satisfy the quality constraint of N1, N2, or N3, simply because it does not contain any of those chemical markers. To solve this multiple-herb extraction system, one additional variable, which can satisfy the quality constraints of N1, N2, and/or N3, needs to be introduced to the system. This additional variable can be obtained by relaxing one of the fixed extraction conditions (such as V, T, or t) or providing one more quality class of herb that contain N1, N2, and/or N3.

Table 3. Summary of Model Parameters of Danshensu and Salvianolic Acid B Extracted at 100°C in 100 mL of Water from Different Quality Classes of Danshen (cited from Lau et al.[1])

	1S	1M	1I
Danshensu			
Φ^0_{DS} (mg/g herb)	29.64	28.25	15.00
$K_{DS}\rho$ (g/cm^3)	0.85	3.65	0.25
$k_{DS}S$ (cm^3/min•g)	2.67×10^{-4}	1.25×10^{-4}	1.63×10^{-3}
Salvianolic acid B			
Φ^0_{SAB} (mg/g herb)	46.56	98.80	15.09
$K_{SAB}\rho$ (g/cm^3)	6.22	0.28	1.05
$k_{SAB}S$ (cm^3/min•g)	1.52×10^{-1}	2.90	8.84×10^{-1}
β_{SAB} (min^{-1})	6.42×10^{-4}	9.73×10^{-4}	6.95×10^{-4}

QA of DG Decoction

Model parameters of chemical markers

Consider extraction of DG with 100 mL of water at 100°C and 120 min. Under these conditions, the extraction of DS, DN, and DeN followed the expected extraction behavior and the SAB undergone decomposition during extraction. All the model parameters of the chemical markers of Danshen and Gegen have been determined in our previous article (for readers who are interested, please refer to our previous article for the details),[1] and are summarized in Tables 3 and 4, respectively. These model parameters were used to execute the QA of DG extraction.

Multiple-herb-extraction effect on DS

During DG extraction, multiple-herb-extraction effect was observed on the extraction of DS, as the extraction behavior was different when Danshen and Gegen were extracted together. Figure 7a shows the extraction kinetics of DS extracted at 100°C in 100 mL of water with a fixed amount (7 g) of 1M quality class of Danshen and different amounts of 2M quality class of Gegen. It can be seen that the C^{∞}_{DS} increased with the amount of Gegen added together in DG extraction, which corresponded to the increase of Φ_{DS}. The change of Φ_{DS} was correlated with the amount of 2M Gegen present during extraction. The expression of the multiple-herb-extraction effect on 1M Danshen by 2M Gegen is defined as

$$\Phi_{DS,1M}=\Phi^0_{DS,1M}r_{DS,1M,2M} \tag{14}$$

where $r_{DS,1M,2M}$ (dimensionless) is the correlation function of $\Phi_{DS,1M}$ as a function of M_{2M}. Based on the experimental data (Figure 7b), a linear correlation function was used

Table 4. Summary of Model Parameters of Daidzin and Daidzein Extracted at 100°C in 100 mL of Water from Different Quality Classes of Gegen (cited from Lau et al.[1])

	2S	2M	2I
Daidzin			
Φ^0_{DN} (mg/g herb)	0.33	3.82	5.41
$K_{DN}\rho$ (g/cm^3)	0.64	1.57	0.57
$k_{DN}S$ (cm^3/min•g)	2.11×10^{-2}	2.35×10^{-3}	1.04×10^{-2}
Daidzein			
Φ^0_{DeN} (mg/g herb)	0.28	2.26	1.24
$K_{DeN}\rho$ (g/cm^3)	0.49	1.04	0.66
$k_{DeN}S$ (cm^3/min•g)	1.08×10^{-2}	2.89×10^{-2}	6.01×10^{-2}

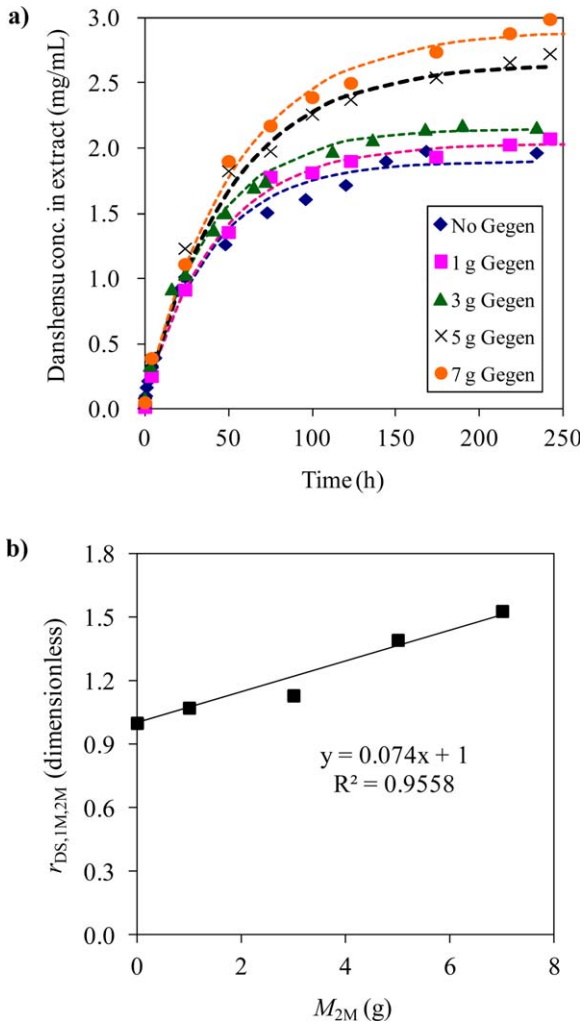

Figure 7. a: Extraction kinetics and (b) correlation plot between $r_{DS,1M,2M}$ and M_{2M} of DS extracted at 100°C in 100 mL of water with 7 g of 1M quality class of Danshen and different amounts of 2M quality class of Gegen.

The curves are based on the QA model in the article. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$r_{DS,1M,2M} = 0.074 M_{2M} + 1 \quad (15)$$

It is assumed that the same correlation function of multiple-herb-extraction effect from 2M Gegen is applicable to the extraction of DS from any other quality class of Danshen.

The effect of extraction solvent on the multiple-herb-extraction effect was also studied. Figure 8 shows the extraction kinetics of DS extracted in 50% ethanol and water mixture solution (50% EtOH) with a fixed amount (7 g) of 1M Danshen and different amounts of 2M Gegen. All the extraction conditions were the same as we discussed in the previous aqueous extraction case, except that the solvent was changed to be 50% EtOH and the temperature was at 70°C. In this experiment, no significant change of C_{DS}^{∞} was observed, indicated that the amount of Gegen present did not affect the value of Φ_{DS}.

To understand the difference between these two experimental results, the pH value of the DG extracts in the two experiments were measured. Table 5 shows the pH value in the aqueous DG extracts. In general, the pH value was lower with extraction time and the initial pH value, which was the

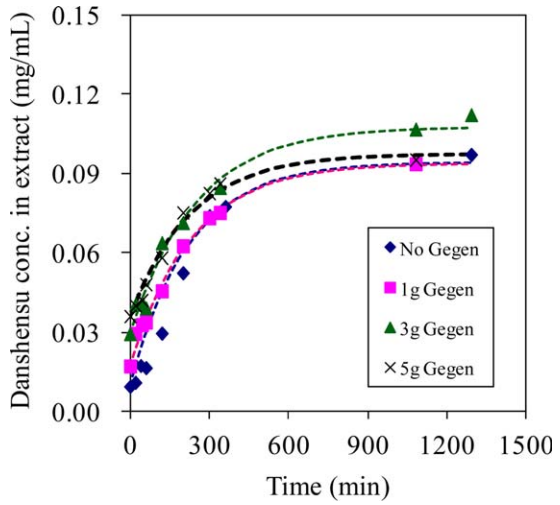

Figure 8. Extraction kinetics of DS extracted at 70°C in 100 mL of 50% ethanol and water mixture solution with 7 g of 1M quality class of Danshen and different amounts of 2M quality class of Gegen.

The curves are based on the QA model in this article. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

pH value of the extract at $t = 0$, decreased with the increase of Gegen amount present. This change of initial pH value was not observed in the 50% EtOH DG extracts that the pH value unchanged as pH 5.5 for all the 50% EtOH DG extracts at any time of the extraction. Based on the observed evidence, it is speculated that an unknown chemical component from Gegen can only be extracted by water but not by 50% EtOH. This unknown chemical component acidified the aqueous extract, as indicated by the decrease in initial pH value.

To confirm the importance of initial pH value on the multiple-herb-extraction effect, an aqueous single-herb extraction of 7 g of 1M Danshen was performed with the same extraction conditions as mentioned, except that the initial pH value was deliberately adjusted from 6.50 to 6.00, which was the same as that in the aqueous DG extraction with 7 g of 1M Danshen and 5 g of 2M Gegen. As it can be seen from Figure 9, the extraction kinetic of DS of this single-herb extraction was similar to that of aqueous DG extraction. Thus, it was concluded that the change of initial pH value is highly possible to be the mechanism of multiple-herb-extraction effect in the aqueous DG extraction.

Practical accuracy of QA of DG extract

Based on the solution strategy, this DG extraction system can be decomposed into one exactly specified subsystem and

Table 5. pH Value in Different Danshen–Gegen Extract Samples During Extraction at 100°C in 100 mL of Water with 7 g of 1M Quality Class of Danshen

	pH value			
Time (h)	No Gegen	1 g Gegen	5 g Gegen	7 g Gegen
0	6.50	6.25	6.00	5.75
4	6.25	6.00	5.75	5.50
24	5.75	5.00	4.75	4.50
75	5.50	4.50	4.25	4.00
100	5.50	4.50	4.25	4.00

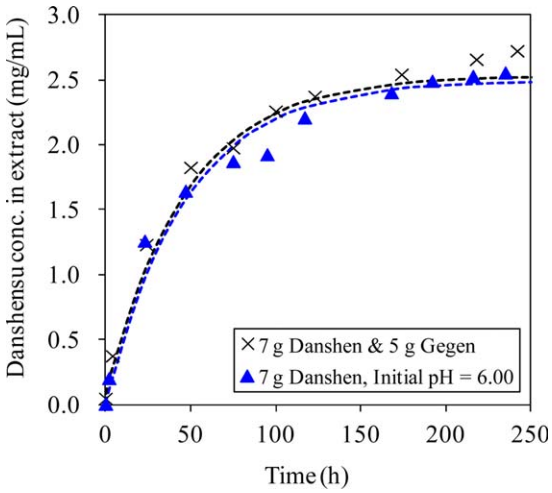

Figure 9. Extraction kinetics of DS from Danshen extraction with initial pH value equals 6.00 with 7 g of 1M quality class of Danshen and DG extraction with 7 g of 1M quality class of Danshen and 5 g of 2M quality class of Gegen at 100°C in 100 mL of water.

The curves are based on the QA model in this article. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

one underspecified subsystem with two degrees of freedom, therefore this DG extraction system is underspecified. By including the correlation function in the QA model, the solutions for manufacture DG extract with quality satisfying the product specifications were determined. Since this DG extraction system was underspecified, there were infinitely many solutions. Minimization of material cost of herbs was performed on top of the QA purpose. Among all the solutions obtained by exhaustive iterations, it was found that the minimum and maximum costs are HK$ 7944/1000 L extract and HK$ 10390/1000 L extract, respectively. The most expensive solution, which was a solution without using 1I Danshen and 2I Gegen, was 30.8% more expensive than the most cost effective solution, which was a solution without using 1S Danshen and 2S Gegen.

To experimentally verify the practical accuracy of the determined solutions, three DG extracts corresponding to the most expensive (DG-1), the most cost effective (DG-2), and the intermediate-cost (DG-3) solutions were produced. The extract qualities of all the three DG extracts were measured (Table 6). In general, good accuracies of the extract quality were observed that the concentrations of the four chemical markers were within ±10% of the desired concentrations in product specifications in every DG extract. The results showed that the modified QA model is reliable to ensure a consistence of product extract quality. Also, the analysis of cost minimization by this QA model was successful to find the most cost effective solution.

Efficacy of consistent quality DG extracts against cytotoxicity

LDH is an intracellular housekeeping enzyme present in almost all body tissue cells. Under normal physiological condition, the level of extracellular LDH is very low. After menadione challenge, the oxidative damage causes the

Table 6. The Amount of Different Quality Classes of Danshen and Gegen used to Prepare the DG Extract Samples DG-1, DG-2, and DG-3 and the Corresponding Concentrations of Danshensu (DS), Salvianolic acid B (SAB), Daidzin (DN), and Daidzein (DeN) Extracted at 100°C in 100 mL of Water with 120 min Extraction Time.

	Danshen amount (g)			Gegen amount (g)			Quality of extract (mg/mL) [% difference]				Material cost of herbs (HK$/1000 L extract)
Sample	Superior	Medium	Inferior	Superior	Medium	Inferior	DS	SAB	DN	DeN	
DG-1	4.14	2.20	0.00	7.66	2.92	0.00	0.130 [−7.4]	3.113 [−9.2]	0.121 [−2.4]	0.068 [−2.9]	10390 (Most expensive)
DG-2	0.00	3.72	5.79	0.00	3.11	0.24	0.130 [−7.1]	3.139 [−8.5]	0.126 [+1.5]	0.063 [−9.8]	7944 (Most cost effective)
DG-3	3.12	2.58	1.42	3.59	2.99	0.15	0.133 [−4.9]	3.120 [−9.0]	0.131 [+5.9]	0.064 [−8.6]	9167 (Intermediate-cost)

The product specifications are: $C_{DS}^{QA} = 0.140$ mg/mL, $C_{SAB}^{QA} = 3.430$ mg/mL, $C_{DN}^{QA} = 0.124$ mg/mL, and $C_{DeN}^{QA} = 0.070$ mg/mL

Table 7. The Percentage of Lactate Dehydrogenase Release (% LDH release) of H9c2 Cells with or Without Menadione Challenge by Different Dose Concentrations (100, 200, and 300 μg/mL) of the Three Consistent Quality Danshen–Gegen (DG) Extract Samples

Sample	Dose conc. (μg/mL)	% LDH release of cell **without** menadione challenge (mean ± S.E.M.)	% LDH release of cell **with** menadione challenge (mean ± S.E.M.)
Control	N/A	23.8% ± 1.7%	54.1% ± 1.9%
DG-1	100	27.4% ± 1.6%	52.7% ± 1.5%
	200	25.5% ± 2.1%	50.2% ± 3.2%
	300	28.8% ± 2.4%	52.5% ± 0.3%
DG-2	100	25.2% ± 1.3%	50.3% ± 0.6%
	200	25.5% ± 1.9%	49.4% ± 1.7%
	300	27.5% ± 2.0%	50.0% ± 3.1%
DG-3	100	24.3% ± 1.4%	48.8% ± 0.5%
	200	24.6% ± 1.8%	48.6% ± 1.5%
	300	24.9% ± 2.2%	48.0% ± 0.9%

leakage of intracellular LDH and increases the extracellular LDH level, which can be detected in the culture medium.

The efficacy of the three consistent quality DG extracts (DG-1, DG-2, and DG-3) was examined by an H9c2 cell assay. This assay investigated the effect of DG extract pretreatment on oxidant injury. In this study, H9c2 cell line was used as an *in vitro* model for investigating the effect of drug treatment on cellular injury by menadione challenge. The extent of menadione-induced cytotoxicity was assessed by the measurement of LDH leakage in the culture medium from the injured cells. The leakage of LDH from the injured cells to the culture medium is an indicator of irreversible cell death due to cell membrane damage. Table 7 summarizes the % LDH release of H9c2 cells challenged with or without menadione treated by different dose concentrations (100, 200, and 300 μg/mL) of the three consistent quality DG extract samples. In general, the % LDH release of each sample with menadione challenge was much higher (more than 20%) than that without menadione challenge. These results supported that this assay was sensitive to menadione-induced toxicity in H9c2 cells.

For each sample, the menadione-induced LDH release (men LDH release) and the percentage protection (% protection) were calculated as follows

$$\text{men LDH release} = \%\ \text{LDH release with men} - \%\ \text{LDH release without men} \tag{16}$$

where % LDH release with men means the % LDH release of cell with menadione challenge and % LDH release without men means the % LDH release of cell without menadione challenge, and

$$\%\ \text{protection} = \frac{\text{control men LDH release} - \text{sample men LDH release}}{\text{control men LDH release}} \times 100\% \tag{17}$$

where control men LDH release means the menadione-induced LDH release of control and sample men LDH release means the menadione-induced LDH release of sample.

Figure 10 shows the percentage protection generated by different dose concentration (100, 200, and 300 μg/mL) of the three consistent quality DG extract samples. The three extracts generated significant percentage protection under all the investigated dose concentrations. It can be seen that higher dose concentration generated higher percentage protection. Also, it is clearly demonstrated that the differences of percentage protection among the three extracts were statistically insignificant. Therefore, we can conclude that the three extracts, which have consistent chemical compositions of the four chemical markers (Table 6), provided consistent biological efficacy against menadione-induced toxicity in H9c2 cells.

Conclusions

QA in extraction is one of the most important unresolved problems in the modernization of traditional Chinese medicines. The root cause of difficulty is that the amount of chemical markers in raw herbs can differ significantly depending on the source and history of these raw herbs, resulting in CHM products with very different quality (i.e., chemical markers in specified proportions as verified by clinical trials). Recently, a QA procedure was developed by Lau et al.,[1] which ensures to produce consistent quality single-herb CHM product. The key idea of this procedure is to use herbs from different sources to smooth out the variations in composition. This study extends the developed QA procedure to multiple-herb extraction by designing proper modifications of the herbal extraction model to take into account the existences of common chemical marker and multiple-herb-extraction effect in multiple-herb extraction. As summarized in Figure 11, our modified QA procedure is based on a model of herbal extraction, which describes the

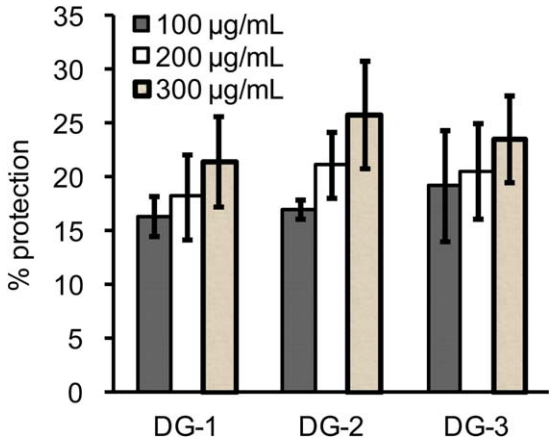

Figure 10. Comparison on percentage protection (% protection) generated by dose concentration of 100, 200, and 300 μg/mL of the three consistent quality DG extract samples.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

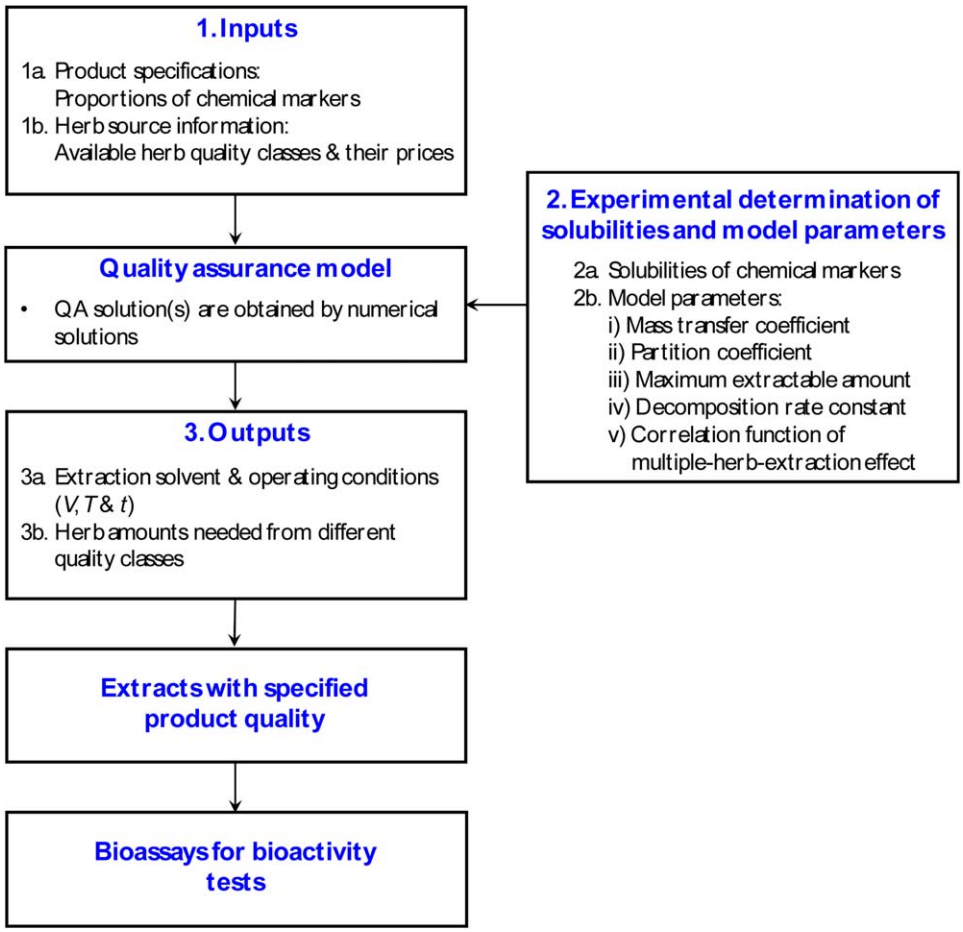

Figure 11. The quality assurance (QA) procedure for producing Chinese herbal medicinal (CHM) products with consistent quality.

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physicochemical phenomena during herbal extraction. Model parameters are defined to account for the maximum extractable amount, the partition of chemical markers between the herb and the extraction solvent, the mass-transfer coefficient, the decomposition of chemical markers, and so forth. A correlation approach was introduced to this QA procedure to quantify the interaction of multiple-herb-extraction effect and the experimental method for determining related correlation function was designed. In addition, a systematic solution strategy was developed to appropriately decompose the multiple-herb extraction system into several subsystems for obtaining solution(s) and determining the overall behavior of the system. With this modified model and the companion experiments, the amount of herbs needed from different quality classes to produce a multiple-herb formula CHM product decoction with consistent quality can be exactly determined.

The application to multiple-herb extraction was illustrated by the extraction of DG. It was observed that a multiple-herb-extraction effect was present in the aqueous extraction of DS and this effect was depended on the extraction solvent. The possible mechanism of this multiple-herb-extraction effect in the aqueous DG extraction was speculated to be the change of initial pH value of the aqueous extraction solvent by an unknown component from Gegen. The experimental chemical marker concentrations fall within $\pm 10\%$ of the specified chemical marker compositions by using the amount of herb from each herb class as predicted by the QA model. Furthermore, an H9c2 cell assay was used to test the efficacy of three consistent quality DG extracts, which were produced by different herb combinations with different material costs of herbs. The results showed that the three DG extracts provided consistent biological efficacy against menadione-induced toxicity.

In future, the present QA method can be extended in different directions. First, it is not uncommon that the herbal formula of a CHM product involves five or more types of herb. The multiple-herb interactions of such multiple-herb extraction system are complicated and a lot of experimental workload is needed to study them one by one. Design of experiments approach like two-level factorial design can be utilized to reduce the experimental workload.[7,8] Second, it is possible that the internal diffusion may dominate if the herb is in slice form. Also, the extractable amount of a chemical marker may depend on whether maceration is included in the process recipe. A general QA procedure can be developed by modifying the existing procedure to include such factors. Third, the current QA procedure is not limited to bioactive markers. It can also be used to ensure that toxic substances should be kept below a specified concentration in a CHM product. Fourth, since the QA procedure offers the production of a CHM product with chemical markers in any

desired proportions, we can systematically produce a number of samples each with a different proportion, and then using bioassays to test the efficacy of each of the samples as a way to identify the bioactive markers. Fifth, different parts of a plant may exhibit different extraction behaviors. For example, the nature of transport of a chemical marker in a seed is expected to be different from that of a leaf. The QA procedure can be suitably modified to account for such differences. Efforts in these directions are underway. In addition, the presented QA method indeed is not limited to CHM production, it can be applied to solve QA problem with similar problem nature that the quality of raw material can differ significantly.

Acknowledgment

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Notation

C_i = concentration of chemical marker i in extraction solvent, mg/mL
C_i^{∞} = expected equilibrium concentration of chemical marker i in extraction solvent, mg/mL
C_i^{QA} = concentration of chemical marker i in extraction solvent desired in product specifications
$C_{i,j}$ = concentration of chemical marker i in extraction solvent extracted from herb of quality class j, mg/mL
$\dot{C}_{i,j}$ = rate of extraction of chemical marker i extracted from herb of quality class j, mg/mL•min
$K_{i,j}$ = partition coefficient of chemical marker i at interface between herb particle of quality class j and extraction solvent, dimensionless
$k_{i,j}$ = mass-transfer coefficient of chemical marker i from herb of quality class j to extraction solvent, cm/min
M_j = herb mass of quality class j, g
n = number of quality classes
$r_{i,j,h}$ = correlation function of maximum exactable amount of chemical marker i from herb of quality class j as a function of herb mass of quality class h, dimensionless
S_j = specific surface area of herb particle of quality class j, cm^2/g
T = extraction temperature, °C
t = extraction time, min
V = volume of extraction solvent, mL
x = number of chemical markers desired in product specifications
$\beta_{i,j}$ = decomposition rate constant of chemical marker i extracted from herb of quality class j, min^{-1}
ρ_j = density of herb of quality class j, g/cm^3
$\Phi_{i,j}$ = maximum extractable amount of chemical marker i of herb of quality class j, mg i/g herb of quality class j
$\Phi_{i,j}^0$ = maximum extractable amount of chemical marker i of herb of quality class j from extraction without multiple-herb-extraction effect and decomposition of chemical maker i during extraction, mg i/g herb of quality class j
$\boldsymbol{D}$,$\boldsymbol{b}$ = matrix and column vector of model parameters
$\boldsymbol{c}$ = column vector of concentrations of chemical markers in extraction solvent
$\boldsymbol{c}^{QA}$ = column vector of concentrations of chemical markers in extraction solvent specified in product specifications
$\boldsymbol{m}$ = column vector of herb masses of quality classes used

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Appendix: Assumption of Insignificant Absorption of Chemical Marker

For multiple-herb extraction, a chemical marker from one herb may not exist in some of other herbs and the chemical marker concentration in the extraction solvent is higher than that in these other herbs, which builds up a concentration gradient driven the diffusion of chemical marker towards the inside of these other herbs. However, as the size of the herb particle used was so small (equal to 1.0 mm or less), the amount of chemical marker extracted from one herb absorbed by other herbs is insignificant.

To verify this assumption, 4.0 mg of pure standard of DS was dissolved in 50 mL of water to form a DS solution. A volume of 50 mL of water was used to macerate 3 g of 2M quality class Gegen for 60 min at room temperature. After maceration, the 50 mL DS solution was added and the system was heated under reflux at 100°C for 120 min. Then, the concentration of DS in the extract was determined using HPLC analysis as aforementioned. According to the results, the concentration of DS was equal to 0.0397 mg/mL, which was 99.2% of the original amount. The results provided evidence that there was no significant amount of chemical marker extracted from one herb absorbed by other herbs.

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