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Review

Biopotency Assays: an Integrated Application to Quality Control of Chinese Materia Medica

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ABSTRACT

The current quality control (QC) pattern for Chinese materia medica (CMM) lacks suitable methods and indicators to evaluate their safety and efficacy effectively, which impedes the smooth development of CMM. In this review, main problems of the current QC pattern for CMM, principally focused on the content determination of constituents, were summarized and the inspiration from the QC of biological products was introduced. With the aim at introducing a suitable tool to the QC of CMM, biopotency assay and its feasibility in the QC pattern for CMM were analyzed and confirmed by relevant researches with years of practice. From the applications of biopotency assays in the QC of CMM in the last 10 years, we propose that biopotency assays should be an integral part of the QC pattern for CMM, for these assays can make the QC indicators related to the clinical safety and efficacy, supplementing the existed QC system of CMM.

Key words

biopotency assays; Chinese materia medica; efficacy; indicator; quality control

1. Introduction

Chinese materia medica (CMM), as an important part in the global medicincal market, has been used to prevent and treat diseases by a large portion of the population for more than 3000 years (Peppin et al, 2008; Zhang et al, 2007). During the last decades, CMM has become more and more popular around the world for its huge medicinal and economic values. However, due to the difficulty in the establishment of a feasible quality control (QC) pattern for CMM which can be used to evaluate the safety and efficacy effectively, the development of CMM is approaching a bottleneck.

In this article, a major topic on the current QC pattern for CMM is discussed. Here, we put forward the QC pattern and methods for CMM centered on biopotency assays with many years of experiences in this field, in order to supply and improve the existed QC system for CMM. We hope this pattern can provide a new research idea on the establishment of QC system for the development of world traditional medicines, especially for herbal medicines.

2. Limitations of existed QC pattern for CMM

At present, medicines in the world can be classified into three kinds as follows: CMM, chemical synthetic drug (CSD),

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and biological product (BP). Among them, CSD is the mainstream in the clinical use because of its quick efficacy directly acting on the target organ. While CMM, a system of ancient medical practice that quite differs in substance, methodology, and philosophy from modern medicines, owns its distinguishing features and plays a crucial role in the health maintenance for about 1.5 billion people worldwide (Cheung, 2011; Hosbach et al, 2003). However, also due to those special features such as systematism, holism, multi-target, and multi-channel resulted from complex chemical constituents, it is often hard to control the quality of CMM in an effective way (Jiang et al, 2010).

In the process of globalization and modernization of CMM, a key point is to keep the controllability and consistency of the quality of CMM (Jiang et al, 2010). Meanwhile, it is also indispensable to serve the clinic with the guidance of QC standards. But the current QC of CMM patterned on the QC method of CSDs, which is mainly focused on the content determination of single constituent or a limited number of constituents, can not actually meet those needs for its poor correlation with the efficacy, specificity, and representativeness. The reasons are given as follows.

2.1 Efficacy

The effective substances of most CMM are not clear. So in the establishment of QC standards for CMM, one or several constituents were chosen as indicators to analyze qualitatively and quantitatively, which was to detect the existence of these constituents and determine their contents. However, there was little direct and internal relevance between those constituents and the clinical efficacy as well as the safety. For example, in *Chinese Pharmacopoeia 2010* (Pharmacopoeia Committee of P. R. China, 2010), the QC indicator for *Cordyceps* which is a famous and precious CMM and more expensive than the gold is to determine the content of adenosine. But adenosine is not the active constituent in *Cordyceps*, which can be poorly related to the clinical efficacy.

2.2 Specificity

Some indicators of CMM may be the active ones, but they are nonexclusive which often widely exist in various kinds of herbal medicines. For instance, ginsenoside, the indicator of *Ginseng Radix* et *Rhizoma* (Pharmacopoeia Committee of P. R. China, 2010), also exists in *Notoginseng Radix* et *Rhizoma* and American ginseng. Even in different parts of the same medicinal plant, such indicator is still widely distributed. Another example is that ginseng, ginseng fibrous roots, and ginseng leaves all contain ginsenoside. More confusingly, the part of ginseng used as medicine in the history is its main root, with total ginsenosides of 4% in general with the current market price of 50 dollars per kilogram; Ginseng fibrous roots contain total ginsenosides of 10% with the price of 40 dollars per kilogram; Ginseng leaves contain total ginsenosides of 12% with the price of 10 dollars per kilogram in the market (Yu et al, 2002). We evaluate the quality of *Ginseng Radix* et *Rhizoma* according to the content of total ginsenosides, ginseng fibrous roots and ginseng leaves which are obviously more cost-effective than ginseng, and it is contrary to the clinical experiences and actual application for thousands of years.

2.3 Representativeness

Since there are complex and various kinds of constituents in CMM, especially in compound preparations, it is difficult to determine the authenticity and evaluate the quality of CMM just by detecting the existence or content of one or several constituents. Taking the famous Chinese patent drug, Liuwei Dihuang Pill, as an example, it is made up of six Chinese herbal medicines (prosecced *Rehmanniae Radix*, *Corni Fructus*, *Moutan Cortex*, *Dioscoreae Rhizoma*, *Alismatis Rhizoma*, and *Poria*) containing thousands of components. But the current QC standard for the drug is to determine the contents of loganin and paeonol (Pharmacopoeia Committee of P. R. China, 2010). Judged by the number of constituents, the representativeness of several constituents could be less than 1/1000; By the total amount of substances, the representativeness could be less than 1/10000. Obviously, the QC mode based on the qualitative and quantitative determination of single constituent can hardly evaluate the quality of CMM objectively and comprehensively.

Therefore, it is necessary to establish a feasible and effective QC pattern combined with clinical demands, especially driven by translational medicine and new development tendency of bioscience in the world (Milne and Kaitin, 2009; Sarkar, 2010). The development of QC pattern for CMM should not only guarantee the stability and consistency of drugs, but also be associated with the clinical efficacy and safety in order to serve the clinic.

3. Inspiration from QC pattern of BP

Observing the substances of CSD, BP, and CMM, and the effect of manufacturing techniques made on them, we can find that CMM are more similar to BP.

(1) By substances, CMM is more similar to BP compared with CSD. CSD often consists of single constituent, and is different from CMM and BP. CMM is composed of one or multiple classes of compounds, while BP is usually made up of polypeptides or proteins with molecular weights within limits.

(2) According to the effect of manufacturing techniques made on substances of the three kinds of drugs, CMM is also more similar to BP. For a CSD, the production methods and conditions may be various, but its substance is almost invariable. For example, although production techniques for vitamin C (VC) include Reichstein Procedure (Reichstein and Grüssner, 1934), one-step synthesis method (Anderson et al, 1985), two-step synthesis method (Hancock and Viola, 2002), and so on, the final constituent and structure of VC are hardly changed. While for BPs, their substances closely relate to the manufacturing process. When the production techniques and conditions change, the substances of a BP also change, as well

as its properties and efficacies. Like BP, the quality and substances of CMM are also influenced by production conditions such as varieties, producing areas, harvesting times, and processing technologies.

Nevertheless, the current QC pattern of CMM was mainly patterned on the CSD but not BP. For CSD, the content of constituents can be directly related to its efficacy and safety. For BP, biopotency assays are major QC methods while the content detection is generally used for the purity examination. For CMM, the morphological identification and content detection of constituents are used to control its quality, without the provision of biopotency assays. Therefore, such a QC pattern of CMM raises the question: Why not use the QC pattern of BP for reference when the two kinds of drugs are so similar? In fact, biopotency assays are more practically valuable and conducive to CMM to some degree compared with the content determination of chemical constituents.

In the development, registration, and control of biological and biotechnology-derived products, biopotency assays play a key role and have already been maturely applied in the establishment of QC standards for BP. Biopotency assays can reflect the bioactivity of drugs, and are consistent with the clinical efficacy to a large extent. Some physical and chemical analysis methods can just represent one aspect of physicochemical properties of drugs, which may not be corresponding to the efficacy (Figure 1). The introduction of biopotency assays to the QC pattern and system of CMM can not only assist the chemical analysis to identify the authenticity of CMM, but also evaluate its quality by efficacy researches. Biopotency assays, associated with the safety and efficacy, are superior in the QC of drugs with unknown and complex constituents which can not be detected with chemical analysis methods.

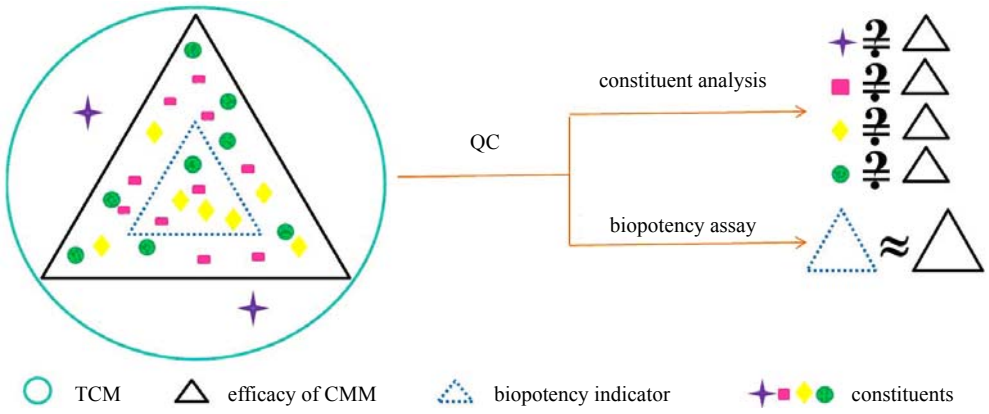

Figure 1 QC of CMM by constituent analysis and biopotency assay

4. Biopotency assay and application

Biopotency assay, as a quantitative and bioidentity test, plays a key role in the development, registration, and control of biological and biotechnology-derived products (Dai et al, 2013; Xiao et al, 2013). It detects the intensity of drug effect on living organisms, in which the response intensities of the drug and the control acting on living organism, organs, or tissues are compared to calculate the dose standard of the drug. Biopotency assays include tests *in vivo* and *in vitro*. Biopotency assay *in vivo* based on animals can reflect the mode of drug action in human body, which is the most classic method. In *Chinese Pharmacopoeia* (Pharmacopoeia Committee of P. R. China, 2010) and *United States Pharmacopoeia* (United States Pharmacopeial Convention, 2012), the potency of recombinant human glucagon and somatropin must be determined in the manufacture process. Biopotency assay *in vitro* can be used to evaluate the bioactivity of drugs on organs, tissues, microorganisms, enzymes, and cells with a series of detection methods (You et al, 2010; Pauly et al, 2009; Coombes et al, 2009). Each of the two assays has its own characteristics. The biospecificity and effect on living organisms of drugs can be better demonstrated *in vivo*. While the repeatability, reliability, and precision of biopotency assay *in vitro* are superior to that *in vivo*. Therefore, the selection of assay can be determined according to different purposes and subjects. The major selection principle is that the biological assay should be correlated to the clinical efficacy or modern pharmacological effects of the drug. What's more, the testing result can be repeatable and the index can sensitively reflect the dose change of the drug. A point should be noted that the repeatability and sensitivity of biopotency assay are evaluated from the angle of biostatistics but not the requirement in physicochemical test. Last but not least, a feasible biopotency assay is an efficient one easy to generalize. Besides cell-based assays, enzymatic reaction assays, and other routine potency detection methods, microcalorimetry based on biothermodynamics has also been developed to determine the drug potency during these years. It presents the energy transduction for the interaction between drug and organisms in real-time, on-line, and efficient way, which can be used as a quantitative and bioidentity test (Figure 2).

At present, biopotency assays are mainly applied to QC fields of biochemical drugs, BPs, and so on (Zhou et al, 2005). They are suitable for drugs with unclear active constituents, complex and varied structures, and the content or bioactivity of which can not be detected by physicochemical

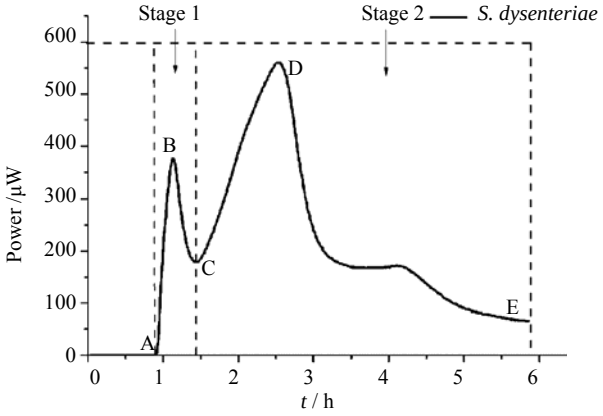

Figure 2 Heat flow power-time curve for *Shigella dysenteriae* at 37 °C

A−B: first exponential growth phase; B−C: transition phase; C−D: second exponential growth phase; D−E: decline phase

analysis (Janzen and Popa-Burke, 2009; Mire-Sluis, 2001; Sun et al, 2008; Thangam Sudha et al, 2007). For CMM, the quality should be reflected not only by the chemical constituents, but also by the efficacy and safety related to the materials. Since biopotency assay is available in different assay formats depending on the action mode of drugs, and can reliably predict safety and efficacy to some extent (DeStefano and Morris, 2011), the QC pattern of CMM may gain ground with biopotency assays as a part.

Now there have already been researches and reports about biopotency assays used in CMM. In *Chinese Pharmacopoeia 2010*, the quality and the toxicity of leech and digitalis are controlled by the use of biopotency assays (Pharmacopoeia Committee of P. R. China, 2010). Researches about the QC of other CMM or their products such as garlic oil (Zhang, 2005), *Leonuri Herba* (Yang and Wang, 2004), *Sparganii Rhizoma* (Chen et al, 2012), *Coicis Semen* (Li et al, 2011), Huangqin Decoction (Tilton et al, 2010), and Gegen Qinlian Decoction (Xu et al, 2013) with the application of biopotency assays have also been reported.

By this token, it is possible and feasible to establish a novel QC pattern for CMM which can not only guarantee the stability and controllability of the standards, but also be directly related to the safety and efficacy of medicines. In other words, the establishment of a QC pattern for CMM which is based on both chemical analysis and biopotency assays is beneficial to the development and modernization of CMM (Figure 3).

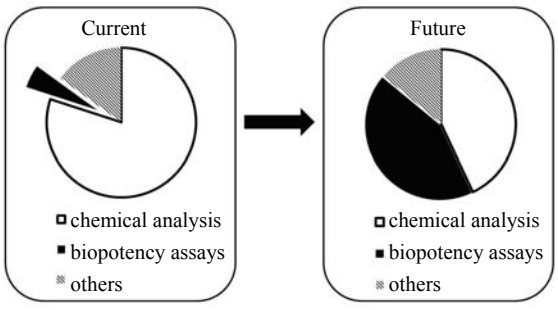

Figure 3 Development of QC pattern for CMM

5. Biopotency assay applied in QC of CMM

Our research group first proposed to establish a QC pattern for CMM with appropriate biological assays, in order to evaluate the quality and efficacy of CMM more objectively and flexibly. Meanwhile, we have devoted ourselves to the establishment of the mode since 1999 and applied it to CMM with unclear active constituents, CMM with toxic constituents, valuable CMM, CMM injections, and so on, obtaining satisfactory results.

5.1 CMM with unclear active constituents

There are numbers of CMM with favorable curative effects containing unclear active constituents, among which the quality is hard to control with routine physical and chemical analysis. So it is essential to carry out suitable researches of biopotency assays. Taking *Isatidis Radix* as an example, one of the QC indicator is to detect the existence of arginine (Pharmacopoeia Committee of P. R. China, 2010), which is neither the main active constituent nor the specific one only existed in *Isatidis Radix*. Therefore, the detection of arginine is almost meaningless to the QC of *Isatidis Radix*.

According to major efficacies and modern pharmacological actions (antiviral activity and bacteriostatic activity) of *Isatidis Radix* (Lin et al, 2005), we used biopotency assays to detect its antiviral and bacteriostatic activities (Li et al, 2009a; 2009b; 2008; Tang et al, 2010; Wei et al, 2008), and finally optimized the biopotency assay for *Isatidis Radix* based on the detection of hemagglutination activity. In this research, we established two antiviral activity (hemagglutination activity and influenza virus neuraminidase activity) detection methods for *Isatidis Radix*. After the contrastive analysis, we found that both methods with good repeatability could be used to evaluate the bioactivity of different *Isatidis Radix*. The relationship between the agglutination activity and antiviral effect of *Isatidis Radix* was further verified. And the agglutination detection method is superior for its safety, inexpensiveness, easiness, and practicality. This novel method could reflect the pharmacodynamic effect of anti-influenza

virus of *Isatidis Radix* and provide a reference for the QC of CMM with appropriate biopotency assays.

5.2 CMM with toxic constituents

After the toxicological cases of pyrrolizidine alkaloids and aristolochic acids (Xiao and Liu, 2004), global concern over the quality and safety of CMM has sky-rocketed and worldwide researches have begun to focus on the QC of CMM. Many Chinese herbal medicines are highly toxic and have a narrow margin of safety between therapeutic and toxic doses (Friese et al, 1997; Gutser et al, 1997), and their toxicity is often attributed to toxic constituents in them. For example, the hypertoxicity in the herbs of *Aconitum* L. is deemed to the existence of diester diterpenoid alkaloids (DDAs), such as aconitine, mesaconitine, and hypaconitine (Ameri, 1998; Chan, 2009; Singhuber et al, 2009). But the current QC method for the herbs of *Aconitum* L., which is the quantitative analysis of its three toxic DDAs by liquid chromatography (Chen et al, 2008; Pharmacopoeia Committee of P. R. China, 2010; Csupor et al, 2009; Jiang et al, 2005; Lu et al, 2009; Xie et al, 2005), can not meet the needs of the clinical practice, for many other constituents are the toxic ones besides the three DDAs in the herbs of *Aconitum* L. (Bisset, 1981; Dong et al, 1981). Moreover the toxicity of the three DDAs is different (aconitine > mesaconitine > hypaconitine), so samples with the same total DDAs content often have different total toxicity (Bisset, 1981; Dong et al, 1981). Thus only limiting the total content of the three DDAs is not enough to ensure the safety in the herbs of *Aconitum* L. in clinic. In order to investigate the toxicity in the herbs of *Aconitum* L., our research group established a biopotency assay to compare its total toxicity with the three DDAs (Qin et al, 2012). In this study, the minimum lethal doses (MLDs) of test samples and standard were determined, respectively. And the toxic potency was calculated by comparing the MLDs. Then the three major DDAs in the herbs of *Aconitum* L. were analyzed using an LC method, which was the current method to evaluate the toxicity in the herbs of *Aconitum* L. We found that the total toxicity of the extract in the herbs of *Aconitum* L. was greater than that of the three alkaloids. In other words, the content of the three DDAs failed to represent the total toxicity in the herbs of *Aconitum* L., and the biopotency assay could characterize their total toxicity by determining their toxic potencies (Table 1). This study may reveal that biopotency assays are the powerful methods for the safety assessment and QC for CMM with toxicity.

6. Valuable and rare CMM

Many valuable and rare species have been applied to CMM since thousands of years ago (Alves et al, 2008; 2010; Leal et al, 2005; Mishra et al, 2011), for their clinical effects have not only got the validation of the history, but also promoted the development of CMM. Nevertheless, it is often hard to evaluate the quality of these CMMs with conventional methods such as the chromatographic and spectrum analysis. For example, the existing QC for *Cordyceps* is to detect the content of adenosine (Pharmacopoeia Committee of P. R. China, 2010) which is neither the mainly active constituent nor the exclusive constituent. Moreover, valuable medicinal animal horns in the market are often sold in the form of powder, hard to be identified with routine morphological identification (Yan et al, 2010a). Accordingly, methods for determining the bioactivity of valuable and rare CMM would

Table 1 Results of UPLC and biopotency assay on herbs of *Aconitum* L. (Qin et al, 2012)

Samples	UPLC			Bioassay			TPT/TPA
	aconitine /(mg·g^{-1})	mesaconitine /(mg·g^{-1})	hypaconitine /(mg·g^{-1})	TPA /(U·g^{-1})	TPT /(U·g^{-1})	*FL* / %	
Crude Fuzi 1	0.0264	0.1840	0.3027	273.25	466.21	6.33	1.71
Crude Fuzi 2	0.0549	0.2116	0.2414	297.83	518.30	9.32	1.74
Crude Fuzi 3	0.0511	0.2448	0.3199	347.70	541.82	6.00	1.56
Baifupian 1	0.0006	0.0196	0.0202	22.23	30.49	8.68	1.37
Baifupian 2	0.0007	0.0070	0.0264	15.75	46.50	7.82	2.95
Baifupian 3	0.0021	0.0067	0.0241	16.13	26.74	5.91	1.66
Baifupian 4	−	0.0017	0.0004	1.38	10.69	7.22	7.75
Baifupian 5	0.0026	0.0208	0.0093	20.97	50.66	7.80	2.42
Baifupian 6	0.0111	0.0605	0.0187	61.26	56.10	7.06	0.92
Baifupian 7	−	0.0001	0.0112	4.38	23.80	9.81	5.43
Yanfuzi	0.0082	0.0259	0.0390	41.56	106.15	9.58	2.55
Heishunpian	0.0001	0.0017	0.0050	3.29	33.33	6.91	10.14
Shufupian	0.0011	0.0009	0.0072	4.54	16.01	8.12	3.53
Huangfupian	0.0274	0.0938	0.0823	125.52	149.48	7.49	1.19
Crude Caowu	0.0161	0.0749	0.0691	95.76	164.20	6.78	1.71
Zhicaowu	0.0458	0.0004	0.0054	48.24	108.52	8.26	2.25
Crude Chuanwu	0.0588	0.2193	0.2117	295.70	356.82	8.69	1.21
Zhichuanwu	0.0006	0.0055	0.0333	17.24	55.46	7.44	3.22

TPT: toxic potency of test sample; *FL*: percentage of fiducial limits; TPA: sum of toxic potency of three alkaloids (aconitine, mesaconitine, and hypaconitine). TPA = 1000 × aconitine content + 709.90 × mesaconitine content + 383.86 × hypaconitine content.

be valuable (Chen et al, 2006; Kim et al, 2004; Tang et al, 2005; Yan et al, 2007). For this reason, our group investigated the metabolic action of natural *Cordyceps* and its cultured mycelia on the growth of *Escherichia coli* with microcalorimetry−one method of the biopotency assays (Zhou et al, 2009). The result showed that *Cordyceps* from disparate producing areas and species made different influence on the growth metabolism of *E. coli*. This difference could be used to distinguish *Cordyceps* of different grades and evaluate their quality. In addition, Yan et al (2010b) studied the bioactivity of *Cervi Cornu Pantotrichum*, *Cervi Cornu*, and *Saigae Tataricae Cornu* on *E. coli* growth using biopotency assays to find the heat change regularity of microbial growth. And they found that this method could qualitatively and quantitatively reflect the bioactivity of these medicines. Those methods based on biopotency assays could provide a paradigm for the QC of other valuable and rare CMM.

7. CMM injections

CMM injections have been widely used in China (Ji et al, 2009; Xue and Roy, 2003) owing to their powerful and rapid therapeutic effects. In order to control the quality of CMM injections, modern advanced methods such as HPLC (Yan et al, 2006), GC (Qi et al, 2004), MS (Zeng et al, 2007), capillary electrophoresis (Ganzera, 2008) and their hyphenated techniques (Fan et al, 2006) have been applied to identify more compounds in them. However, many constituents can not be detected for a lack of the absorption in spectra, especially those biopollutants, pharmaceutical adjuvants, and biological active constituents (Huang et al, 2009), which resulted in adverse drug reactions and adverse drug events (Zeng and Jiang, 2010). Hence, it is necessary to establish novel approaches for the comprehensive QC of CMM injections from a biological viewpoint (Boyle et al, 2011; Jiang et al, 2010; Liu et al, 2010). Ren et al (2011) in our group combined biopotency assays with the chemical analysis to detect the fluctuation in the quality of Yinzhihuang Injection from both chemical and biological aspects (Figure 4).

HPLC and thermal activity monitoring were applied to develop the chemical fingerprint and biological fingerprint of normal samples bought from the regular factory and artificially abnormal samples exposed to different conditions including the temperature, light, air, and sterilization, respectively. They found that all abnormal samples could be correctly distinguished when the two analysis techniques combined. This method could be used for the early prediction

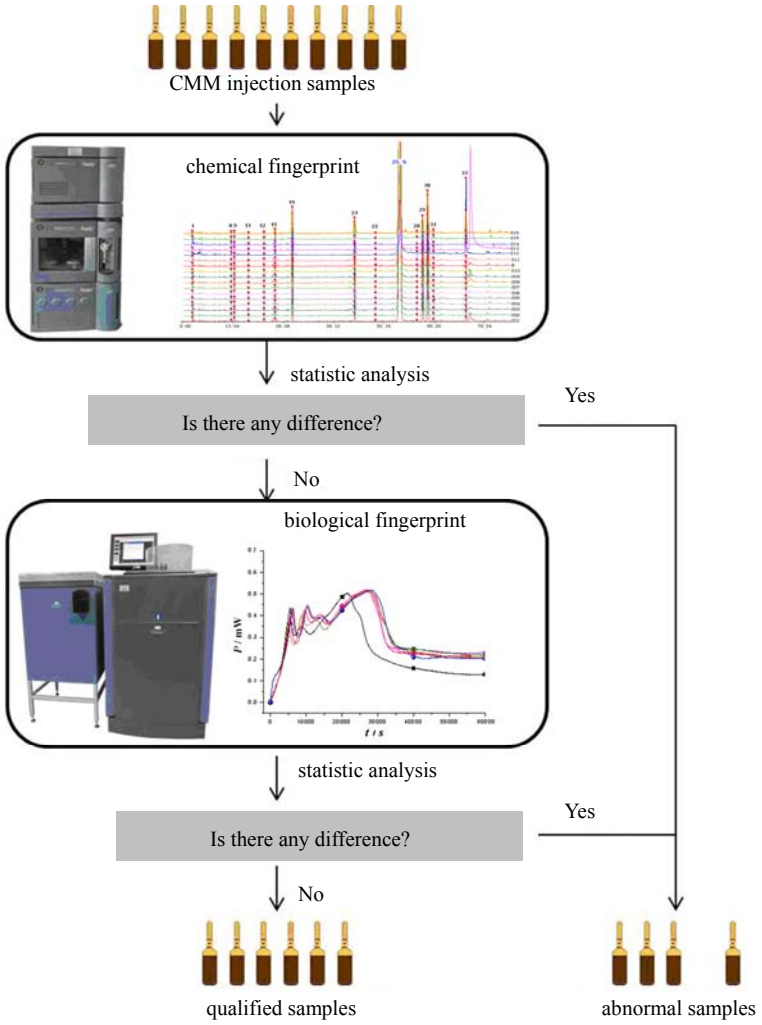

Figure 4 Quality monitoring of CMM injection samples based on chemical and biological analyses (Ren et al, 2011)

of adverse drug events, which might help improve the safety of CMM injections.

8. Conclusion

Currently, the pharmacological activities of some chemical constituents from CMM have been well explored. Therefore, the content determination of the known active constituents of CMM is still an important way for the QC of CMM. To introduce biopotency assays into the QC of CMM does not mean to replace the chemical analyzing methods completely. Biopotency assay has its own advantage to relate the effect, but the focus on one or two pharmacological activities is also not enough and can not be thought as totally relating to the clinical efficacy. Meanwhile, from the aspect of precision and reproducibility, some biopotency assays may be not as good as the chemical analysis. In fact, the chemical analysis is more likely used to determine the authenticity of CMM, while biopotency assay is more suitable to be applied for the evaluation. Therefore, neither of them can be excluded in the QC pattern of CMM.

The introduction of biopotency assays in the QC of CMM reveals a combination of multiple disciplines, technologies, and methods, which takes the advantages of biological detection and chemical analysis to promote the development of QC pattern for CMM. It is a beneficial attempt for the modernization, normalization, and standardization of CMM. Even though there would be quite a few technical problems such as the definition of high-quality CMM, the selection of biological detection methods and the rationality of *in vitro* activity evaluation. We believe that the integrated application of biopotency assays will be solved with continuous researches and unremitting efforts of scientific and technological workers. The QC pattern of CMM based on both chemical analysis and biopotency assays can also provide some beneficial research ideas and methods for the QC of CMM and the development of botanical drugs in the world.

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