Journal of Chromatography A, 1218 (2011) 5618–5627

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Ruggedness and robustness of conversion factors in method of simultaneous determination of multi-components with single reference standard

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ARTICLE INFO

Article history:
Received 30 March 2011
Received in revised form 13 June 2011
Accepted 14 June 2011
Available online 22 June 2011

Keywords:
Robustness
Conversion factor
Salvia miltiorrhiza
Botanical
Single standard to determine multi-components (SSDMC)
HPLC

ABSTRACT

Single standard to determine multi-components (SSDMC) is a novel and rational method for quality control of botanical products and traditional Chinese medicines (TCMs). However, it is restricted to wide application due to unknown fluctuation in conversion factors when it is performed in different laboratories. To evaluate the fluctuations of conversion factors, we selected *Salvia miltiorrhiza* as an example to determine three components of tanshinones by SSDMC method. Then ruggedness and robustness test were adopted to comprehensively investigate three kinds of factors that may influence stability of conversion factors, which were related with environmental parametric variables, operational parametric variables and peak measurement parametric variables. Nested-factorial-design was used to perform ruggedness tests. One-variable-at-a-time (OVAT) procedure and Plackett–Burman (PB) design were both used in robustness test. The results showed that stability of conversion factors was principally related with accuracy of wavelength of UV detector, peak measurement parameters and concentration of standard solution. The acceptable range of conversion factors was obtained from robustness test. Our results showed that conversion factors were inevitable to change, but when key parameters were well controlled, the range of its fluctuation was acceptable and the SSDMC method could be used widely in different laboratories.

1. Introduction

Traditional Chinese medicines (TCMs) have been widely used to treat various diseases for thousands of years in China. Recently, an increasing appreciation of the health-promoting benefits of herbal preparations has been observed in the United States [1]. To control the quality of the complex botanical products and traditional Chinese medicines (TCMs), determination of multi-components was considered to be one of the key methods by Chinese Pharmacopoeia and United States Pharmacopoeia. During the revision of Chinese Pharmacopeia 2005 edition (Ch.P. 2005), Chinese Pharmacopoeia Commission directed that analytical method and testing items of monograph being revised should embody the idea of comprehensive quality control of TCMs, which multi-components or fingerprint should be analyzed rather than single marker compound [2]. For the moment, there were two main methods for the quantitative determination of multi-components in herbal products. The first method is to use multiple reference standards for the analysis of multiple components. For instance, in monographs of Ch.P. 2010 edition, three reference standards (aconitine, hypaconitine and mesaconitine) were used to determine three alkaloids in *Aconitum carmichaelii* Debx (Chuanwu) [3], and four reference standards (polyphyllin I–IV) were used to determine four components in *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand-Mazz. (Chonglou) [4]. In comparison, the second method only requires a single reference standard to simultaneously determine the contents of multi-components, which could be abbreviated as SSDMC (single standard to determine multi-components.) method. In the second method, the content of each component could be obtained directly or calculated by multiple conversion factors. Due to the difficulties and expenses to prepare bulk of all reference standards, the application of the first method was limited, and it would be especially difficult to determine more than five components. SSDMC method only needs the minimum number of reference standard with low cost. And it enables to determine more than ten components simultaneously. Thus, the use of SSDMC method was ideally explored.

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doi:10.1016/j.chroma.2011.06.058

Conversion factors of SSDMC method could be divided into two types based on UV detection. The first type is that the values of conversion factors are considered as the value of 1, which could only be used when the molar absorptivity and molecular weights of all analytes possess high similarities. Such as in USP 33, the quality of Cat's Claw was controlled by this method and isopteropodine is used as single reference standard to directly determine the other five components [5]. The reason was that six components had the same structure belonging to pentacyclic indole alkaloids with the same molecular weight. The only difference was the configuration of those six components. Another example was that 15 anthocyanins in Bilberry Extract were determined because they had similar chromospheres structure [6]. However, the use of this method was limited since results obtained were just an approximation, which may deviate from the true value. The second type, which conversion factors of all analytes are different, could avoid this deviation. Molar absorptivity (ε) of analytes with different chromophores is generally different at the same wavelength. (Such as the log(ε) of tanshinone II_A, cryptotanshinone and tanshinone I are 4.47, 4.39 and 4.30 respectively at 270 nm.) Therefore the content of other components should be calibrated when they are determined by single reference standards. The conversion factors were just used to calibrate the contents, which were also called as relative response factors in some literatures. It could be defined as response ratio of reference standard and analyte at the same unit mass concentration. Such as in USP 33, the total phenols in *Echinacea pallida* (Nutt.) Nutt. were determined by conversion factors. The chlorogenic acid was used as one reference standard, and the contents of other three phenol acids (caftaric acid, chicoric acid and echinacoside) were calibrated by 0.881, 0.695 and 2.220, respectively [7]. Tu et al. analyzed seven anthraquinones in rhubarb rhizome by conversion factors. The emodin was selected as single reference standard, and maximum conversion factor was aloe-emodin that the value was 1:0.0759 [8]. The results clearly showed that it was absolutely necessary to calibrate the content by conversion factors.

As mentioned above, quality control of botanical products (herb drugs or TCMs) by multi-component quantification has reached a consensus. SSDMC with conversion factors has been applied in 20 of 108 monographs of herbal dietary supplements in USP 33 (such as red clover, *Echinacea angustifolia*, St. John's Wort., etc.). In European Pharmacopoeia 7.0 (EP7.0), 6 herbal drugs were assayed by this method (such as Ginkgo dry extract and Purple coneflower herb and its root) in 232 herbal drugs. While in the latest version of Ch.P. 2010 edition (volume I), only Coptides Rhizoma (Huanglian) was controlled by SSDMC method in all 593 Chinese crude drugs recorded, in which conversion factor was not used. The berberine hydrochloride was selected as single reference standard, and the contents of four alkaloids (epiberberine, coptisine, palmatine and berberine) were determined respectively without calibration by conversion factors [9]. The above facts showed that SSDMC method with conversion factors was not widely used in Ch.P. 2010 and EP7.0. One of the most important reasons was that the potential fluctuation in conversion factor in different laboratories was not fully taken into consideration [8]. Thus SSDMC method was heavily restricted by the ruggedness and robustness of conversion factors. And the factors which would influence the fluctuation of conversion factors have not been reported up to now. Therefore it should be investigated systematically.

In this study, to investigate the ruggedness and robustness of conversion factors, we selected Salvia Miltiorrhizae Radix et Rhizoma (Danshen) as an example. Known from our previous works in fingerprint of Danshen [10], the quality of lipophilic part in Danshen can be controlled by determining three main components – tanshinone I, cryptotanshinone and tanshinone II_A. A number of methods on the simultaneous determination of the three components had been reported [11–13]. There have no reports regarding the simultaneous determination of three tanshinones with SSDMC method as a compendia procedure. Therefore analytical procedure adopting a SSDMC method with conversion factors was validated initially. The three tanshinones (tanshinone II_A, tanshinone I and cryptotanshinone) of Danshen were simultaneously determined by tanshinone II_A as single reference standard, which is easy to obtain and abundant in the material, on high-performance liquid chromatography with diode-array detector (HPLC-DAD). And then all factors which might have influence on the conversion factors were studied comprehensively. (1) Whether it was related with environmental parameters, such as different days, analysts, instruments, and columns. If this was the case, what were the reasons? (2) Whether it was related with operational parameters, such as different acid concentration in mobile phase, ratio of components in mobile phase, wavelength of UV detector, column length, injection volume, column temperature and concentration of reference standard. (3) Whether it was related with peak measurement parameters which were rarely reported, such as different slit width, bandwidth and integration parameters. (4) What is the acceptable range of conversion factors? (5) Finally, the values of conversion factors and the analytical procedure were verified and confirmed by Shanghai Institute for Food and Drug Control (SIFDC) of China which has Laboratory Accreditation Certificate. These results provided a firm foundation for SSDMC method with conversion factors to use as compendia procedures.

2. Experimental

2.1. Chemicals and materials

Tanshinone II_A (A), cryptotanshinone (C) and tanshinone I (I) (Fig. 1) were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of tanshinone I was determined to be 97% by peak area normalization method on HPLC, and purities of the other two compounds were more than 98%. Acetonitrile for HPLC was obtained from Honeywell (UV, NJ, USA). Methanol for analytical grade was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Phosphoric acid for HPLC was obtained from Tedia (USA). High purity deionised water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

Salvia Miltiorrhizae Radix et Rhizoma (Danshen) were collected from Shandong Province of China, and identified by one of the coauthors (Dr. De-An Guo). Voucher specimens were deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.2. Apparatus

Analyses were primarily performed by an Agilent 1100 HPLC System, comprised a quaternary solvent delivery system, an on-line degasser, an auto-sampler, a column temperature controller and a diode-array detector (DAD) coupled with an analytical workstation (Chemstation For LC 3D Systems A10.02) (Agilent Technologies, Palo Alto, CA, USA). Two additional different HPLC instruments were used. One is Agilent 1100 HPLC System comprised a variable wavelength detector (VWD) (Agilent Technologies, Palo Alto, CA, USA), another was a Waters 2996 HPLC System comprised a quaternary solvent delivery system, an on-line degasser, an auto-sampler, and photodiode array detector coupled with an analytical workstation (Empower 2 software) (Waters Corp, Milford, MA, USA). A BRANSON B3500S-DTH ultrasonic bath (140 W, 42 kHz) (BRANSON Ultrasonic, Shanghai, China) was used for sample preparation. Samples were primarily separated on a Zorbax Extend-C_{18} column (5 μm particles, 4.6 mm i.d. × 250 mm; Agilent) with a guard column (5 μm particles, 4.6 mm i.d. × 10 mm; Agilent).