Strategies for Integral Metabolism Profile of Multiple Compounds in Herbal Medicines: Pharmacokinetics, Metabolites Characterization and Metabolic Interactions

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Abstract: Herbal medicines (HMs) are gaining more and more attention all over the world, because of their significant curative effect in treating multi-factorial diseases. Recently, the *in vivo* and *in vitro* metabolism study of HMs has become an important issue because these data can help us to better understand the efficacies and toxicities of HMs. However, the integral metabolism profile of HMs is confronted with many challenges: 1) HM is a multi-component system; 2) most components are unknown (nontarget); 3) trace of components in HM. Given the challenges described above, the demand for more powerful bioanalytical tools and strategies that are adequate for integral metabolism profile of HMs' multi-components has increased. In the past few years, newer methods, or adaptations to methods, have been published, and this review will attempt to discuss new improvements in strategies and methodologies for HMs' multi-component ADME evaluation. In particular, improvements have been reported for experimental approaches to pharmacokinetics study of HMs, as well as strategies applied to metabolites characterization of HMs' multi-components, and the metabolic interactions between ingredients in HMs, including advance and proposed strategy: "chemical fishing" based strategy for metabolic interactions of HMs.

Keywords: Herbal medicines, multiple components, pharmacokinetics, metabolites characterization, metabolic interactions, strategy.

1. INTRODUCTION

For centuries people have used plants for healing [1]. However, the strong historic bond between plants and human health began to unwind in 1897, when synthetic acetyl salicylic acid (aspirin) was introduced to the world [2]. And the synthetic-chemistry-dominated pharmaceutical industry entered a triumph in the twentieth century, which attempts to treat complex diseases with a 'single golden molecular bullet' [3]. Unfortunately, it is incompetent to resist the multifactorial nature of many complex diseases, such as diabetes, heart disease, cancer and psychiatric disorders. Therefore, many researchers gradually shift their focus to multi-component drug therapies for resisting chronic and complex diseases.

Herbal medicines (HMs), generally prepared with a specific combination of different herbs, are becoming increasingly popular as a multi-component drug therapy [4, 5]. As is known, the most outstanding feature of HMs is that they produce curative effect through a concerted pharmacological intervention of multiple compounds interacting with multiple targets [6]. Such an approach might provide a superior therapeutic effect and decrease in side effect profile compared to the action of a single selective ligand, especially in the treatment of some chronic and complex diseases [7-9]. Recently, the efficacy of HMs has been gradually documented by pharmacological and clinical study, and the active constituents of HMs have been revealed endlessly during these assays.

Besides the pharmacological evaluation, the integral metabolism profile of HMs' multi-components is of certain importance for explaining and predicting their efficacy and toxicity, and the widely reported herb-drug interactions [10-12]. Unfortunately, the current research on integral metabolism of HMs is still in its infancy. As pointed out by WHO [13], there is very limited knowledge about pharmacokinetics (PK) and metabolomics, of HMs,because of the following challenges: (1) HM is a complex system, in most cases medicinal plants comprise hundreds of different constituents with diverse physicochemical properties; (2) most components are unknown (nontarget); (3) trace components in HMs result in low plasma concentration after oral administration, which makes global qualitative and quantitative analysis difficult; (4) due to the fact that HMs are complex, multi-component systems and adequate or acceptable methodologies are lacking for their integral metabolism research and evaluation, especially in the aspects of sensitivity and throughput.

Given the challenges described above, the demand for more powerful bioanalytical tools and strategies that are adequate for integral profiling of metabolism of HMs has increased. This review will attempt to discuss new improvements in strategies and methodologies for integral metabolism profile of HMs' multi-components, and comprises the following aspects:

1. Introduction to the most recent improvements in strategies and methods for PK study of HMs:

- turbulent flow chromatography coupled online to fast high performance liquid chromatography and mass spectrometry (Online TFC-LC/MS);
- relative exposure approach to herbal PK independent of standards;
- segmental and selected ion monitoring strategy;
- 'LC-electrolyte effects' and pulse gradient chromatography;

2. Strategies for metabolites characterization:

- metabolic fingerprinting technique;
- fragmentation behavior-based strategy;

3. Metabolic interactions between ingredients in HMs: advances and proposed strategy.

2. SOME ASPECTS OF NEW METHODS FOR PHARMACOKINETICS OF HMS

PK plays an important role, and represents an integral part throughout the whole pipeline of new drug discovery [14]. The extensive PK study of HMs is critical for better understanding their pharmacologic activities and clinical effects and guiding the device of clinical administration dose. Due to the highly complexity and

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diversity of chemical constituents in HMs, the PK analysis of HMs has been a difficult subject to study and lacks for sufficient technological support for a long time. Fortunately, new technologies in chromatography and mass spectrometry (MS) have greatly facilitated the extensive PK study of HMs. There mainly are, at present, three kinds of chromatography coupled to MS methods applied in the PK assays of HMs, namely, liquid chromatography-MS (LC-MS), gas chromatography-MS (GC-MS), and capillary electrophoresis-MS (CE-MS) methods. Generally, LC-MS or LC-MS/MS-based assays were and still are commonly used for analyzing multiple constituents of HMs in complex biological fluids [15]. These conventional assays have been reviewed in the literatures [16, 17], and will not be rehashed here. Herein, we will attempt to discuss new improvement in the methods for PK study of HMs' minor- and multi-components.

2.1. Online Turbulent-Flow Chromatography-LC/MS

Turbulent flow chromatography (TFC), as a separation system, has been demonstrated to be a rugged and time-efficient pretreatment method for biological samples [18]. By using large material-packed column in TFC, biological samples could be directly injected into a high-flow rate aqueous mobile-phase stream. Consequently, large protein molecules in the biological samples can easily pass through the TFC column as waste, while the targeted compounds for analysis are retained. This makes TFC a fast and rugged extraction technique. However, using large packing materials considerably decreases the separation efficiency. It is thus necessary to use a TFC column and an analytical column in tandem to maintain sufficient resolving power and avoid potential interference among the multiple compounds and signal suppression in MS detection.

Recently, our research team has developed a method based on the on-line TFC and fast HPLC/MS (TFC-LC/MS) for sensitive and high throughput PK study of HMs [19]. A schematic diagram of the on-line TFC-LC/MS instrument set-up based on column-switching and fast HPLC is shown in Fig. **1**. The operating procedure was briefly described as following: after injection of sample, the switching valve was switched to the L position, and the PUMP 1 started to deliver solvent at 4.0 mL/min to load the sample onto a hydrophilic-lipophilic balanced (HLB) extraction column and subsequently to clean the sample (see Fig. **1A**), this loading and washing step was completed after 1min when the switching valve was switched to the E position (see Fig. **1B**); the extraction column was then in the flow path of the PUMP 2, and the PUMP 2 started to deliver a gradient flow to elute the analytes from the extraction column and to perform the separation on a fast HPLC column; this elution step was completed within 7 min when the switching valve was switched back to the L position. This method was successfully applied for pharmacokinetic study of verticine, verticinone and isoverticine, the chemical markers of *Fritillaria thunbergii*, after oral administration of total steroidal alkaloids extract of *F. thunbergii* to rats. The authors also demonstrated that the sensitivity of the on-line TFC-LC/MS method was much improved, about 100-fold more sensitive over those reported previously by other methods [20, 21]. This study has shown the potential of TFC on-line coupled to fast HPLC and MS for high throughput and sensitive analysis of multiple and trace constituents of HMs in complex biological fluids in regard to quantification of target analytes.

2.2. Relative Exposure Approach

It has been well acknowledged that most components of HMs are unknown (nontarget), which results in lacking of authentic standards for globally assessing herbal PK. To address such a critical problem, more recently, Wang's group proposed a 'relative exposure approach' (REA) that entails the use of sequentially diluted original herbal preparations to prepare the 'mixed calibration curves' for assessing herbal PK independent of specific authentic compounds for each component [22]. The linear regression equation for each component was expressed as: $y = bx + a$, where y represents the mass response ratio of targeted analytes to internal standard, and x represents the concentrations of rude extract. The relative plasma concentrations of certain components can be calculated from the corresponding calibration curves and the data are expressed as the concentration of rude extract. This means that the actual plasma concentration of certain component is equivalent to its content contained in the rude extract at measured concentration. Pharmacokinetic parameters were then calculated from the relative concentrations versus time data.

In this study, the authors took *schisandra* lignans extract (SLE) as an example to interpret and validate the potential of REA strategy in global assessing herbal PK. The authors developed a LC-IT-

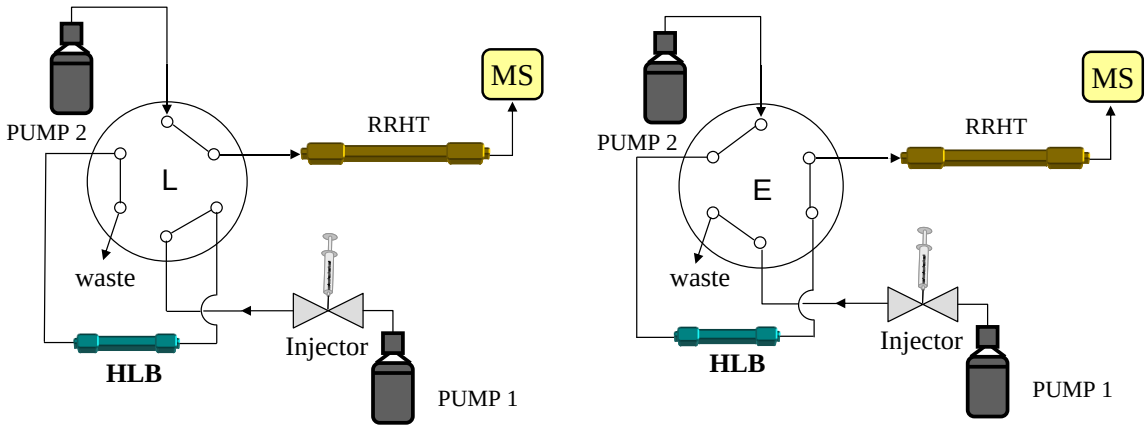

Fig. (1). Schematic representation of on-line turbulent-flow column-switching system. (Reproduced from [19] with kind permission from Elsevier).

TOF/MS assay based methodology for following the PK of all potential plasma components after SLE ingestions. And the suggested experimental workflow is schematically depicted in Fig. **2**. First, the components contained in SLE were detected and identified by LC-IT-TOF/MS. Second, both lignan compounds and potential metabolites in the rat plasma after oral administration of SLE were identified by comparing the retention time and accurate mass data with those of the lignans in SLE. Third, the potential quantitative capacity of LC-IT-TOF/MS was fully validated and compared with a well-established typical LC-Q/MS assay. Fourth, the REA was developed on the basis of calibration curves prepared by sequential dilution of rude extracts (SLE in this study). Finally, the REA was applied to PK assays of lignans in rats and the PK parameters from the relative concentrations versus time data were calculated.

Based on the powerful LC-IT-TOF/MS assay and REA, the plasma PK profiles of 21 out of a total of 31 lignans contained in SLE were successfully characterized. From these plasma profiles, the relative PK parameters including AUC, C_{max}, T_{max}, and $t_{1/2}$ were calculated. And the authors found that all these parameters are comparable among different components. To confirm the reliability of this approach, the above important PK parameters for five target lignans were compared with those obtained from the LC-Q/MS assay calibrated by the authentic standards. The results proved that the REA based on LC-IT-TOF/MS assay provides almost identical results of all the PK parameters, except for the absolute plasma concentrations. The present REA strategy would be fairly useful for addressing the critical problems underlined in the field of herbal PK study, including the qualitative and quantitative analysis of multiple targets and nontarget components, and the lacking of authentic standards for verification and calibration.

2.3. Segmental and Selected Ion Monitoring Strategy

As is well known, most quantitative analysis in the PK study of HMs were carried out by LC-MS or LC-Q/MS-based assays, which could provid satisfactory performances on quantifying single or several constituents of HMs in complex biological fluids [23-25]. However, it is reported that the detecting sensitivity based on LC-MS or LC-Q/MS always decreased dramatically when several ions were detected simultaneously. Such a sensitivity compromise would lead to a critical problem for quantitation of herbal components in plasma at the same time, because for most herbal components the plasma concentration is extremely low. To address such problem, Liang *et al*. proposed a simple and universal strategy, namely segmental and selected ion monitoring (SSIM), for simultaneous detection of multi-components in bio-sample based on LC-Q/MS [26]. SSIM could be implemented easily by using an instrument control language (ICL) procedure written to allow the mass spectrometer to switch between the analyte masses at specific retention times [27]. Herein, the authors set simultaneous detection of 16 saponins in rat plasma as an example to investigate the influence of SSIM on quantitation of multi-component using LC-Q/MS.

Based on the premising chromatographic separation, four types of SSIM modes were designed and compared: (1) all the 16 saponins ions were detected in one segment (1-SM, control group); (2) divided into two groups (2-SMs) and the detection time was separated into two time segments; (3) three segments (3-SMs) were set; (4) the detection time was separated into five segments (5-SMs), and <5 ions were determined in each time window. ICL procedures were written to allow the mass spectrometer to switch between the saponins masses at specific retention times. The signal-to-noise (S/N) ratios and lower limits of quantification (LLOQs) were compared under the four types of SSIM modes using the above optimized chromatographic conditions. Their results showed: the noise was reduced significantly with fewer detected ions within one segment (shown in Fig. **3**); the 5-SMs LLOQs were 4-10-fold lower than those under 1-SM, and most of saponins could be quantitated at 1-5 ng/mL in 0.1mL rat plasma. Upper limits of quantification (ULOQs), as a direct factor affecting the dynamic range, were also compared. ULOQs of all the saponins determined under 5-SMs mode were much higher than those determined under 1-SM mode, indicating that a proper SSIM mode could not only provide much higher sensitivity for all the target analytes, but also dramatically broaden their dynamic ranges. Subsequently, a systematic investigation on accuracy, precision, stability, recovery and matrix effects was performed to verify that the method based on 5-SMs mode could meet the quantitative criterion for 16 saponins in biological sample. And a positive result was revealed.

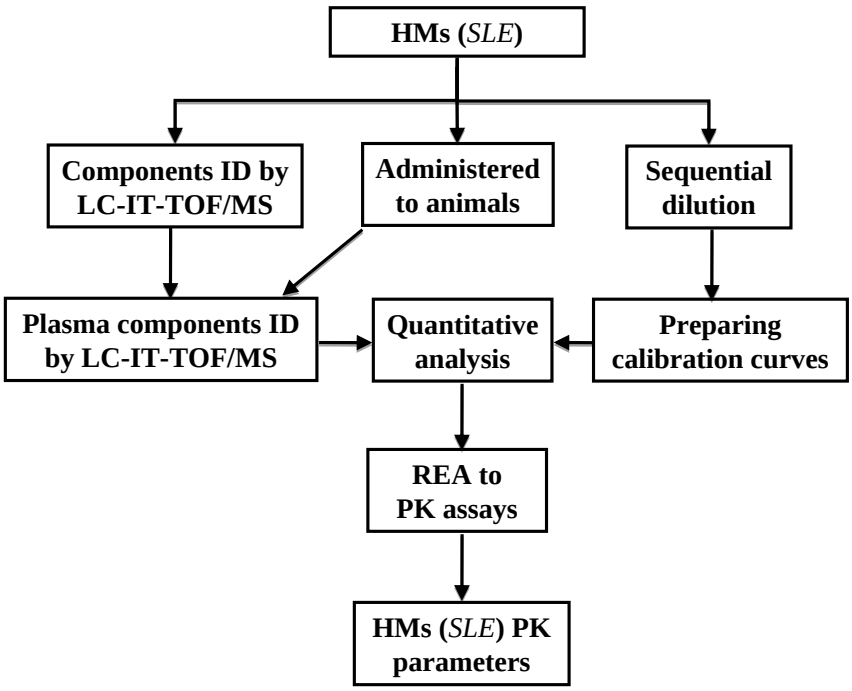

Fig. (2). Suggested herbal PK analysis platform and workflow based on LC–IT-TOF/MS.

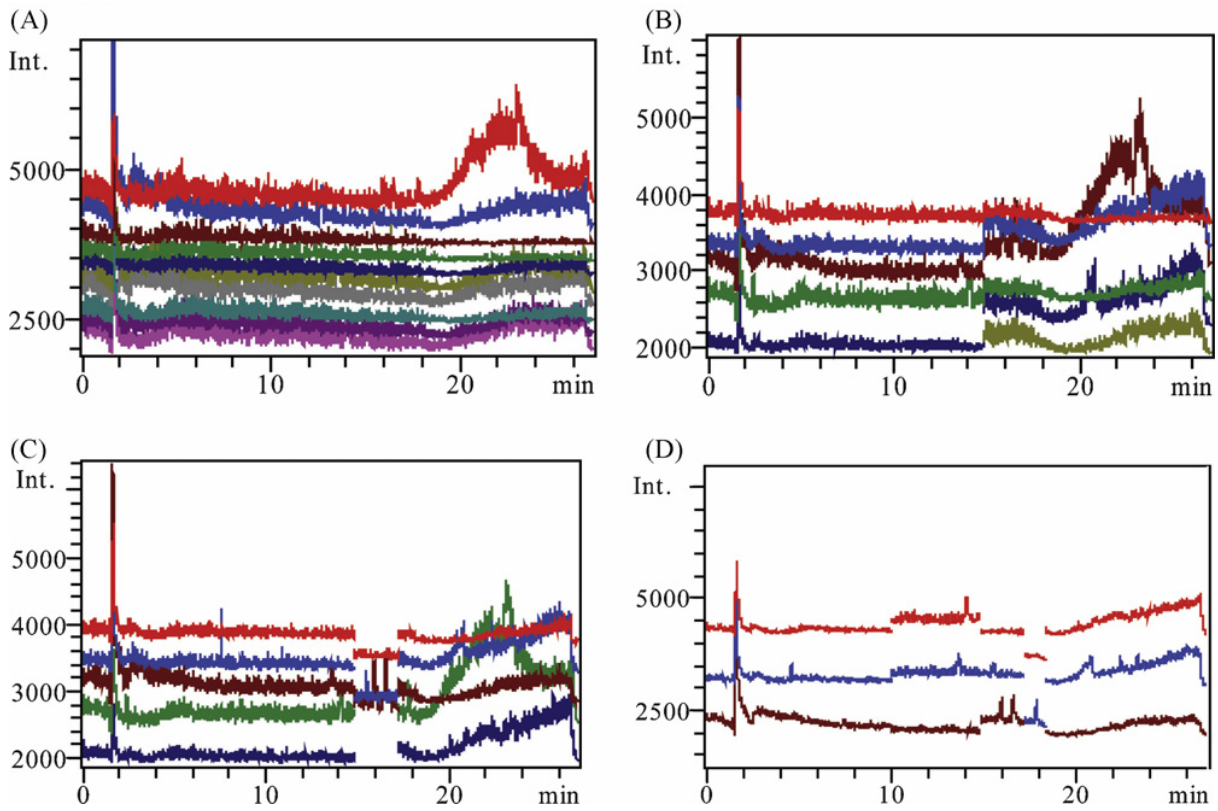

Fig. (3). The baseline of the 16 saponins determined by LC–Q/MS under four different SSIM modes (A: 1-SM; B: 2-SMs; C: 3-SMs; D: 5-SMs). (Reproduced from [26] with kind permission from Elsevier).

In Summary, for quantitation of herbal multiple compounds in biological sample, SSIM possesses higher sensitivity and wider dynamic range when compared with 1-SM. To some extent, SSIM breaks the application bottleneck of LC-Q/MS on the quantitative analysis of multiple components.

2.4. 'LC-electrolyte Effects' and Pulse Gradient Chromatography

Li's group has reported that the use of a mobile phase containing an extremely low concentration (0.01-0.02‰) of ammonium formate or formic acid increased analyte electrospray ionization (ESI) response and controlled against matrix effects [28]. They termed these favorable effects as 'LC-electrolyte effects'. Herein, the authors set simultaneous detection of eight ginkgo flavonoids and terpenoids in plasma as an example to investigate the influence of LC-electrolyte effects on quantitation of multi-component using LC-MS/MS. Modifying the LC mobile phase with $HCOONH_4$ (0.2 mM) increased both ESI efficiency and capacity. This significantly enhanced analytical performance of the developed method, *i.e.*, improved sensitivity, negligible matrix interference and cross-interference, wide linear dynamic ranges, high sample throughput, and quite small initial sample size. Therefore, 'LC-electrolyte effects' is beneficial for global assessing herbal PK.

Pulse gradient chromatography, similar to solid-phase extraction (SPE) for sample clean-up [29], first retains the analytes with a mobile phase containing ~100% water (start proportion) allowing the unretained sample organic solvent and matrix components to be washed from the column. The analytes then elute from the column in a small volume with a strong mobile phase containing ~100% CH_3CN (elution proportion), which also results in favorable band compression [30]. The working parameters of the pulse gradient method include the start proportion (SP; %B, v/v), the start proportion segment (SPS; min), the elution proportion (EP; %B, v/v), the elution proportion segment (EPS; min), and the column equilibrium segment (CES; min). The pulse gradient method can be optimized by simply tuning the above parameters, generally just tune the SPS is adequate. Li' group has combined LC-electrolyte effects and pulse gradient chromatography to develope a sensitive LC-MS/MS-based method for the detection and quantification of ginkgo flavonols in dog plasma [31]. The developed assay provided lower limits of quantification of 1.3, 1.3 and 0.4 pg on-column for quercetin, kaempferol and isorhamnetin, respectively, which is more sensitive than any previously reported method for determining ginkgo flavonols. These results indicated that the pulse gradient method, combined with LC-electrolyte effects, can further control against matrix effects and elutropic effects.

3. STRATEGIES FOR METABOLITES CHARACTERIZATION

3.1. Metabolic Fingerprinting Technique

Since serum pharmacochemistry was introduced to screen the bioactive compounds from HMs [32, 33], some analogical methods based on serum pharmacochemistry have been proposed [34, 35]. The representative method is metabolic fingerprinting technique (MFT). By comparing the chromatography fingerprinting of a HM with its metabolites profile, MFT not only reflects what might happen *in vivo*, but also projects the integrity of HMs. Zhang *et al.* analyzed the chemical fingerprint and metabolic fingerprint of *Salvia miltiorrhiza* injection with HPLC-UV and HPLC-MS, and defined polyphenolic acids as the main bioactive constituents [36]. Based on the MFT and LC/Q-TOF-MS/MS, Wang *et al.* [37] established a method for rapid screening and analysis of the multiple absorbed bioactive components and metabolites of Yin Chen Hao Tang (YCHT) in rat plasma. Consequently, 45 compounds in YCHT and 21 compounds in rat plasma after oral administration of YCHT were detected. Thirty compounds in YCHT and all the compounds detected in rat plasma were identified either by comparing the retention time and MS data with that of reference compounds or

by MS analysis and retrieving the reference literatures. The MFT has provided significant advantages in straightforward screening *in vivo*, giving a high probability of hitting, and being consonant with characteristics of HMs. However, this method may suffer from the laborious and time-consuming sample preparation.

3.2. Fragmentation Behavior-based Strategy

In the past few years, reports on the identification of constituents in HMs and the profiling of their metabolites have been steadily increasing, especially for the recently accelerated development of various hyphenated and hybrid MS techniques such as MS/MS, IT-TOF/MS, TOF/MS, QTOF-MS/MS [38-41]. Generally, the components contained in HMs could be structurally classified into several families and a same family of components usually contain same carbon skeleton or substructures, from which the same fragment ions can be produced in collision induced dissociation (CID) mode.

Recently, a strategy of profiling the constituents in HMs and their metabolites in biological fluids based on the combination of fragmentation behavior and metabolic pathways was reported by Geng *et al* [42]. In their study, flavonols in the active fraction of *Gossypium herbaceam* L. (AB-8-2) and bile samples from rats were analyzed using LC-MS^n. The *in vivo* metabolic reactions of flavonols were mainly methylation, glucuronidation and sulfation [43-45]. Thus, losses of 15, 80 and 176 Da in MS/MS spectra were used to characterize methyl, sulfate and glucuronide conjugates, respectively. In comparison with the blank sample, 31 quercetin-based and 12 kaempferol-based compounds were characterized in the bile samples.

Li's group has also applied this approach in profiling of the integral metabolism of Danggui Buxue Tang (DBT), a well-known Chinese herbal formula, with LC-TOF/MS [46]. Metabolites of DBT were identified using dynamic adjustment of the fragmentor voltage to produce structure-relevant fragment ions. By using this approach, a total of 68 compounds including 13 parent compounds and 55 metabolites were detected in the drug-containing urines compared with blank urines, and enabled the identification of 43 metabolites including 27 isoflavonoids and 16 phthalide metabolites. Mass spectrometry fragmentation behavior and metabolic pathway complement each other in structural identification and correlating the metabolites and their parent forms. This study provides a basis for research into profiling the integral metabolism of HMs.

4. METABOLIC INTERACTIONS BETWEEN INGREDIENTS IN HMS

Metabolic interactions refers to the influences on metabolism when two or more different drugs (multi-component drug) are used simultaneously or successively, which can result in an enhancement in curative effect or toxic and side-effect, or decreased efficacy and therapeutic failure. HMs, as a multi-component drug therapy, is increasingly used for treating complex disease. Therefore, it is of great clinical significance to study the metabolic interactions of HMs.

4.1. The Advances in Metabolic Interactions Studies of HMs

As HMs comprise rich chemical libraries, metabolism-mediated component and component interactions will occur when HMs were used. The greater the number of components involved, the greater the potential for component-component interactions. It is worthy of note that the *in vitro* or *in vivo* studies on metabolic interactions between ingredients in herbs are limited, and just a few of reports about this work were published.

Our group has studied the interaction property of multi-components in DBD with BSA by microdialysis coupled with HPLC-DAD-MS [47]. By comparison of the binding degrees of single reference solution with that in the extract of DBD, potential interactions between multi-compounds in DBD were observed. For example, the binding degrees of ononin with BSA was 36.8% in each solution while 29.2% in the decoction. The decrease might be caused by competitive effect. However, the binding degrees of chlorogenio acid, ferulic acid and calyocosin with BSA were increased by synergistic effect.

Bi *et al*. have assessed the interactions of BSA with four anthraquinones from herbs (emodin, rhein, aloeemodin and aloin) by employing spectroscopic techniques [48]. In their study, the mutual influences on the interactions of anthraquinone-BSA for these compounds were investigated. By fixing the concentration of one compound in a series of test tubes containing BSA and adjusting the concentrations of another compound, the apparent binding constant K'_A of the latter was obtained. With aloe-emodin or rhein in the BSA solution, the apparent binding constant K'_A of emodin and BSA decreased with the concentration of aloe-emodin or rhein increasing. Similarly, the K'_A of aloe-emodin-BSA dropped rapidly when there was a fixed concentration of emodin in the BSA solution. These results demonstrated that emodin, aloe-emodin and rhein possessed a common binding site in BSA and there was a competition binding with BSA between emodin and rhein or aloe-emodin.

Mizuhara *et al*. have demonstrated the pharmacokinetic interactions of glycyrrhetic acid (GA) derived from glycyrrhizinic acid (GL) in licorice and anthraquinones derived from rhubarb [49]. When GL was orally co-administrated with a non-effective dose of sennoside A to rats, the $AUC_{(0\text{-lim})}$ and C_{max} of GA decreased. In the examination using an in situ loop of rat colon, the remaining ratio of GA rose drastically by the co-administration of sennoside A, sennidin A and rhein. The inhibition activity of these anthraquinones on GA absorption was also observed and found to be dose-dependent. The maximum inhibition ratio was approximately 75% by rhein, 60% by sennoside A and 25% by sennidin A.

Microsomal system, as the major carrier of cytochrome P450 enzymes (CYPs), is generally selected as *in vitro* model for prediction of metabolism-based interactions of herbs [50]. Iwata *et al*. have ever reported that schisandra extract has a potent inhibitory effect on human liver microsomal erythromycin *N*-demethylation activity mediated by CYP3A4, and known components of schisandra fruit, gomisins B, C, G, and N and γ-shizandrin, also inhibited the N-demethylation activity [51]. Wang's group has examined the potential for the metabolism-based drug interaction arising from seven active components of danshen (the root of *Salvia miltiorrhiza*) extract [52]. Probe substrates of cytochrome P450 enzymes were incubated in human liver microsomes with or without each component of danshen extract. The results indicated that cryptotanshinone, tanshinone I, and tanshinone IIA were potent competitive inhibitors of human CYP1A2, cryptotanshinone and danshensu were moderate competitive inhibitors of human CYP2C9, tanshinone I and cryptotanshinone were weak inhibitors of human CYP2D6, and danshen multiple components had complicated effects on CYP3A4. These findings provided some useful information for safe and effective use of danshen preparations in clinical practice.

In summary, the common feature of the above studies is that some available ingredients of HMs were selected to evaluate the component-component interactions in herbs. In this way, some potential metabolic interactions between ingredients in herbs were successfully proved. However, these methods ignored two facts: 1) Whether these ingredients are bioavailable (absorbable); 2) and whether these results are in accordance with the *in vivo* actual conditions of bioavailable herbal components. Thus, the properties of component-component metabolic interactions of HMs obtained through this way are not integral.

4.2. Proposed Strategy: "Chemical Fishing" Based Strategy for Metabolic Interactions of HMs

More recently, our research team has reported a strategy for holistic activity and interaction evaluation of the components in HMs, namely chemical markers' fishing and knockout technology. As shown in Fig. **4**, an HPLC technique was used to separate constituents in an herb extract. Through the valve switching technique, ingredients were thus divided into two parts: the target compounds and others without the target. This technology has been successfully applied in evaluating the interactions of the bioactive components in *Lycoris radiate* [53]. This successful application suggested that we can utilize the method for the study of metabolism-mediated interactions between ingredients in herbs. Thus, we herein propose a "chemical fishing" based strategy for predicting metabolic interactions of HMs (Fig. **5**).

The first step of this strategy is to screen and qualitatively identify the bioavailable herbal components (what are absorbed). As reported, the commonly used models in the field of drug permeability and absorption are mainly Caco-2 cells [54-56] and liposomes [57-62]. These models coupled with analytical technologies, such as LC-TOF/MS, LC-QTOF/MS and LC-IT-TOF/MS, are potent tools for screening and identifying bioavailable components from HMs. In fact, these methods have been successfully used to screen compounds with good permeability (bioavailable herbal components) in HMs [63-68].

Then, the key step of this strategy is to obtain different collections of bioavailable components in herbs. Herein, we utilize the chemical markers' fishing and knockout technology mentioned above to prepare these collections, including total bioavailable components, single bioavailable components, and different combination of these components. The collections obtained then can be applied to integral elucidation of potential metabolic interactions between ingredients in herbs. As for the methods for prediction of metabolic interactions, the commonly used *in vitro* models, such as HSA, BSA, and microsomes, is sufficient. Moreover, the chemical markers' fishing technology can be scaled up by using preparative or semi-preparative column and electric flow switcher, which facilitate preparing collections on large scale for animal-based evaluation (PK interactions).

5. SUMMARY AND TRENDS

The chemical constituents of an HMs are highly complex, mostly unknown and varying in content and physico-chemical property. The integral metabolism profile of the multiple compounds in complex system of HMs is a formidable challenge. There have been several innovations in the area of HMs' multi-components metabolism evaluation in the last few years.

This review has focused on several areas of awareness that can enhance the quality of HMs' metabolism profiling, including improvements in strategies for PK study, metabolites characterization, and prediction of metabolic interactions between ingredients in HMs. It should be concluded from this review that researchers have made great progresses in global metabolism profiling of HMs especially in developing new methods for breaking the critical bottleneck of multi-component PK study of HMs. The advantages and drawbacks of new methods for PK study of HMs' multi-components have been discussed and summarized in Table **1**.

In this review, we also proposed a strategy, namely "chemical fishing" based strategy, for predicting metabolic interactions of HMs. The strategy has attractive points, such as the ability to analyze multi-components in HMs extracts, the ability to screen bioavailable herbal components, the ability to quickly collect the bioavailable components.

There are other important aspects with regard to strategies for integral profiling of metabolism of HMs not discussed in this review (*e.g.*, metabolomics strategy and herb-durg interactions). However, many new hurdles will need to be overcome in the future before researchers could even begin to contemplate how to deal with integral metabolism research of complex system as HMs. For example, trace of active components in HMs is commonly much less absorbed in circulation, thus better detection systems are needed; HM is a complex system, and more powerful computa-

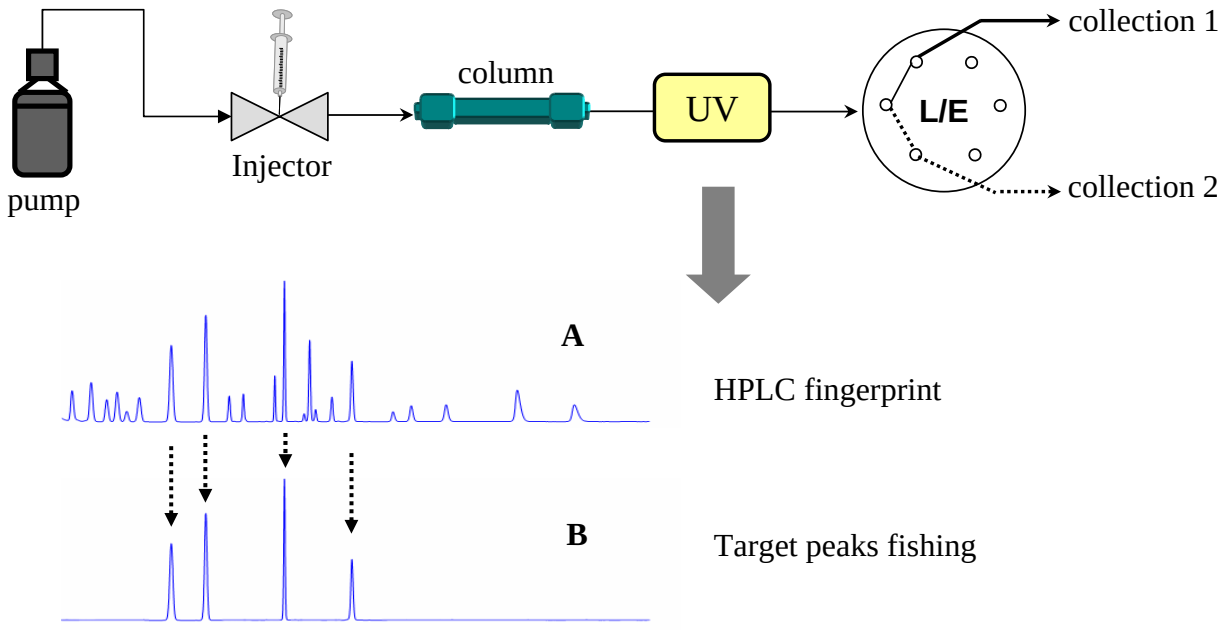

Fig. (4). Schematic diagram of the chemical markers' fishing and knockout technology.

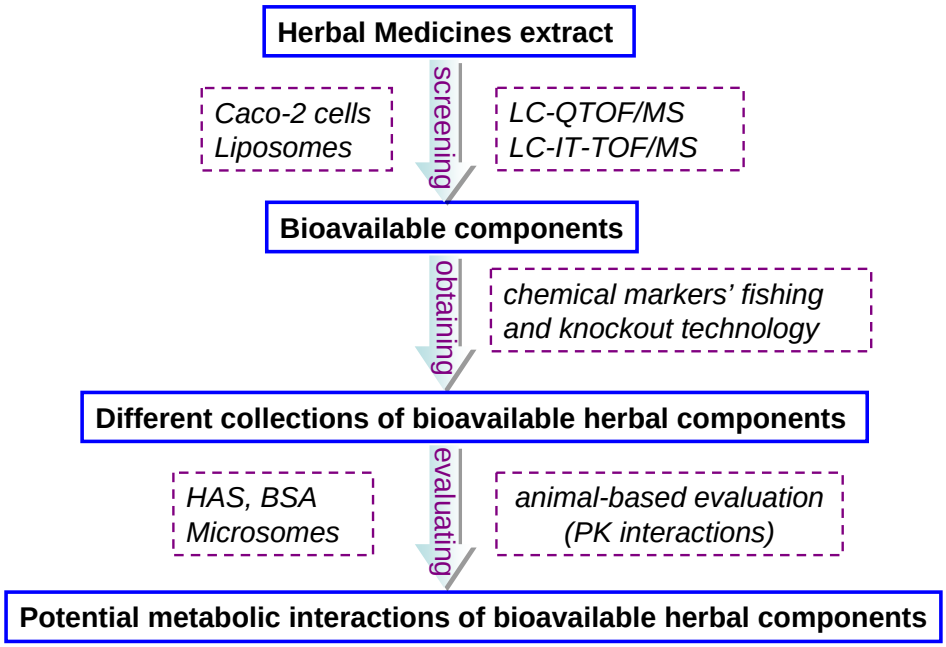

Fig. (5). The proposed platform and workflow of "chemical fishing" based strategy.

Table 1. The Advantages and Limitations of New Methods for PK Study of HMs' Multi-components

Approaches		Advantages	Limitations
New improvement in strategies and methods for PK of HMs	TFC–LC/MS	High throughput and sensitive; Online sample extract and direct injection.	Required delicate instrument alignment, not often available in a routine laboratory.
	REA	Independent of specific authentic compounds, suitable for PK study of nontarget components in HMs.	Unavailable of absolute plasma concentrations of specific components.
	SSIM strategy	More sensitive and wider dynamic range; could be implemented easily by using an ICL.	Relatively dependent on good chromatographic separation.
	'LC-electrolyte effects' and pulse gradient chromatography	Increase both ESI efficiency and capacity; controll against matrix effects and elutropic effects; improved sensitivity, wide linear dynamic ranges; suitable for PK study of multiple and trace components in HMs.	Further low concentration of electrolyte (eg. 0.04mM HCOOH) will lead to converse results, thus, particular optimization is imperative.

tional and statistical tools are crucial for dealing with large and complex data sets. In conclusion, it is likely that the demand for powerful bioanalytical approaches and integral evaluation strategies will continue to be urgent.

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