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

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A procedure for herbal extraction has been developed for producing a Chinese herbal medicine (CHM) with consistent quality regardless of the source of the herb. The quality assurance (QA) procedure is based on a model which accounts for the physicochemical phenomena governing herbal extraction. With this model and the companion experiments for determining the relevant model parameters, the amount of each herb needed from different herb quality classes to produce a CHM decoction with consistent quality can be determined. The procedure was illustrated by the extraction of Danshen and that of Gegen. For both examples, the experimental chemical marker concentrations fell within ± 10% of the specified concentrations by using the amount of herb from each herb class as predicted. © 2013 American Institute of Chemical Engineers *AIChE J*, 59: 4241–4254, 2013
Keywords: Chinese herbal medicine, consistent quality, quality assurance, single-herb extraction, product design

Introduction

Modernization of traditional Chinese medicines (TCM) has been actively pursued over the past decades.[1] Topics of investigation include DNA fingerprinting of herbs,[2] identification of chemical markers,[3] chromatographic fingerprinting and chemometrics,[4,5] separation and purification of active pharmaceutical components from herbs,[6,7] pharmacology and bioactivity screening,[8] toxicology,[9] among others. Despite the advances, surprisingly little has changed in the way a Chinese herbal medicinal (CHM) product is manufactured. Typically, the process begins with the extraction of raw herbs in specified proportions according to a traditional recipe. Figure 1 shows such a *recipe* for Danshen-Gegen extraction. Raw herbs in fixed proportions by weight, referred to as a *formula*, are macerated in water at room temperature for a certain period of time. This is followed by extraction at high temperature and spray drying of the extract. The final product is in the form of a tablet or capsule containing the dried powder.

The overall pharmacological performance of a CHM product is believed to be the integrative result of a number of active pharmaceutical ingredients (to be referred to as *bioactive markers*) extracted from the herbs. For a given CHM, there is a sense in the CHM community of what the bioactive markers might be, but there is often no consensus on exactly what they are as well as the proper proportions of the bioactive markers in the CHM product. To avoid controversy, these ingredients are sometimes referred to as *chemical markers* to reflect the fact that some chemical markers may not be bioactive markers. Commonly, the *quality* of a CHM product is quantified by a number of chemical markers in proportions verified by clinical trials.

Even if a recipe is strictly followed in the extraction process, there is no guarantee that a CHM product with the chemical markers in the desired proportions can be produced. It is well-known that the concentration of chemical markers in an herb from different *classes* and sources can be very different.[10–13] (In the herbal market, the herbs are traditionally classified as superior, medium or inferior class based on the judgment of the herbalist). For example, an analysis of 74 Danshen samples showed that the amount of two chemical markers—danshensu and salvianolic acid B—in the raw herb can differ by an order of magnitude (Figure 2). With such variations, it is highly unlikely that the same quality (i.e., proportions of chemical markers) can be obtained in the final CHM product using a fixed formula and a fixed recipe. In addition, the problem is compounded by the fact that the extraction phenomena can be highly complex. For example, some extracted compounds are unstable undergoing reactions such as hydrolysis during extraction. How to

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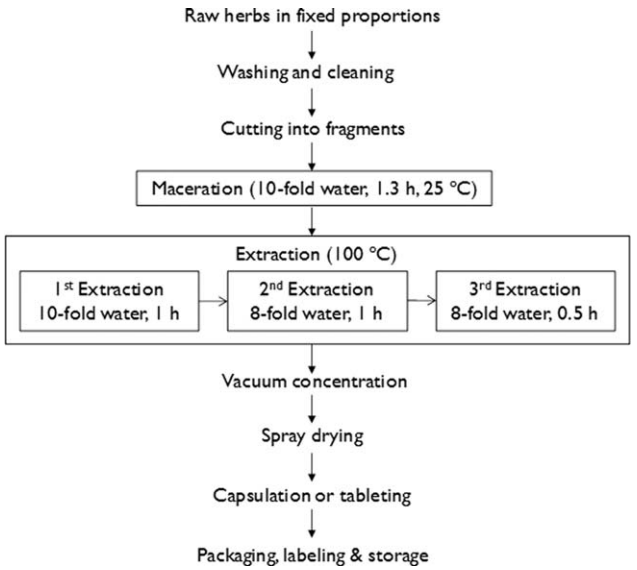


Figure 1. Traditional practice of recipe-based method of Danshen-Gegen extraction.

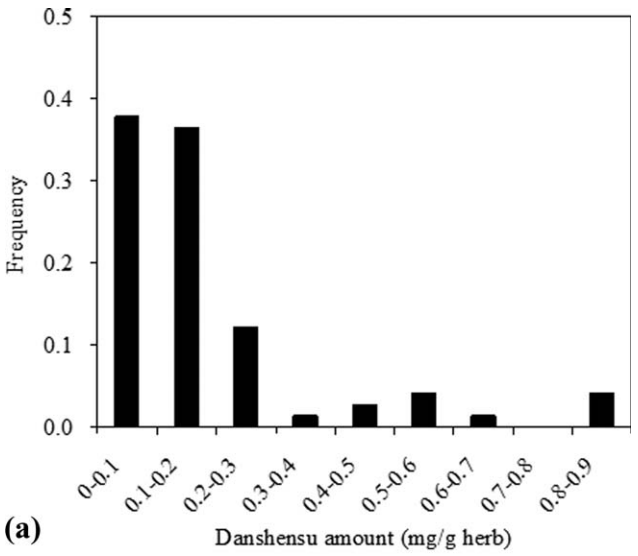
produce a CHM with consistent quality has been identified to be a critical issue awaiting solution.[14]

The objective of this work is to develop a quality assurance (QA) procedure for producing systematically and effectively a consistent quality CHM product regardless of herb sources. The QA procedure begins by specifying the proportions of the chemical markers in the product extract (Step 1a), and collecting the different classes and sources of the raw herb on the market (Step 1b). It includes a model of herbal extraction, which describes the physicochemical phenomena governing herbal extraction. Model parameters are identified for this model and the experimental methods for determining such parameters are formulated (Steps 2a and 2b). Solution of this model provides the operating conditions and the amount of each herb needed from different quality classes to produce a CHM decoction with consistent quality (Steps 3a and 3b).

Quality Assurance of CHM Product

The herbal extraction model

A schematic diagram of herbal extraction by heat and reflux in a well-mixed stirred tank with an extraction solvent of volume V (mL), and at temperature T (°C), is shown in Figure 3. It should be noted that in this model T is fixed. The herb particles are shaded differently to represent different quality classes of the same herb. Several chemical markers are simultaneously extracted from these herb particles. The amount of chemical marker i in herb of quality class j at a given time t (min) is $\Omega_{i,j}$ (mg i/g herb of quality class j), and the concentration of this chemical marker at the interface is $C^s_{i,j}$ (mg i/mL). C_i (mg i/mL) is the concentration of chemical marker i in the extraction solvent. It is assumed that the mass-transfer rate inside the small herb particles is fast compared with that external to the herb particles. This assumption is supported by the fact that the extraction profile was independent of particle size (Appendix A). Of course, this assumption of relatively fast internal mass transfer may not be valid if a raw herb is in the form of large slices instead powders.



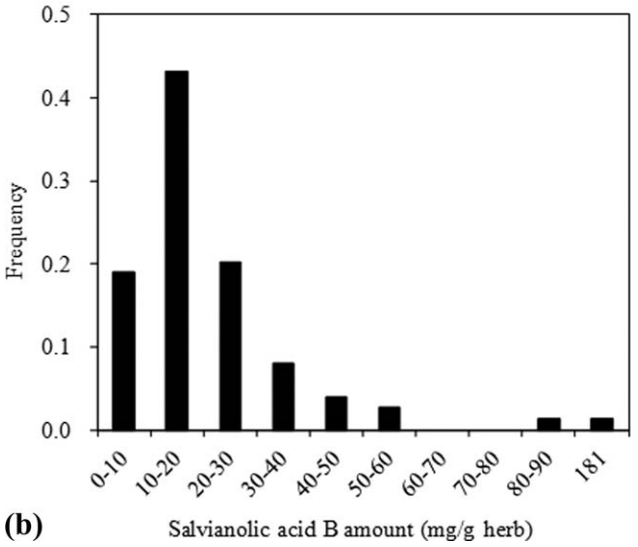


Figure 2. Amount of (a) danshensu, and (b) salvianolic acid B in 74 Danshen samples from different sources.

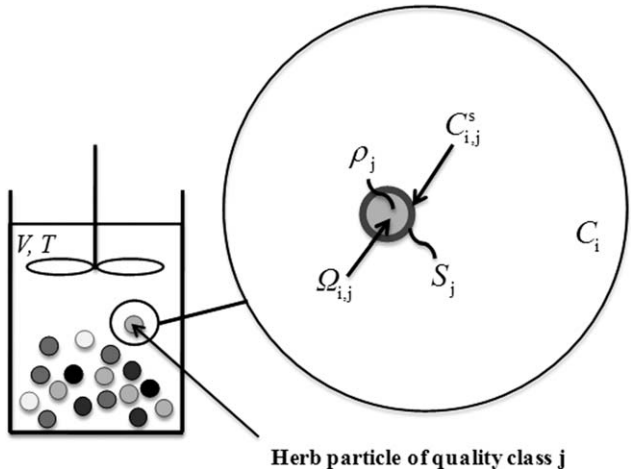


Figure 3. Illustration of chemical marker i extraction from herb of quality class j.

The rate of extraction of chemical marker i from an herb particle of quality class j can be expressed as

$$\frac{d\left(VC_{\mathrm{i,j}}\right)}{dt}=k_{\mathrm{i,j}}M_{\mathrm{j}}S_{\mathrm{j}}\left(C_{\mathrm{i,j}}^{\mathrm{s}}-C_{\mathrm{i}}\right) \tag{1}$$

where $k_{\mathrm{i,j}}$ (cm/min) is the mass-transfer coefficient, S_{j} (cm^2/g) is the specific surface area of herb of quality class j, M_{j} (g herb of quality class j) is the herb mass of quality class j and $C_{\mathrm{i,j}}$ (mg i/mL) is the concentration of chemical marker i extracted from herb of quality class j in the extraction solvent.

The interfacial concentration $C_{\mathrm{i,j}}^{\mathrm{s}}$ is related to $\Omega_{\mathrm{i,j}}$ by a partition coefficient $K_{\mathrm{i,j}}$ (dimensionless) defined as

$$K_{\mathrm{i,j}}=\frac{C_{\mathrm{i,j}}^{\mathrm{s}}}{\rho_{\mathrm{j}}\Omega_{\mathrm{i,j}}} \tag{2}$$

where ρ_{j} (g/cm^3) is the density of herb of quality class j. It is assumed that the chemical marker concentration inside the herb particle and that at the interface can be described by a partition coefficient. This assumption was verified by a set of extraction experiments at equilibrium (Appendix B). Mass balance of chemical marker i for herb quality class j is expressed as

$$M_{\mathrm{j}}\Phi_{\mathrm{i,j}}=M_{\mathrm{j}}\Omega_{\mathrm{i,j}}+VC_{\mathrm{i,j}} \tag{3}$$

where $\Phi_{\mathrm{i,j}}$ (mg i/g herb of quality class j) is the maximum extractable amount of chemical marker i for herb quality class j. The absolute amount of a chemical marker i in an herb particle of a given quality class is of course fixed. However, $\Phi_{\mathrm{i,j}}$, for a given extraction method and under specified extraction conditions can be smaller than the absolute amount. For example, ultrasound is known to affect $\Phi_{\mathrm{i,j}}$. In addition, $\Phi_{\mathrm{i,j}}$ may decrease during extraction because a chemical marker decomposes under the extraction conditions. Equation 3 can be rearranged to give

$$\Omega_{\mathrm{i,j}}=\Phi_{\mathrm{i,j}}-\frac{V}{M_{\mathrm{j}}}C_{\mathrm{i,j}} \tag{4}$$

Substituting Eq. 4 into Eq. 2 gives

$$C_{\mathrm{i,j}}^{\mathrm{s}}=K_{\mathrm{i,j}}\rho_{\mathrm{j}}\Phi_{\mathrm{i,j}}-\frac{K_{\mathrm{i,j}}\rho_{\mathrm{j}}V}{M_{\mathrm{j}}}C_{\mathrm{i,j}} \tag{5}$$

Equation 5 is substituted into Eq. 1 to eliminate $C_{\mathrm{i,j}}^{\mathrm{s}}$

$$\frac{dC_{\mathrm{i,j}}}{dt}=\frac{k_{\mathrm{i,j}}M_{\mathrm{j}}S_{\mathrm{j}}}{V}\left[\left(K_{\mathrm{i,j}}\rho_{\mathrm{j}}\Phi_{\mathrm{i,j}}-\frac{K_{\mathrm{i,j}}\rho_{\mathrm{j}}V}{M_{\mathrm{j}}}C_{\mathrm{i,j}}\right)-C_{\mathrm{i}}\right] \tag{6}$$

With n quality classes, the mass of chemical marker i in the extraction solvent is

$$C_{\mathrm{i}}=\sum_{h=1}^{n}C_{\mathrm{i},h}=C_{\mathrm{i},1}+C_{\mathrm{i},2}+\cdots+C_{\mathrm{i},n} \tag{7}$$

Substituting Eq. 7 into Eq. 6 and rearranging, we get

$$\frac{dC_{\mathrm{i,j}}}{dt}=\frac{k_{\mathrm{i,j}}M_{\mathrm{j}}S_{\mathrm{j}}}{V}\left\{K_{\mathrm{i,j}}\rho_{\mathrm{j}}\Phi_{\mathrm{i,j}}-\left[\left(1+\frac{K_{\mathrm{i,j}}\rho_{\mathrm{j}}V}{M_{\mathrm{j}}}\right)C_{\mathrm{i,j}}+\sum_{\substack{h=1\\h\neq\mathrm{j}}}^{n}C_{\mathrm{i},h}\right]\right\} \tag{8}$$

Equation 8 can be written in matrix form as

$$\frac{d\mathbf{c}_{\mathrm{i}}}{dt}=\mathbf{A}_{\mathrm{i}}\mathbf{c}_{\mathrm{i}}+\mathbf{g}_{\mathrm{i}} \tag{9}$$

where

$$\mathbf{c}_{\mathrm{i}}=\begin{bmatrix}C_{\mathrm{i},1} & C_{\mathrm{i},2} & \cdots & C_{\mathrm{i},n}\end{bmatrix}^{T} \tag{10}$$

$$\mathbf{A}_{\mathrm{i}}=\begin{bmatrix}\left(-\frac{k_{\mathrm{i},1}M_1S_1}{V}\right)\left(1+\frac{K_{\mathrm{i},1}\rho_1V}{M_1}\right) & -\frac{k_{\mathrm{i},1}M_1S_1}{V} & \cdots & -\frac{k_{\mathrm{i},1}M_1S_1}{V}\\ -\frac{k_{\mathrm{i},2}M_2S_2}{V} & \left(-\frac{k_{\mathrm{i},2}M_2S_2}{V}\right)\left(1+\frac{K_{\mathrm{i},2}\rho_2V}{M_2}\right) & \cdots & -\frac{k_{\mathrm{i},2}M_2S_2}{V}\\ \vdots & \vdots & \ddots & \vdots\\ -\frac{k_{\mathrm{i},n}M_nS_n}{V} & -\frac{k_{\mathrm{i},n}M_nS_n}{V} & \cdots & \left(-\frac{k_{\mathrm{i},n}M_nS_n}{V}\right)\left(1+\frac{K_{\mathrm{i},n}\rho_nV}{M_n}\right)\end{bmatrix} \tag{11}$$

$$\mathbf{g}_{\mathrm{i}}=\begin{bmatrix}\left(\frac{k_{\mathrm{i},1}M_1S_1}{V}\right)\left(K_{\mathrm{i},1}\rho_1\Phi_{\mathrm{i},1}\right)\\ \left(\frac{k_{\mathrm{i},2}M_2S_2}{V}\right)\left(K_{\mathrm{i},2}\rho_2\Phi_{\mathrm{i},2}\right)\\ \vdots\\ \left(\frac{k_{\mathrm{i},n}M_nS_n}{V}\right)\left(K_{\mathrm{i},n}\rho_n\Phi_{\mathrm{i},n}\right)\end{bmatrix} \tag{12}$$

Equation 9 can be solved by standard method as

$$\mathbf{c}_{\mathrm{i}}=\sum_{h=1}^{n}p_h\varepsilon_{\mathrm{i}}^{(h)}e^{\lambda_{\mathrm{i},h}t}+\left(\mathbf{A}_{\mathrm{i}}^{-1}\right)\left(-\mathbf{g}_{\mathrm{i}}\right) \tag{13}$$

where $\lambda_{\mathrm{i},h}$ is an eigenvalue, $\varepsilon_{\mathrm{i}}^{(h)}$ is the corresponding eigenvector, and p_h is a constant that can be determined by substituting the initial conditions $\mathbf{c}_{\mathrm{i}}(0)=\mathbf{c}_{\mathrm{i}}^{0}$, which are determined experimentally, into Eq. 13.

The concentrations of the x chemical markers can be represented as

$$\mathbf{c}=\begin{bmatrix}C_1 & C_2 & \cdots & C_x\end{bmatrix}^{T} \tag{14}$$

Each element in $\mathbf{c}$ can be obtained from Eqs. 7 and 13. For a product extract that satisfies the product specifications, we have

$$\mathbf{c}=\mathbf{c}^{\mathrm{QA}}=\begin{bmatrix}C_1^{\mathrm{QA}} & C_2^{\mathrm{QA}} & \cdots & C_x^{\mathrm{QA}}\end{bmatrix}^{T} \tag{15}$$

Since the extraction conditions (V, T and t) are fixed, the only variable in Eqs. 7, 13, and 15 is $\mathbf{m}$, the mass of each herb class used in extraction

$$\mathbf{m}=\begin{bmatrix}M_1 & M_2 & \cdots & M_n\end{bmatrix}^{T} \tag{16}$$

Table 1. Summary of Model Parameters, Specified Conditions and Unknown Variables of Herbal Extraction Model

Model parameters	Specified conditions	Unknown variables
Maximum extractable amount $\Phi_{i,j}$	Concentrations of x chemical markers $\mathbf{c}=\begin{bmatrix} C_1^{QA} & C_2^{QA} & \cdots & C_x^{QA} \end{bmatrix}^T$	Masses of n herb quality classes $\mathbf{m}=\begin{bmatrix} M_1 & M_2 & \cdots & M_n \end{bmatrix}^T$
Density-lumped partition coefficient $K_{i,j}\rho_j$	Extraction temperature T	
Area-lumped mass transfer coefficient $k_{i,j}S_j$	Extraction time t	
Decomposition rate constant β_i (optional)	Volume of solvent V	

which can be found by numerically solving the aforementioned set of equations. The model parameters, specified conditions and unknown variables of the herbal extraction model are summarized in Table 1.

Existence and uniqueness of solution

At a specified set of extraction conditions, if the quality constraint is satisfied, $C_i=C_i^{QA}$ is a constant and $\frac{dC_{i,j}}{dt}=\dot{C}_{i,j}^{QA}$ is of a given value. Eq. 6 can be rearranged as

$$\left(\frac{\Phi_{i,j}}{V}-\frac{C_i^{QA}}{K_{i,j}\rho_j V}\right)M_j=C_{i,j}+\frac{\dot{C}_{i,j}^{QA}}{\left(k_{i,j}S_j\right)\left(K_{i,j}\rho_j\right)} \tag{17}$$

Summing Eq. 17 over j and using Eq. 7, we get

$$\sum_{j=1}^{n}\left[\left(\frac{\Phi_{i,j}}{V}-\frac{C_i^{QA}}{K_{i,j}\rho_j V}\right)M_j\right]=C_i^{QA}+\sum_{j=1}^{n}\left[\frac{\dot{C}_{i,j}^{QA}}{\left(k_{i,j}S_j\right)\left(K_{i,j}\rho_j\right)}\right] \tag{18}$$

As there are x chemical markers, we have x sets of Eq. 18, which can be represented as a system of linear equations

$$\mathbf{Dm}=\mathbf{b} \tag{19}$$

where

$$\mathbf{D}=\begin{bmatrix} \frac{\Phi_{1,1}}{V}-\frac{C_1^{QA}}{K_{1,1}\rho_1 V} & \frac{\Phi_{1,2}}{V}-\frac{C_1^{QA}}{K_{1,2}\rho_2 V} & \cdots & \frac{\Phi_{1,n}}{V}-\frac{C_1^{QA}}{K_{1,n}\rho_n V} \\ \frac{\Phi_{2,1}}{V}-\frac{C_2^{QA}}{K_{2,1}\rho_1 V} & \frac{\Phi_{2,2}}{V}-\frac{C_2^{QA}}{K_{2,2}\rho_2 V} & \cdots & \frac{\Phi_{2,n}}{V}-\frac{C_2^{QA}}{K_{2,n}\rho_n V} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\Phi_{x,1}}{V}-\frac{C_x^{QA}}{K_{x,1}\rho_1 V} & \frac{\Phi_{x,2}}{V}-\frac{C_x^{QA}}{K_{x,2}\rho_2 V} & \cdots & \frac{\Phi_{x,n}}{V}-\frac{C_x^{QA}}{K_{x,n}\rho_n V} \end{bmatrix} \tag{20}$$

$$\mathbf{b}=\begin{bmatrix} C_1^{QA}+\sum_{j=1}^{n}\frac{C_{1,j}^{\bullet QA}}{\left(k_{1,j}S_j\right)\left(K_{1,j}\rho_j\right)} \\ C_2^{QA}+\sum_{j=1}^{n}\frac{C_{2,j}^{\bullet QA}}{\left(k_{2,j}S_j\right)\left(K_{2,j}\rho_j\right)} \\ \vdots \\ C_x^{QA}+\sum_{j=1}^{n}\frac{C_{x,j}^{\bullet QA}}{\left(k_{x,j}S_j\right)\left(K_{x,j}\rho_j\right)} \end{bmatrix} \tag{21}$$

Equation 19 is a set of linear equations that allows ready determination of the existence of solutions. The number of rows of **D** is the number of chemical markers (x) which represents the number of quality constraints and the number of columns of **D** is the number of quality classes (n), which represents the number of variables. In general, as long as $\det(\mathbf{D})\neq 0$, there are three possible scenarios. When $x=n$ (scenario 1), the system is exactly specified and a unique solution of **m** exists which can be used to produce an extract with the desired quality. When $x<n$ (scenario 2), the system is underspecified and has infinitely many solutions of **m**. The degree(s) of freedom, which is equal to $n-x$, can be utilized for purposes such as cost minimization. When $x>n$ (scenario 3), the system is overspecified and there is no exact solution. However, one can minimize the error or deviation from the specified proportions of chemical markers. A convenient way to achieve error minimization is to relax the specified extraction conditions such as T and t, thereby introducing additional variables to the system. In other words, either T or t is allowed to change in the model. Of course, a feasible solution of **m** must consist only non-negative elements.

Determination of model parameters

It can be seen in the aforementioned equations that the model parameters are the maximum extractable amount ($\Phi_{i,j}$), the density-lumped partition coefficient $\left(K_{i,j}\rho_j\right)$ and the area-lumped mass-transfer coefficient $\left(k_{i,j}S_j\right)$. These parameters can be experimentally determined by performing extraction experiments for each of the quality classes. For a single quality class and if the chemical marker does not decompose during extraction, Eq. 6 can be expressed as

$$\frac{dC_i}{dt}=k_i'\left(C_i^{\infty}-C_i\right) \tag{22}$$

where

$$k_i'=k_iS\left(K_i\rho+\frac{M}{V}\right) \tag{23}$$

and

$$C_i^{\infty}=\frac{\Phi_i^0}{\left(\frac{V}{M}+\frac{1}{K_i\rho}\right)} \tag{24}$$

Note that Eq. 24 is obtained by setting

$$\Phi_i=\Phi_i^0 \tag{25}$$

In Eq. 6 Φ_i^0 (mg i/g herb) is a constant representing the maximum extractable amount of chemical marker i. Equation 22 has been used empirically for tea and coffee extraction.[15,16] C_i^{∞} is the concentration of chemical marker i of the extraction profile when t tends to infinity, and the rate of extraction approaches zero. Integration of Eq. 22 gives

$$C_i=C_i^{\infty}-\left[\left(C_i^{\infty}-C_i^0\right)e^{-k_i't}\right] \tag{26}$$

where C_i^0 (mg i/mL) is the initial concentration of chemical marker i in the extraction solvent at $t=0$.

k_i' (Eq. 23), and C_i^∞ (Eq. 24) can be obtained by fitting the experimental data with Eq. 26. Rearranging Eq. 24 gives

$$\frac{1}{C_i^\infty} = \left(\frac{V}{\Phi_i^0}\right)\left(\frac{1}{M}\right) + \frac{1}{K_i \rho \Phi_i^0} \tag{27}$$

By plotting $\frac{1}{C_i^\infty}$ against $\frac{1}{M}$, Φ_i^0 can be determined from the slope and $K_i\rho$ can be determined from the y-intercept. Similarly, rearranging Eq. 23 gives

$$k_i' = (k_i S)(K_i \rho) + \left(\frac{k_i S}{V}\right) M \tag{28}$$

By plotting k_i' against M, k_iS can be determined by the slope or the intercept since $K_i\rho$ is known.

Sometimes, a chemical marker undergoes decomposition reaction such as hydrolysis both inside the herb particle and outside in the extraction solvent during extraction. The decomposition can be quantified by first-order kinetics. Thus, the maximum extractable amount of chemical marker i decreases exponentially during extraction and can be expressed as

$$\Phi_i = \Phi_i^0 e^{-\beta_i t} \tag{29}$$

where β_i (min^{-1}) is the decomposition rate constant of chemical marker i. It is assumed that the decomposition of the chemical marker i in maceration is negligible. This assumption is supported by experimental results that there was minimal decomposition of salvianolic acid B, a key chemical marker in Danshen, in water at 25°C for 60 min, the same as the maceration conditions (Appendix C).

Substituting Eq. 29 into Eq. 6 and then integrating it gives

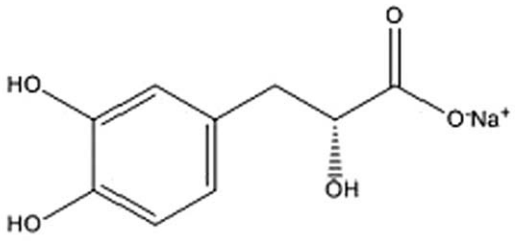
$$C_i = \left[\frac{k_i' C_i^\infty}{(k_i' - \beta_i)}\right] e^{-\beta_i t} - \left\{\left[\frac{k_i' C_i^\infty}{(k_i' - \beta_i)}\right] - C_i^0\right\} e^{-k_i' t} \tag{30}$$

Therefore, β_i along with C_i^∞ and k_i' can be obtained by fitting of the experimental extraction data to Eq. 30. The corresponding values of the model parameters (Φ_i^0, $K_i\rho$ and k_iS) can be determined by the same method mentioned previously.

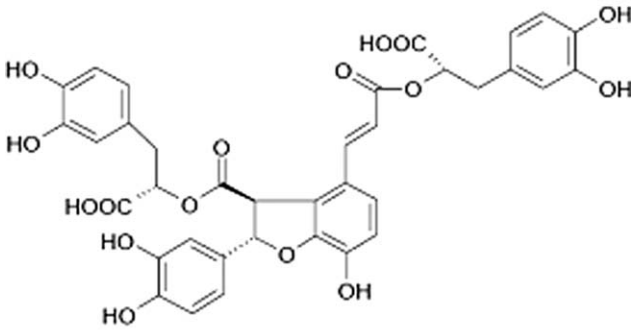
Example 1: Quality Assurance of Danshen Decoction

Danshen (*Radix salviae miltiorrhiza*) is a medicinal herb widely used in China and other Asian countries. Raw Danshen and Gegen and their isolated compounds have been shown to produce beneficial effects on cardiovascular functions in animals, humans and cultured human endothelial cells.[17–19]

In this example, danshensu (DS) and salvianolic acid B (SAB) (Figure 4) were selected as chemical markers. Step 1a of the QA procedure is to specify the product specifications. The product quality of Danshen decoction was specified as the ratio of DS to SAB = 1:24.5 (or equivalently the concentrations of DS in the extract $C_{DS}^{QA} = 0.14$ mg/mL and that of SAB $C_{SAB}^{QA} = 3.43$ mg/mL). These values were given by the medical researchers of a 10-year long project supported by the University Grants Council in Hong Kong. All standards of the chemical markers were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), with purities higher than 98%. Step 1b of the QA procedure is to obtain the herb source



Danshensu, $C_9H_9O_5Na$, MW.: 220.15



Salvianolic acid B, $C_{36}H_{30}O_{16}$, MW.: 718.63

Figure 4. Molecular structure of danshensu and salvianolic acid B.

information. Three different quality classes (categorized as superior, medium or inferior class) of Danshen were used. Based on the market price in Hong Kong in spring 2010, the cost of Danshen were HK$ 100/kg for superior class (1S), HK$ 100/kg for medium class (1M) and HK$ 40/kg for inferior class (1I). Here, "1" refers to Danshen in order to distinguish it from "2" (Gegen) to be discussed later. Raw herb was ground into powder form and sieved such that all the herb powders used in this study had particle size equal to 1.0 mm or less. All the herb powders were stored in a 4°C refrigerator awaiting later 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.

Selection of extraction solvent and temperature based on solubility information

Step 2a of the QA procedure is to experimentally measure the solubilities of the chemical markers. Chemical marker solubility is important for selecting an extraction solvent and extraction temperature. The solubility should be sufficiently high so that the chemical marker is far away from saturation. The solubilities of DS in water, 50% ethanol solution (50% EtOH) and ethanol (EtOH) were measured using the isothermal solid-disappearance method[20] (Figure 5). The corresponding solubilities of SAB were not measured, as they are much higher than that of DS. First, a known amount of DS and solvent was heated in a double-walled glass vessel to a temperature that was 5°C higher than the target temperature. After 10 min of equilibration, a known amount of solvent was slowly added to the glass vessel until the solid chemical marker dissolved completely and the mixture became a

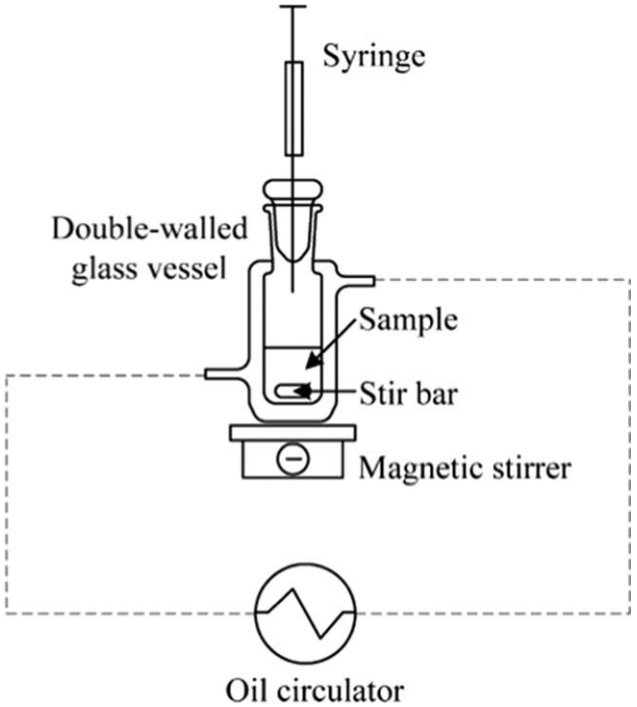


Figure 5. Schematic diagram of setup for solubility measurement.

homogeneous solution. Afterward, the homogeneous solution was cooled until the solute was observed to crystallize from the solution. The temperature was then raised to the target temperature and kept there for 10 min. Next, a small but known amount of solvent was added to the system again every 5 min until all of the solute was dissolved. The solubility of the chemical marker at the target temperature was calculated from the amount of solute and the total amount of solvent in the glass vessel.

The measured solubility data of DS are shown in Figure 6. In general, the solubility of DS increased with increasing temperature for all the three solvents. For example, the DS solubility in water was 12.12 mg/mL at 60°C, which was much higher than that at 20°C. In addition, it can be seen that the solubility of DS decreased with an increase of ethanol in the other two solvents. For example at 60°C, the DS solubility in water (12.12 mg/mL) was more than six-fold and two hundred-fold higher than that in 50% EtOH (1.85 mg/mL) and EtOH (0.06 mg/mL), respectively. According to the solubility data of DS, water was chosen as the extraction solvent and 100°C (i.e., the highest temperature for water) was chosen as the extraction temperature, since both conditions could maximize the extraction efficiency.

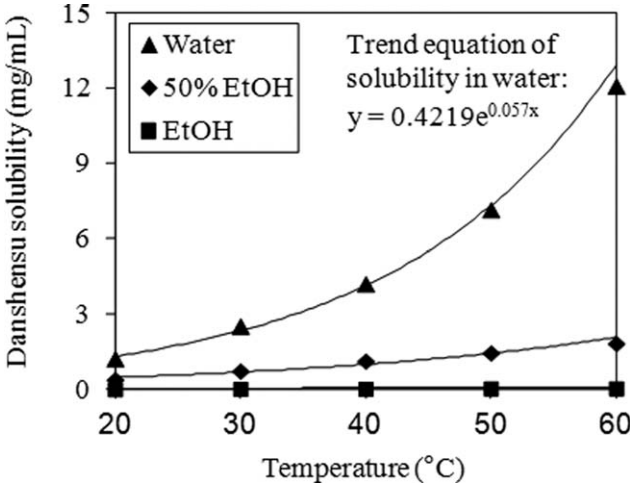


Figure 6. Solubility of danshensu in water, 50% ethanol-water solution (50% EtOH) and ethanol (EtOH) at different temperatures.

Extraction kinetics and model parameters

Step 2b of the QA procedure is to experimentally determine the model parameters of the chemical markers. The extraction method used in this project was heat-and-reflux extraction. The schematic diagram of the experimental setup is shown in Figure 7. For simplicity, V was fixed at 100 mL. For each extraction experiment, a certain amount of herb powder 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 high-performance liquid

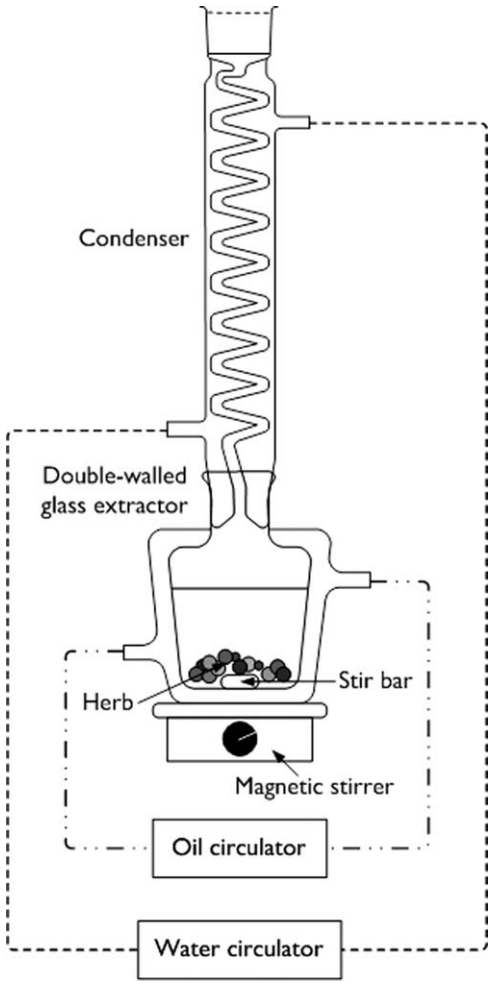


Figure 7. Schematic diagram of setup for heat-and-reflux extraction.

chromatography (HPLC) analysis on a Waters Alliance HPLC system which comprises the Waters 2956 Separation Module and the Waters 2996 Photodiode Array Detector. An ODS-2 Hypersil 5 μm (4.6 mm $\times$ 250 mm) column was used. The mobile phase was water and acetonitrile (with 0.1% acetic acid) at a flow rate of 1 mL/min with gradient mobile phase ratio. A sample of 40 μL was injected and the elution was monitored by UV absorbance at 270 nm.

A series of experiments were conducted to obtain the extraction profiles of DS and SAB from different amounts (3, 5 and 7 g) of 1S, 1M and 1I quality classes Danshen at 100°C in 100 mL of water. The extraction profiles of DS and SAB from 1M quality class Danshen are shown in Figure 8. Extraction kinetics of DS and SAB from the other two quality classes of Danshen (available in online supplement) exhibit similar behavior. It can be seen that the extraction kinetics of DS followed the expected extraction behavior. In contrast, decomposition of SAB was observed. This could be due to the hydrolysis of SAB in water during extraction.[21] Thus, the model with a first-order decomposition constant was used to treat all the extraction data of SAB. It should be noted that there was no interconversion of DS and SAB during extraction as verified by an experiment. In this experiment, a certain amount of pure SAB was dissolved in 100 mL of water and the solution was kept at 100°C for 8 h. No significant amount of DS was observed in the solution, showing that the conversion of SAB to DS does not occur under the specified extraction conditions. The values of C^{∞} and k' for each data set were determined.

According to Eqs. 27 and 28, plots of $\frac{1}{C^{\infty}}$ against $\frac{1}{M}$ and k' against M for DS and SAB were prepared. The plots for 1M quality class Danshen are shown in Figures 10 (for DS) and 9 (for SAB) as example. The plots for the other two quality

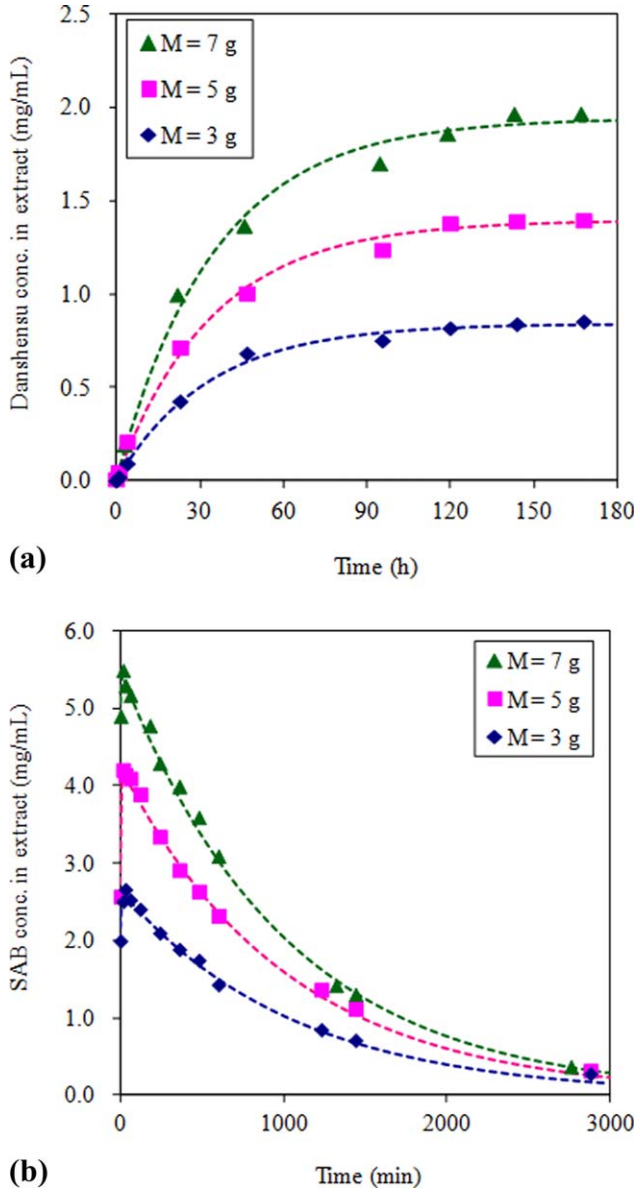


Figure 8. Extraction profiles of (a) danshensu, and (b) salvianolic acid B (SAB) at 100°C in 100 mL of water from different amounts of 1M quality class of Danshen.

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.]

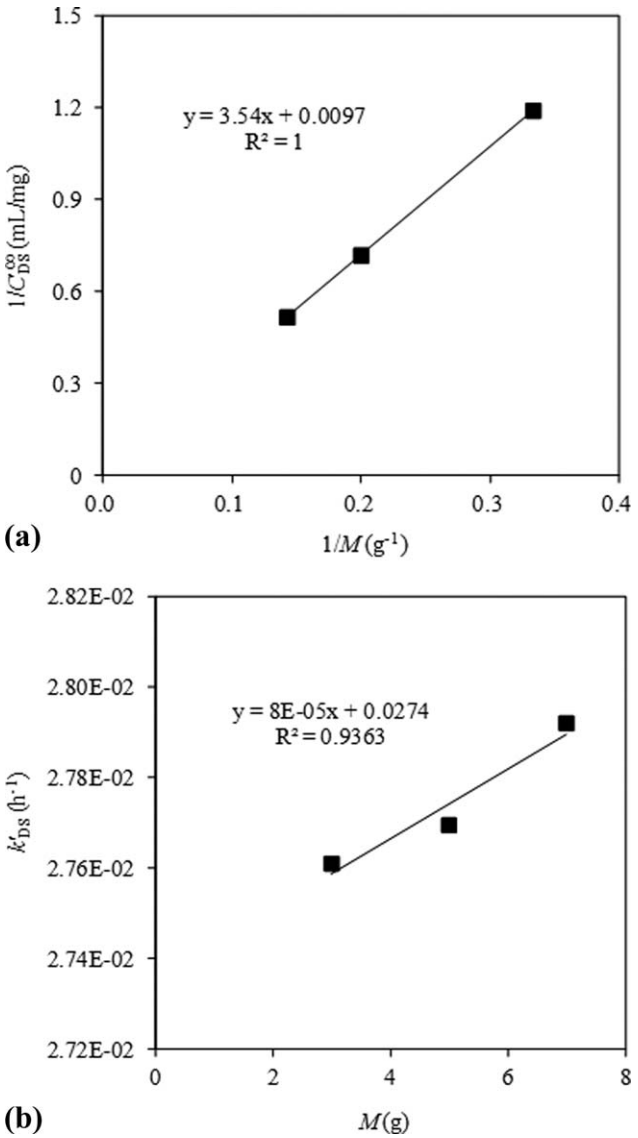


Figure 9. Plots of (a) $\frac{1}{C^{\infty}_{DS}}$ against $\frac{1}{M}$, and (b) k'_{DS} against M of danshensu (DS) extracted at 100°C in 100 mL of water from 1M quality class of Danshen.

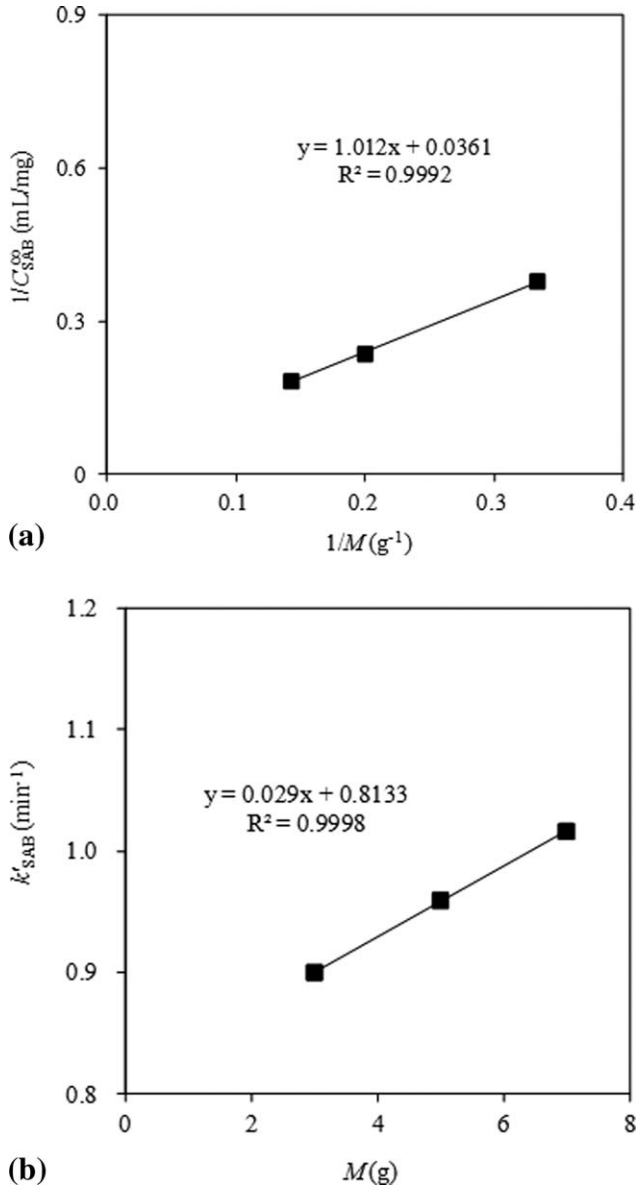


Figure 10. Plots of (a) $\frac{1}{C^{\infty}_{SAB}}$ against $\frac{1}{M}$, and (b) k'_{SAB} against M of salvianolic acid B (SAB) extracted at 100°C in 100 mL of water from 1M quality class of Danshen

classes of Danshen (not shown here) exhibit a similar behavior with very good linear fit. The values of model parameters were obtained, which are summarized in Table 2. The value differences of model parameters from different quality classes Danshen indicated that the physicochemical behaviors of DS and SAB extracted from different quality classes were not the same. For example, the maximum extractable amount of DS extracted from 1S quality class Danshen was the highest at 29.64 mg/g herb. Regarding the extraction rate, DS was extracted faster from 1I quality class Danshen than that from 1S and 1M quality classes Danshen. Next, these model parameters were used to execute the QA of Danshen decoction.

Table 2. 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

Danshensu	1S	1M	1I
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	**1S**	**1M**	**1I**
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}

Scenario 1: Danshen extraction system with unique solution ($x = n$)

Step 3a of the QA procedure is to specify the extraction solvent and a set of operating conditions of V, T and t. As mentioned, water was chosen as the extraction solvent, V was fixed at 100 mL, and T was chosen as 100°C. Here, the extraction time t was arbitrarily fixed to be 120 min. Step 3b of the QA procedure is to use the quality assurance model to determine the herb amounts needed from the different quality classes. In the given product specifications, two quality constraints have to be satisfied ($x = 2$). If only two quality classes ($n = 2$) are used, a unique solution can be determined. For the case of using 1S and 1M quality classes Danshen, the solution is $M_{1S} = 5.62$ g and $M_{1M} = 1.37$ g. For the case of using 1M and 1I quality classes Danshen, the solution is $M_{1M} = 3.14$ g and $M_{1I} = 9.87$ g. Interestingly, for the case of using 1S and 1I quality classes Danshen, the solution of $M_{1S} = 9.94$ g and $M_{1I} = -7.55$ g is obviously infeasible.

Scenario 2: Danshen extraction system with infinitely many solutions ($x < n$)

Now, if all three quality classes ($n = 3$) are used to produce Danshen decoction, the extraction system is underspecified. Infinitely many solutions, all of which are able to produce Danshen decoction with consistent quality, can be determined. In order to experimentally verify the accuracy of the QA procedure, three solutions (with solution numbers 1, 2 and 3) were used to produce samples of Danshen decoction. The extract qualities of all three samples were measured (Table 3). For each sample, good accuracies of the extract quality were observed that the concentrations of the two chemical markers were within ±10% of the desired concentrations in the product specifications. The results showed that this QA procedure was reliable to ensure the consistence of extract quality.

Table 3. The Amount of Different Quality Classes of Danshen used in Solution Numbers 1, 2 and 3, and the Corresponding Concentrations of Danshensu (DS) and Salvianolic Acid B (SAB) Extracted at 100°C in 100 mL of Water with 120 min Extraction Time

Solution number	Danshen amount (g)			Quality of extract (mg/mL) [% difference]	
	1S	1M	1I	DS	SAB
1	5.62	1.37	0.00	0.146 [+4.3]	3.324 [-3.1]
2	3.00	2.19	4.62	0.153 [+9.1]	3.722 [+8.5]
3	0.00	3.14	9.87	0.133 [-4.9]	3.763 [+9.7]

The product specifications are: $C^{QA}_{DS} = 0.14$ mg/mL and $C^{QA}_{SAB} = 3.43$ mg/mL

The degree of freedom can be utilized for the minimization of the cost of herb. It was found that among all the solutions obtained after exhaustive iterations, the most cost-effective solution costs HK$ 6988/1000 L extract and the most expensive solution costs HK$ 7093/1000 L extract. The cost difference is small in this case.

Scenario 3: Danshen extraction system with no exact solution ($x > n$) and relaxation of time constraint

If only one quality class is available for the production of Danshen decoction (i.e., $n = 1$), the extraction system is overspecified. Under this situation, no exact solution can be obtained by the model. Instead, an imperfect solution can be found by minimizing the total percentage error of product quality, where

$$\text{Total percentage error} = \sum_{i=1}^{x} \left(\left| \frac{C_i^{QA} - C_i}{C_i^{QA}} \right| \times 100\% \right) \quad (31)$$

Table 4 summarizes the calculated percentage error of product quality, it can be seen that the error is around 15–40%, which cannot be accepted according to the desired quality requirement.

If the extraction time (t) is allowed to vary while keeping V and T constant, there is an additional variable to the system. Now, it is a nonlinear system with two equations (Eq. 26 for DS and Eq. 30 for SAB) and two variables (M and t). Thus, the system becomes exactly specified and a finite number of solution(s) of M and t can be determined. For each quality class of Danshen, a set of solution of M and t was determined by using the QA model (Table 4).

Extraction experiments of 1S and 1M Danshen cases were conducted to check the determined solutions. The experiment of 1I Danshen case was not conducted due to the limitation of controlling a gentle mixing condition for this relatively large herb-to-solvent ratio. The results are also presented in Table 4. Good agreement of predicted extract quality with experiments was observed for the two product decoctions.

Example 2: Quality Assurance of Gegen Decoction

Gegen (*Radix puerariae lobatae*) is a medicinal herb that is widely used with Danshen and has similar pharmacological functions to Danshen. In this example, daidzin (DN) and daidzein (DeN) (Figure 11), both of which were reported to have biological activities,[22,23] were selected as chemical markers. All standards of the chemical markers were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), with purities higher than 98%. Three different quality classes (categorized as superior, medium or inferior class) of Gegen were used. Based on the market price in Hong Kong in spring 2010, the cost of Gegen were HK$ 30/kg for superior class (2S), HK$ 60/kg for medium class (2M) and HK$ 20/kg for inferior class (2I) (Step 1b of the QA procedure). Interestingly, the price of medium class Gegen was set to be more expensive than the superior class one by the herb supplier, showing that the perception of quality by the traditional herbalist is somewhat arbitrary. All the herbs of Gegen were treated with the same methods used in example 1. Except for the materials specifically mentioned, all other materials were the same as example 1.

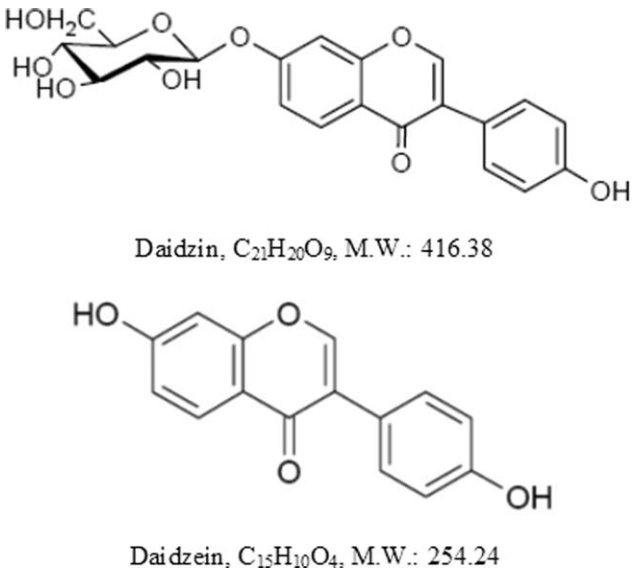


Figure 11. Molecular structure of daidzin and daidzein.

Consider extraction of Gegen with 100 mL of water at 100°C and 120 min. The product quality of Gegen decoction was specified as the ratio of DN to DeN = 1.77:1 (or equivalently the concentrations of DN in the extract $C_{DN}^{QA} = 0.124$ mg/mL and that of DeN $C_{DeN}^{QA} = 0.070$ mg/mL) (Step 1a of the QA procedure). These values were given by the medical researchers of a 10-year long project supported by the University Grants Council in Hong Kong. The solubilities of DN and DeN in water at 100°C were obtained by the same methods as mentioned in example 1, which are 0.24 mg/mL and 0.08 mg/mL, respectively (Step 2a of the QA procedure). The extraction kinetics and model

Table 4. The Amount of Different Quality Classes of Danshen used and the Corresponding Concentrations of Danshensu and Salvianolic Acid B Extracted at 100°C in 100 mL of Water with Fixed Extraction Time

	Fixed $t = 120$ min			Relaxed t			
Quality class	Danshen amount (g)	C_{DS} (mg DS/mL) [Calculated % difference]	C_{SAB} (mg SAB/mL) [Calculated % difference]	Danshen amount (g)	t (min)	C_{DS} (mg DS/mL) [% difference]	C_{SAB} (mg SAB/mL) [% difference]
1S	6.90	0.14 [0.0]	2.94 [−14.17]	7.85	81	0.151 [+7.9]	3.587 [+4.6]
1M	4.53	0.09 [−38.7]	3.43 [0.0]	4.95	199	0.135 [−3.8]	3.485 [+1.6]
1I	17.18	0.14 [0.0]	2.05 [−40.2]	30.53	58	N/A	N/A

The same with relaxed extraction time. The product specifications are: $C_{DS}^{QA} = 0.14$ mg/mL and $C_{SAB}^{QA} = 3.43$ mg/mL

Table 5. Summary of Model Parameters of Daidzin and Daidzein Extracted at 100°C in 100 mL of Water from Different Quality Classes of Gegen

Daidzin	2S	2M	2I
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	**2S**	**2M**	**2I**
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}

parameters were obtained by the same methods as mentioned in example 1 (extraction profiles are not shown here). There was no interconversion of DN and DeN during extraction, as supported by experimental results although DN can be hydrolyzed to DeN under highly acidic conditions.[24] Two samples were prepared by dissolving a certain amount of pure DN in 100 mL of aqueous solution with pH values of 3 and 4, respectively. These pH values are representative of those in this project, and the two solutions were kept at 100°C for 3 h. It was observed that the amount of DN was unchanged and there was no DeN in both samples, showing that DN is not hydrolyzed to DeN under the specified extraction conditions. The model parameters of DN and DeN are summarized in Table 5 (Step 2b of the QA procedure). These model parameters were used to execute the QA of Gegen decoction.

The extraction with 100 mL of water at 100°C for 120 min were used (Step 3a of the QA procedure). Here, all the three quality classes (i.e., $n = 3$) were used to produce the Gegen decoction, the extraction system was underspecified and infinitely many solutions exist. By using the QA model, all the solutions could be determined. The accuracy of the QA procedure on the production of Gegen decoction was justified experimentally. Three solutions (with solution numbers 1, 2 and 3) were used to produce samples of Gegen decoction and their qualities were measured (Table 6) (Step 3b of the QA procedure). For each sample, the concentrations of the two chemical markers were within $\pm10\%$ of the desired concentrations in the product specifications, which further support the accuracy of this QA procedure.

Table 6. The Amount of Different Quality Classes of Gegen used in Solution Numbers 1, 2 and 3 and the Corresponding Concentrations of Daidzin (DN) and Daidzein (DeN) Extracted at 100°C in 100 mL of Water with 120 Min Extraction Time

Solution number	Gegen amount (g)			Quality of extract (mg/mL) [% difference]	
	2S	2M	2I	DN	DeN
1	0.00	2.25	1.88	0.130 [+4.8]	0.074 [+6.0]
2	5.00	2.10	1.76	0.123 [−0.8]	0.073 [+4.6]
3	10.00	2.00	1.61	0.128 [+3.2]	0.069 [−0.9]

The product specifications are: $C^{QA}_{DN} = 0.124$ mg/mL and $C^{QA}_{DeN} = 0.070$ mg/mL

In addition to the QA of Gegen decoction, cost minimization was performed. Among all the solutions obtained by exhaustive iterations, the most expensive solution costs HK$ 22,523/1000 L extract, which is more than 13-fold of that of the most cost-effective solution, which costs HK$ 1726/1000 L extract. The results showed that sometimes the cost difference for different solutions can be very huge, hence, the cost-minimizing ability of the QA model is very useful to ensure the cost effectiveness of the production.

Conclusions

Product quality inconsistency has been one of the most important unmet challenges in the modernization of TCM. The root cause of the problem is the huge variations in the chemical compositions of the raw herbs, which depend on whether the herb is wild or cultured, and on the season of the year when the plants are harvested, among others. The traditional response of the TCM community is to insist on using the same source of herbs, the same formula and the same recipe to prepare the CHM product. While the producer can analyze the product before sales, and discard or recycle the batch that is off spec, this quality control approach is neither satisfying nor practical. It cedes control of the manufacturing process to a belief without true understanding and vastly limits the availability of raw herbs.

Instead of strictly following the traditional recipe, our QA procedure takes advantage of the availability of herbs of different compositions (herb classes) to produce a CHM product with consistent quality. As summarized in Figure 12, it begins with product quality specifications in terms of the proportions among the chemical markers. Then, the model parameters for each herb class including partition coefficient, maximum extractable amount, mass-transfer coefficient, etc. are determined. Solution of the QA model yields the amount of herb needed from each quality class to produce a CHM decoction with the specified amounts of chemical markers. In addition to composition consistency, the product extract can be tested by bioassays for its bioactivity as part of this QA procedure, if desirable.

The application to single-herb extraction was illustrated by the extraction of Danshen and that of Gegen. For both examples, the experimental chemical marker concentrations fall within $\pm10\%$ of the specified chemical marker compositions by using the amount of herb from each herb class as predicted by the QA model. In addition to meeting the product specifications, the model can be used to minimize the product cost if $x < n$; i.e., the number of chemical markers (x) is less than the number of quality classes (n). As illustrated in example 2, the cost of herb of the most expensive solution was more than 13 times of that of the cheapest solution. If $x > n$, the extraction conditions can be relaxed to introduce additional variables for satisfying the quality constraints. In example 1, it was illustrated that the relaxation of extraction time could lead to a solution for an originally overspecified system.

This QA procedure for single herb extraction can be extended in different directions. First, many CHM products involve formulas with multiple herbs. It is well-known that the compounds from the different herbs in the extraction solution interact with one another and affect the overall extraction behavior. These additional physicochemical phenomena can be captured in an expanded multiple-herb QA model. Second, it has been proposed that the use of

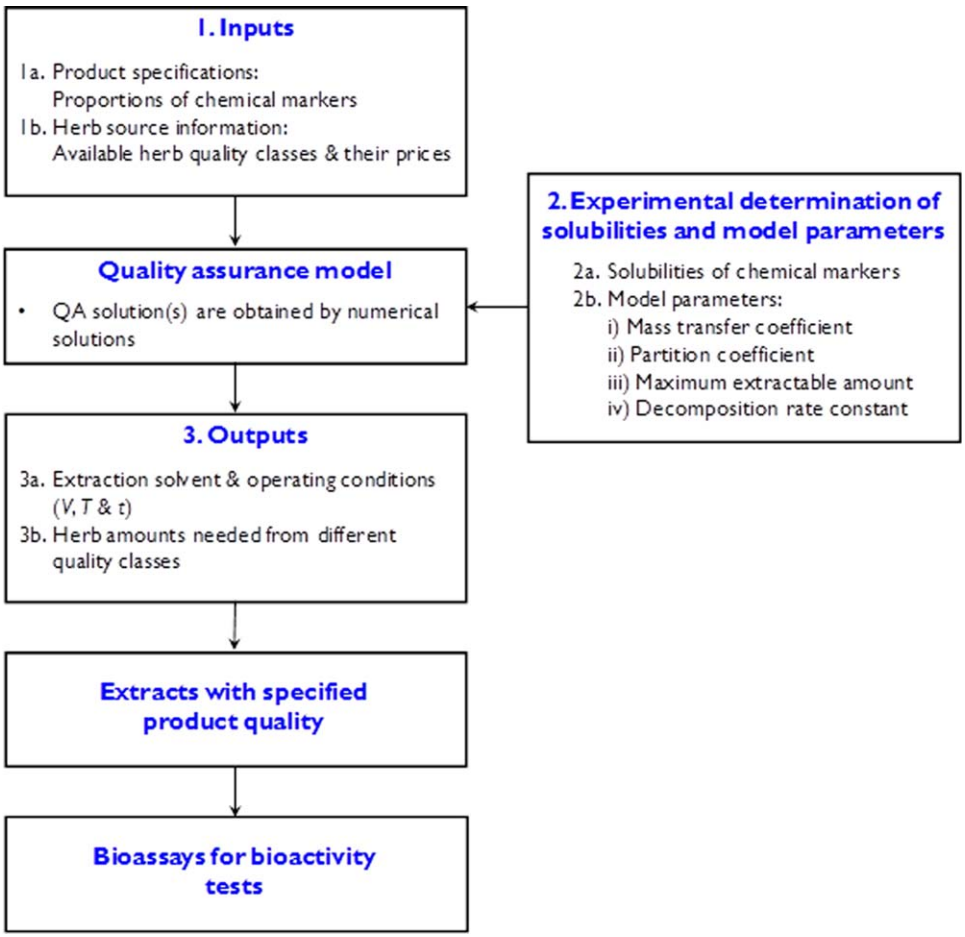


Figure 12. The quality assurance (QA) procedure for producing Chinese herbal medicinal (CHM) products with consistent quality.

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

chromatographic fingerprints of the product extract can better predict the biological responses.[25] Since the current QA model can be used for any number of chemical markers, it would be interesting to carry out a study by using a sufficiently large number of chemical markers to represent the fingerprint, and compare the approaches. A fingerprint can be exactly captured if infinitely many chemical markers are used. However, conceptually, a limited number of chemical markers strategically selected should be sufficient to reproduce the chromatographic profile. Third, this QA model based on heat-and-reflux extraction can be extended to account for advanced extraction methods with enhanced performance, such as ultrasound-assisted extraction,[26] and microwave-assisted extraction.[27] Fourth, the quality error of each chemical marker's concentration was kept within $\pm 10\%$ of the desired value in this project. In future, this error may be further reduced by the readjustment of the model parameter values using the extract quality obtained experimentally under the actual extraction conditions. Efforts in these directions are underway.

Acknowledgments

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Notation

C_i = concentration of chemical marker i in extraction solvent, mg/mL
C_i^{∞} = 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
$C_{i,j}^{s}$ = concentration of chemical marker i in extraction solvent at interface of 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 for herb of quality class j, 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
S_j = specific surface area of herb 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
$\Omega_{i,j}$ = amount of chemical marker i in herb of quality class j, mg i/g herb of quality class j
$\Phi_{i,j}$ = maximum extractable amount of chemical marker i for herb of quality class j, mg i/g herb of quality class j
$\Phi^0_{i,j}$ = constant representing maximum extractable amount of chemical marker i for herb of quality class j, mg i/g herb of quality class j
$\boldsymbol{A}_i$, $\boldsymbol{g}_i$ = matrix and column vector of model parameters of chemical marker i
$\boldsymbol{D}$,$\boldsymbol{b}$ = matrix and column vector of model parameters
$\boldsymbol{c}$ = column vector of concentrations of chemical markers in decoction
$\mathbf{c}^{QA}$ = column vector of concentrations of chemical markers in decoction specified in product specifications
c_i = column vector of concentrations of chemical marker i in decoction from different quality classes of herb
$\mathbf{c}^0_i$ = column vector of initial concentrations of chemical marker i in decoction from different quality classes of herb
$\varepsilon^{(h)}_i, \lambda_{i,h}, p_h$ = eigenvector and its corresponding eigenvalues of chemical marker i and related constant of quality class h
$\boldsymbol{m}$ = column vector of herb masses of quality classes used

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Appendix A: Assumption of Negligible Mass Transfer Resistance Inside an Herb Particle

Both Danshen and Gegen are radix herbs with highly porous matrix structure. It is assumed that the mass-transfer rate inside the herb particle is fast compared with the external rate. Figure A1 shows the extraction profile of SAB from

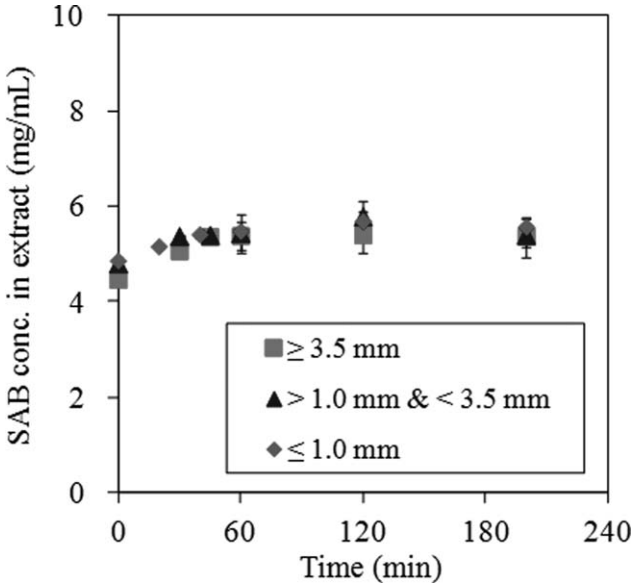

Figure A1. Extraction profile of salvianolic acid B (SAB) at 60°C in 100 mL of 50% ethanol-water solution from 7 g of 1M quality class of Danshen with different particle sizes.

7 g of 1M quality class Danshen with different particle sizes. Before extraction, all the herb particles were macerated in 50 mL of water at room temperature (25°C) for 60 min. After that, 50 mL of ethanol was added to the solvent and the temperature was increased to 60°C to start the extraction. It can be seen that the extraction performance was independent of the herb particle size, providing evidence to support this assumption.

Appendix B: Partition of Chemical Marker Between Herb and Solvent

This assumption was tested with an experiment with two extraction steps. Only one quality class of Danshen or Gegen was used in each experiment. At the beginning, the amount of chemical marker i in the herb was equal to Ω_i^0. A portion of this chemical marker was removed from the herb with short extraction time in the first extraction. According to Eq. 4, we have

$$\Omega_i^1 = \Omega_i^0 - \frac{V}{M} C_i^1 \tag{B1}$$

where Ω_i^1 is the amount of chemical marker i remained in herb, and C_i^1 is the concentration of chemical marker i in the extract. Then, the herb was recovered and was extracted with fresh solvent in the second extraction. The system was given a sufficiently long extraction time to reach equilibrium. Again, we have by materials balance

$$\Omega_i^\infty = \Omega_i^1 - \frac{V}{M} C_i^\infty \tag{B2}$$

where Ω_i^∞ is the amount of chemical marker i remained in the herb, and C_i^∞ is the equilibrium concentration of chemical marker i in the extract after the second extraction.

According to the definition of partition coefficient (Eq. 2)

$$C_i^\infty = K_i \rho \Omega_i^\infty \tag{B3}$$

Different C_i^∞ and Ω_i^∞ pairs were obtained by removing a different amount of chemical marker i from the herb in the first extraction. In first extraction, 3 g of Danshen (for DS) or 7 g of Gegen (for DN or DeN) was extracted in 100 mL of water. Then, the extract was removed and 100 mL of fresh water was added to the remaining herbs for the second extraction again at 100°C until equilibrium. Plots of C_i^∞ against Ω_i^∞ are shown in Figure B1. In general, the data show good linearity, and, thus, the validity of Eq. B3. The value of $K_i\rho$ is different for different chemical markers and different quality classes of the same herb type. The latter implies that cell wall may vary due to the growth condition, development stage,[28] and time of storage.

Appendix C: Assumption of Negligible Decomposition of Chemical Marker in Maceration

In this project, the maceration was performed at room temperature (25°C) for a relatively short time (60 min). It is assumed that there is no significant decomposition of the

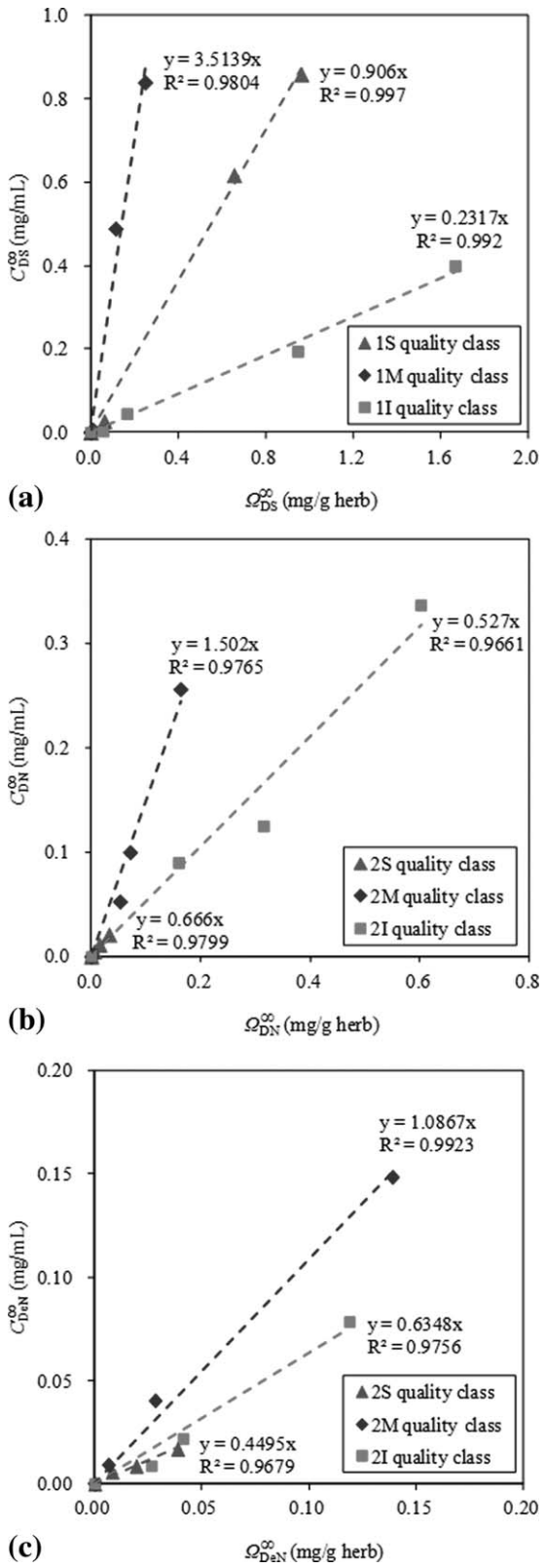

Figure B1. Plots of (a) C_{DS}^∞ against Ω_{DS}^∞ of danshensu (DS) extracted from 3 g of different quality classes of Danshen, (b) C_{DN}^∞ against Ω_{DN}^∞ of daidzin (DN), and (c) C_{DeN}^∞ against Ω_{DeN}^∞ of daidzein (DeN) extracted from 7 g of different quality classes of Gegen at 100°C in 100 mL of water.

chemical marker i in maceration. In order to verify this assumption, 67.5 mg of pure standard of SAB was dissolved in 100 mL of water and the solution was kept at 25°C. The amount of SAB in the solution in 60 min after it was dissolved was determined using HPLC analysis as mentioned previously. According to the results, the amount of SAB was equal to 66.4 mg in 100 mL of water, which was 98.3% of the original amount. This results provided evidence that there was no significant decomposition of SAB under maceration conditions.

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