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Pitfalls of the Selection of Chemical Markers for the Quality Control of Medicinal Herbs

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ABSTRACT

Selection of chemical marker is critical to the quality control and standardization of medicinal herbs. The ideal chemical marker is the active principle with demonstrated clinical efficacy contributing to the therapeutic effect of the herb, while the worst ones are the analytical marker and "phantom" marker whose pharmacological actions are unknown, uncontrollable or unpredictable. In this article, we explore the seven categories of chemical markers, discuss the pitfalls of choosing them, and illustrate the problems using a traditional Chinese medicinal herb *Ligusticum chuanxiong* as an example.

Key words: medicinal herb, quality control, chemical marker, *Ligusticum chuanxiong*

INTRODUCTION

Quality control is a fundamental procedure in the standardization of medicinal herbs and herb-based proprietary products for pharmacological evaluation and therapeutic use. As the biological actions of any herbal materials are solely and directly contributed by their chemical compositions, it is logical that quality control of medicinal herbs is most commonly accomplished by analyzing their chemical profiles. To achieve this goal, the employment of suitable chemical markers is of paramount importance; nevertheless, this is not a straightforward issue and is susceptible to various pitfalls. In this minireview, the most currently utilized chemical markers for the quality control of medicinal herbs, in particular herbs used in traditional Chinese medicine (TCM) practice, are discussed. The chemical markers described in this article are focused on the quality control of the parent herb. The chemical markers for the quality control of TCM herb-based compound formula (Fu Fang in Chinese) are more complicated and not discussed in this article. However, since the chemical markers in compound herbal formula are selected rationally as a combination of various chemical markers from each individual herb, issues reviewed in this article also provide considerable value for the quality control of herbal compound formula. The advantages and limitations of usefulness of these chemical markers for quality control are also discussed. Moreover, using our recent studies on a TCM herb *Ligusticum chuanxiong* as an example, problems in using an inappropriate chemical marker for quality control are illustrated.

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CHARACTERIZATION OF CHEMICAL MARKERS

In a recent minireview article Srinivasan[(1)] has classified chemical markers of herbal drugs into four categories: active principle, active marker, analytical marker and negative marker. In this article, with a modification of Srinivasan's four categories and on the basis of the current and common usage of markers for the quality control of medicinal herbs worldwide, in particular TCM herbs, we classified chemical markers for the quality control of medicinal herbs into seven categories. Although not all seven markers are documented in China Pharmacopoeia[(2)] and/or other authoritative documentations, they are all routinely used for the quality control of herbal materials in manufacture and pharmacological research.

The seven categories of chemical markers are shown in Figure 1. (1) Active principle: the chemical constituent has known clinical activity that contributes to the efficacy of the parent herb. For example, terpene trilactones (i.e., ginkgolides A, B, C, J and bilobalide), flavonoids and proanthocyanidins are the active principles of *Ginkgo biloba* leaves contributing to their therapeutic cardiovascular actions[(3)]. The active principle is the ideal chemical marker for the quality control of reproducible clinical efficacies of the herb and its extract. (2) Active marker: the chemical constituent has known pharmacological actions that may or may not contribute to the clinical effects of the parent herb. The pharmacological activities of this category of markers are mainly demonstrated by *in vitro* and/or *in vivo* studies using either isolated pure compounds or mixed herbal extracts containing the constituents of interest; however, their true values

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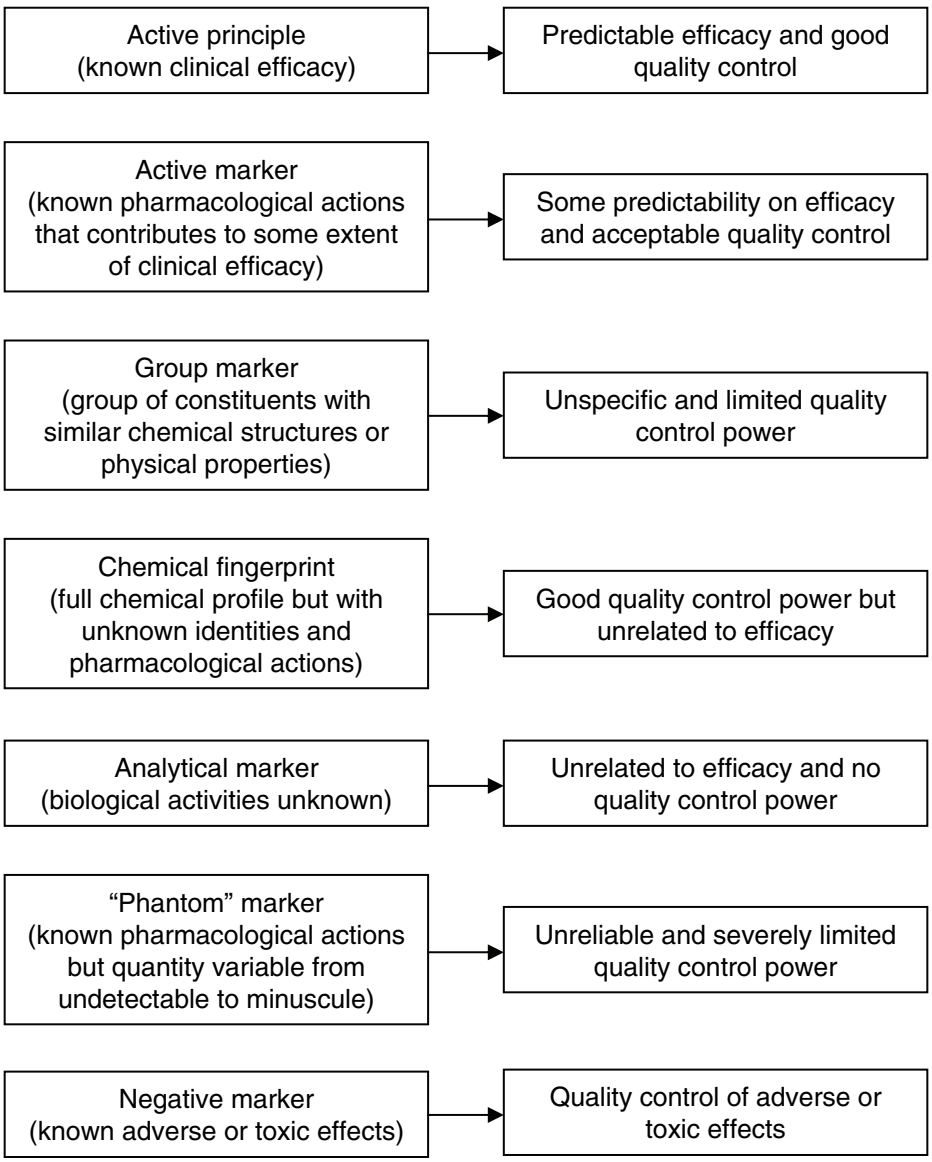

Figure 1. Classification of chemical markers for quality control of medicinal herbs.

contributing to the overall effects of the herb in the body remain unclear. The use of ferulic acid for the quality control of TCM herbs *Angelica sinensis* and *Ligusticum chuanxiong* is a typical example under this category[4-6]. (3) Group marker: group of constituents sharing similar chemical structures and/or physical properties. Some constituents in the group are of known pharmacological actions that may contribute to the clinical effects of the parent herb, while the activities of other constituents in the group remain unknown. The quality control using this category of marker is based on the total contents of the group. For instance, the total amount of polysaccharides, instead of the amount of individual polysaccharide with known activities, is employed as the quantitative tool for Ganoderma deriving from *Ganoderma lucidum* or *G. japonicum*[7]. This category of markers may not reflect the true clinical outcomes of the parent herb. (4) Chemical fingerprint: the spectroscopic pattern (e.g., UV spectrum) or chromatographic profile (e.g., HPLC chromatogram) of the herb. The chemical fingerprint represents a group of constituents with both known and unknown identities in the parent herb. It is often used to compare the similarity of chemical profiles between the herb of interest and the reference herbal material[8]. It has good qualitative control power but with no quantitative values. For example, a chemical fingerprint of three identified and 16 unidentified peaks was established for Flos Carthami (the dried flower of *Carthamus tintorius*) collected from Fenqiu, Henan province, China, where has been traditionally recognized as the indigenous growing place for this herb. This chemical fingerprint has subsequently used as a reference for comparing and qualita-

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tively assessing Flos Carthami obtained from other sources[9]. (5) Analytical marker: the constituent is not known for any biological activities but are simply present in the parent herb. Constituents that either present in a relatively high content in the herb or being easily measured using common analytical methods are normally employed as analytical markers. For example, curculigoside is usually used as a chemical marker for *Curculigo orchioides* on the merit that it is one of the main components, although biological activity reports are scarce[10,11]. (6) "Phantom" marker: the constituent has known pharmacological actions but its quantity in the parent herb is extremely variable to the extent that it may not be detectable. The usage of tetramethylpyrazine as a chemical marker for the TCM herb *Ligusticum chuanxiong* is a good example for this category and is described in details below. (7) Negative marker: the constituent has known adverse or toxic effects. It provides valuable safety prediction on the quality control of the parent herb. One typical example is ginkgo acids, which although constitute relatively low amounts in *Ginkgo biloba* leaves, they are regarded as the major cause for allergic effects of the herb and thus their total contents in *Ginkgo biloba* extracts used for medical purpose must be controlled to be less than 1 ppm[12].

PITFALLS OF CHEMICAL MARKER SELECTION

With established clinical actions that correlate with the therapeutic effects of the parent herb, the active principle serves as the ideal chemical marker for the quality control of medicinal herbs. Nevertheless, the active principles for the majority of medicinal herbs are rarely elucidated, and in most situations the use of active marker is also considered appropriate. Although their clinical effects are yet to be proven, their well-established pharmacological activities may contribute to the therapeutic efficacy of the parent herb to some extents, and thereby make them suitable chemical markers.

Unfortunately in the case of the quality control of TCM herbs, at present the industry and academic usually do not employ the active principles or the active markers, probably due to limited availability, but commonly use analytical markers and/or group markers. However, analytical markers provide no information on the biological effects of the parent herb, which are the primary concerns in the first place. Hence, even if the analytical markers were standardized, the clinical efficacy of the medicinal herb in question would still be impossible to predict. Analytical markers are therefore unacceptable for quality control proposes.

The China Pharmacopoeia has advocated the use of group markers as quality control tools for TCM herbs[2]; nevertheless, the appropriateness of this practice is doubtful. As mentioned above, group markers are not single entities but a group of compounds sharing similar chemical structures and/or physical properties (e.g., alkaloids), and quantification of a particular group marker ignores the relative composition of each of its component. This may be problematic, since constituents sharing similar chemical structures or physical properties are unlikely to elicit identical biological activity, and without knowing the relative composition of each constituent will handicap the prediction of the overall pharmacological effects of the group and thus of the herb. Moreover, the utilization of group markers leaves rooms for adulteration as one can deliberately add any group-member chemical into the herb of interest in order to achieve a required certain amount of the group marker.

Recently, chemical fingerprint becomes increasing popular and is widely accepted method for the quality control of medicinal herbs[13]. Although chemical fingerprint represents a whole chemical profile of the herb in question, currently in most cases, the identities of a large number of constituents in the profile are unknown. Thus this marker is commonly used only for qualitative comparison of chemical patterns between the herb of interest and authenticated reference herbal material. The correlation between the chemical patterns and clinical efficacy of the herb interested is still to be investigated.

Negative markers provide strong power on the control of the adverse and/or toxic effects of the herbal materials. However, the utilization of this marker alone is inadequate due to its lack of prediction of the efficacy of the herb. On the other hand, the use of "phantom" marker may bring serious consequences because this marker is unreliable and may be absent in the herb of interest due to the variable nature of herbal plants. The problem of using "phantom" marker is further discussed below using our research experiences in TCM studies.

RHIZOMA CHUANXIONG AS AN EXAMPLE OF INAPPROPRIATE SELECTION OF CHEMICAL MARKER

Our recent studies on a TCM herb Rhizoma Chuanxiong, which is derived from the rhizome of *Ligusticum chuanxiong* Hort. (Family Umbelliferae), provided a typical example with problems in the selection of chemical marker for the quality control of TCM herbs. Rhizoma Chuanxiong is a widely prescribed medicinal herb for treating cardiovascular diseases in China[2]. More than 100 chemical constituents including alkaloids, organic acids and phthalides have been identified from *Ligusticum chuanxiong* obtained from different places worldwide[4,5,14,23-28]. Among these chemicals, the alkaloid tetramethylpyrazine (also known as ligustrazine and chuanxiongzine, Figure 2) has received the most attention[14]. While it is demonstrated to exhibit some cardiovascular effects[15], its usage as a quality control chemical marker for *Ligusticum chuanxiong* is challengeable because not only other chemical ingredients,

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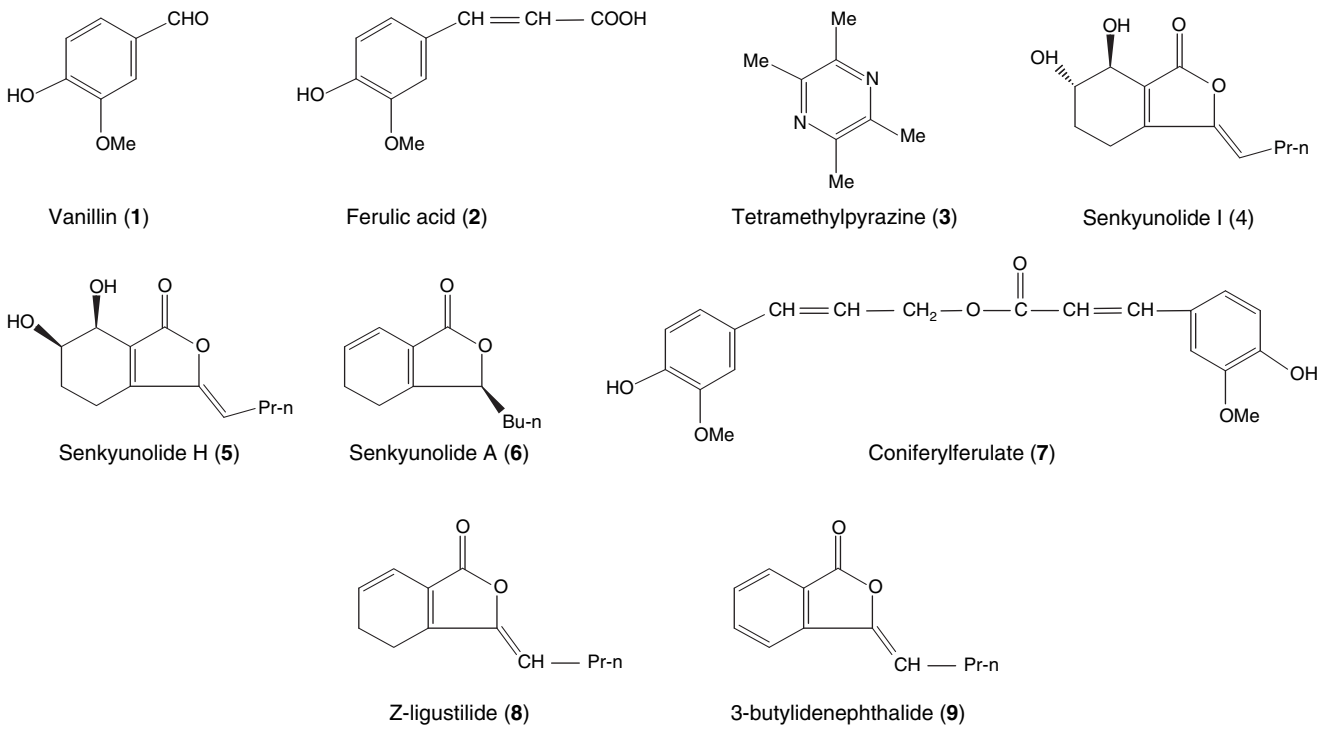

Figure 2. Chemical structures of some compounds isolated from Rhizoma Chuanxiong. **1.** vanillin; **2.** ferulic acid; **3.** tetramethylpyrazine; **4.** senkyunolide I; **5.** senkyunolide H; **6.** senkyunolide A; **7.** coniferyl ferulate; **8.** Z-ligustilide; **9.** butylidenephthalide.

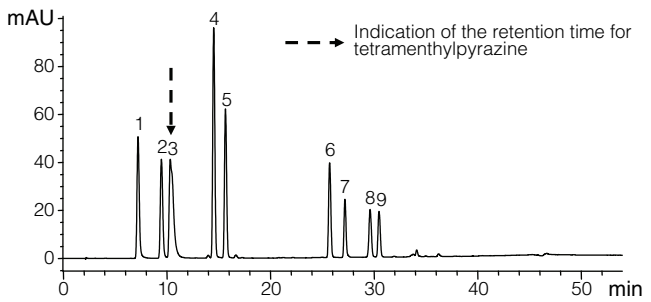

Figure 3. HPLC-UV chromatogram of nine reference compounds of *Ligusticum chuanxiong*. **1.** vanillin; **2.** ferulic acid; **3.** tetramethylpyrazine; **4.** senkyunolide I; **5.** senkyunolide H; **6.** senkyunolide A; **7.** coniferyl ferulate; **8.** Z-ligustilide; **9.** butylidenephthalide.

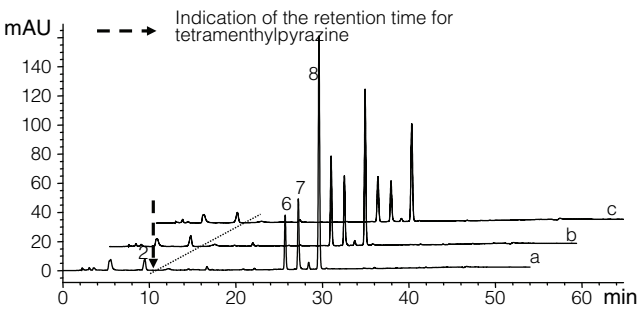

Figure 4. HPLC-UV chromatograms of fresh samples of three GAP cultivars of *Ligusticum chuanxiong* collected on May 9, 2003. **a.** cultivar I; **b.** cultivar II; **c.** cultivar III.

especially phthalide derivatives (Figure 2), have been proven to contribute most of the cardiovascular effects of the herb[16-22] but also mounting discoveries reported negligible quantity or absence of tetramethylpyrazine in numerous *Ligusticum chuanxiong* samples[4,23-28].

The strongest argument against the case of tetramethylpyrazine is its minute and variable quantity found in Rhizoma Chuanxiong. Concurring to reports by other investigators[23], using on-line HPLC-UV-MS method our research group was unable to detect any tetramethylpyrazine in more than 100 fresh, dried and processed herbal samples collected from several Good Agriculture Practice (GAP) cultivating bases in Sichuan province, China[24-28]. The representative HPLC-UV chromatograms of reference constituents, which have been reported to be isolated from Rhizoma Chuanxiong, and several fresh, dried and processed herbal samples are illustrated in Figures 3-8. It was found that the three major components, namely senkyunolide A (**6**), ligustilide (**8**) and coniferylferulate (**7**), and ferulic acid (**2**) (Figure 2) as a minor component were identified in all fresh samples of three cultivars (Figure 4), five different individual samples from the same cultivar (Figure 5), and samples of the same cultivar collected at different times (Figure 6). These four constituents were also found in all dried

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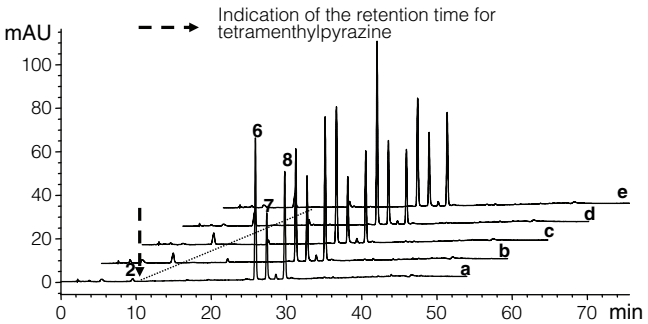

Figure 5. HPLC-UV chromatograms of five fresh plant samples of *Ligusticum chuanxiong* (cultivar I) collected on May 9, 2003.

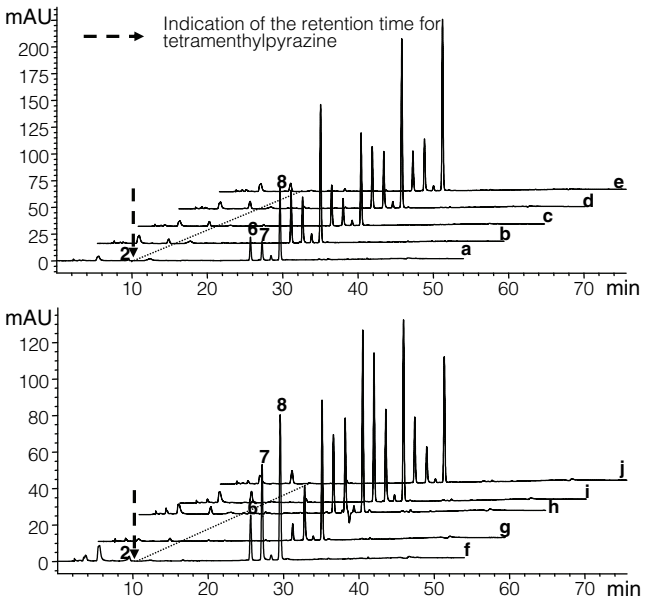

Figure 6. HPLC-UV chromatograms of fresh samples of *Ligusticum chuanxiong* (cultivar I) collected at different times. **a.** Oct 8, 2002; **b.** Oct 30, 2002; **c.** Dec 2, 2002; **d.** Jan 2, 2003; **e.** Feb 11, 2003; **f.** Mar 9, 2003; **g.** Apr 11, 2003; **h.** Apr 24, 2003; **i.** May 2, 2003; **j.** May 9, 2003.

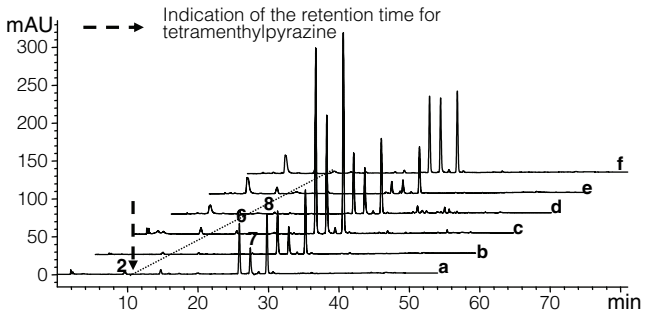

Figure 7. HPLC-UV chromatograms of *Ligusticum chuanxiong* (cultivar I) samples dried by different methods. **a.** oven (60°C) dried whole rhizome; **b.** oven (60°C) dried rhizome slice; **c.** sun dried whole rhizome; **d.** sun dried rhizome slice; **e.** sun dried whole root; **f.** freeze dried rhizome slice. Detailed information on drying procedures is reported in reference 28.

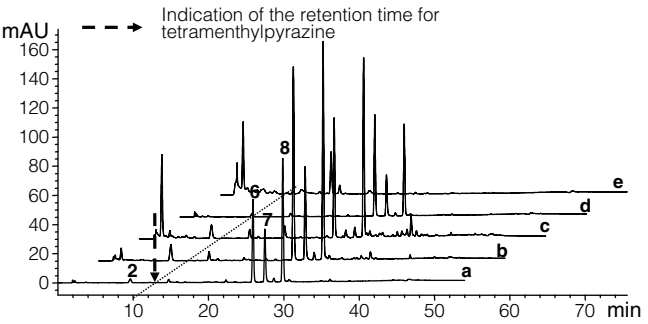

Figure 8. HPLC-UV chromatograms of *Ligusticum chuanxiong* (cultivar I) processed by different methods. **a.** oven (60°C) dried rhizome slice; **b.** roasted slice; **c.** bran-sautéed slice; **d.** wine-pretreated roasted slice; **e.** water extract pellet. Detailed information on processing procedures is reported in reference 28.

herbal samples using different drying methods (Figure 7) and processed samples by different processing procedures (Figure 8). However, no tetramethylpyrazine was detected in all the fresh, dried and processed samples tested. It is now generally accepted that *Ligusticum chuanxiong* either does not contain or has less than 1 μg/g (i.e., < 0.0001%) of tetramethylpyrazine[23,29,30]. Because the presence of tetramethylpyrazine in the herb remains debatable, its biological effects are very improbable to represent to those of Rhizoma Chuanxiong. Subsequently, this "phantom" marker is definitely not suitable for the quality control of Rhizoma Chuanxiong herb.

CONCLUDING REMARKS

Proper selection of chemical marker is essential for the quality control of medicinal herbs. Certainly the active principle is the ideal chemical marker for the quality control of the clinical efficacy of medicinal herbs. However, because of limited availability and information on this category of chemical markers, others in particular a combination of more than one category, such as active marker combined with chemical fingerprint, may also be reasonably considered. A general awareness is much needed to avoid pitfalls leading to inappropriate choices. Furthermore, it is advisable that the name and type of chemical marker selected for the quality control of herbal materials shall be disclosed in all herbal products such that the general public can evaluate the adequacy of the standardization method used.

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