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A bio-activity guided *in vitro* pharmacokinetic method to improve the quality control of Chinese medicines, application to Si Wu Tang

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ABSTRACT

The purpose of this study was to demonstrate the feasibility of using a bio-activity guided *in vitro* pharmacokinetic (BAPK) method in identifying relevant (absorbable and bioactive) markers for quality control (QC) of Chinese medicines (CM), using Si Wu Tang (SWT), a popular CM for women's health, as an example.

A stepwise BAPK approach was utilized for relevant marker determination and evaluating of six SWT products: (1) data mining to identify active components of SWT, (2) quantification of the identified active components in each SWT product, (3) determination of *in vitro* dissolution and metabolism of the components under simulated gastrointestinal conditions, (4) identification of absorbable components or marker(s) via *in vitro* Caco-2 cell model, (5) stability testing of the permeable marker(s).

Our results showed considerable variations in the amount of active components in different SWT products. Of the nine active components identified from data mining, three (ferulic acid, ligustilide, senkyunolide A) were found to be well permeated and stable over three months. Paeoniflorin, the marker designated by Chinese Pharmacopoeia, was poorly permeable and thus could not be considered a relevant marker for SWT. Our preliminary evaluation of the BAPK method appears to be feasible and may offer as a useful approach for identifying relevant markers of other TCM products in the future.

1. Introduction

Chinese medicine (CM), including traditional Chinese medicine (TCM) formulae have been widely used in China, Asia and other parts of the world for several thousand years. Yet relevant method of quality control (QC) for such products is still lacking. One current commonly used QC method for CM or TCM product is to perform stability testing of certain markers chosen arbitrarily or according to the Chinese Pharmacopoeia (CP) (The State Pharmacopoeia Commission of P.R. China, 2005). The more advanced fingerprint technique has also been investigated with the intention to identify a comprehensive profile for the components in the CM (Zhong et al., 2009). However, such markers/components may not be absorbed or pharmacologically active and thus not relevant to the product activity *in vivo*. Without a QC marker that has relevance to product activity, the product's therapeutic value becomes questionable.

Conceptually, all active compounds in a pharmaceutical product that are bioavailable in the systemic circulation can be considered as relevant markers for QC since such markers import *in vivo* relevance. Unlike a conventional pharmaceutical product (western drug) which usually contains one absorbable active compound and is also the designated marker compound for QC, many compounds exist in CM. Thus identification of relevant marker(s) (absorbable and bioactive) for CM becomes difficult especially if they are determined by an *in vivo* approach, e.g. from blood or plasma samples after CM administration. The difficulty is primarily technical as it is virtually impossible to distinguish many such markers from hundreds of endogenous chemicals which are also present in the systemic circulation. In addition, determination of specific activity of these marker compounds *in vivo* not only can be labor intensive but require clinical facility as well as patient informed consent. Thus identification of relevant markers for CM from an *in vivo* approach is complicated and not generally feasible.

When CMs are administered orally (the most common and convenient route of administration) to achieve desirable therapeu-

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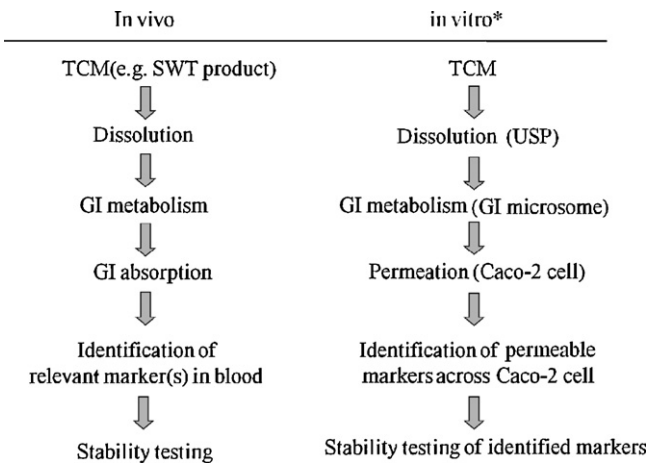

Fig. 1. BAPK approach: *in vitro*/*in vivo* (*iv*/*iv*) comparison.

tic effect, the bioactive components must undergo the following biopharmaceutic/pharmacokinetic (PK) processes in the gut: dissolution, metabolism, and absorption (Shargel et al., 2004). Only those active components (parent compounds and their metabolites) that are absorbable may be considered as relevant markers since they are capable of contributing to *in vivo* activity. Thus, identification of absorbable bio-active components using *in vitro* models that can simulate *in vivo* PK processes may be a practical approach for identifying relevant markers for QC. Based on this premise, we proposed the following sequential steps for an *in vitro* bio-activity guided pharmacokinetic (BAPK) approach that can parallel the *in vivo* PK processes (see Fig. 1). This proposed *in vitro* approach not only circumvents the need for human plasma samples, but more importantly it avoids confounding endogenous chemicals present in the plasma, thus making analytical separation of multiple active components in TCM more feasible.

The purpose of the present report is to evaluate the feasibility of the *in vitro* BAPK approach for identifying relevant markers for QC, using Si Wu Tang (SWT) as an example. SWT is a TCM formulae widely used for the treatment of women's disease in China and Asia. It is composed of four herbs, i.e. *Radix paeoniae alba*, *Rhizoma chuanxiong*, *Radix angelicae sinensis* and *Radix rehmanniae preparata*. There are at least 9 active components, namely gallic acid (GA), paeoniflorin (PF), paeonol (PO), ferulic acid (FA), ligustrazine (CXQ), catalpol (Cat), senkyunolide A (SA), ligustilide (Lig), and butylphthalide (Bu) which are already identified and reported in the literature (The State Pharmacopoeia Commission of P.R. China, 2005; Hsu et al., 1997; Lau et al., 2007; Chan et al., 2007; Li et al., 2004; Ou and Kwok, 2004; Peng et al., 2007; Fu et al., 2007; Kim et al., 2008). Thus, in the present evaluative study, SWT is selected to be a representative TCM formula testing the application of this *in vitro* BAPK method.

2. Materials and methods

2.1. Materials

The authentic standards of the six known active components of SWT, namely gallic acid, paeoniflorin, paeonol, ferulic acid, ligustrazine, catalpol were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The remaining three known active components of SWT, including senkyunolide A, ligustilide and butylphthalide, were obtained from the Hong Kong Jockey Club Institute of Chinese Medicine Ltd. (Hong Kong). The purity of these reference standards was above 98%. Acetonitrile and methanol were of HPLC grade and obtained from Labscan (Labscan Asia, Thailand). All reagents and chemicals (unless specified elsewhere) were of analytical grade and used without further purification. The 4 raw herbs for manufacture of the SWT product, e.g. *R. chuanxiong*, *R. angelicae sinensis*, *R. rehmanniae praeparata* and *R. paeoniae alba*, were purchased from Sichuan, Gansu, Henan and Anhui province in China, respectively, and authenticated by Prof. Zhongzheng Zhao in Hong Kong Baptist University and checked for contamination of heavy metal and pesticides according to the methods stated in the Pharmacopoeia of PRC 2005. To compare various SWT products, two reference SWT products [one in solid dosage form (CU-SWT) and one in solution form (CU-SWHJ)] were manufactured by Hong Kong Institute of Biotechnology (GMP approved manufacturer in Hong Kong) based on the protocol described in Pharmacopoeia of PRC (2005) using the above 4 herbs. Four different SWT commercial products in both solid and liquid formulations (SWT-1, 2, 3 as solid dosage forms and SWHJ as liquid formulation) were purchased from TCM product stores in Hong Kong and Shenzhen, P.R. China.

2.2. BAPK method

The six SWT products were evaluated based on the proposed BAPK approach. The following sequential steps were carried out for identifying and compare the potential relevant QC markers in these SWT products: (1) literature search (data mining) to identify active components, (2) quantitative analysis of the identified active components in each SWT product, (3) determination of *in vitro* dissolution and metabolism of the components under simulated gastrointestinal conditions, (4) identification of absorbable marker(s) via *in vitro* Caco-2 cell model, i.e. determination of parent compound and metabolites with good permeability, (5) stability testing of the identified marker(s) from (4).

For animal and human experiments (obtaining gut homogenates containing gut microsomes), the protocols were approved by the Animal Ethics Committee of the Chinese University of Hong Kong and Human Ethics Committee of the First Affiliated Hospital, Sun Yat Sen University, respectively.

2.2.1. Data mining to identify active components

The major components in the four raw herbs that composed of SWT were identified through both Chinese and English publication databases including China Journal Net (CJN) and Wang Fang Data (for articles written in Chinese), Ovid Web Gateway and SciFinder Scholar (for articles written in English) after careful review. Then two criteria were used in selecting the potential bio-active markers for further evaluation by the BAPK approach: (1) the compounds chosen must have reported activity from either *in vivo* or *in vitro* studies and (2) the content of these compounds could be quantitatively detected with LC/MS/MS assay method based on previous published report on structure and content. Since the initial components selected were based on their activity already reported in the literature, if these compounds are found to be absorbable, they could possess potential activity *in vivo*.

2.2.2. Quantitative analyses of bio-active components in SWT products

To identify the bioactive components, a sensitive and selective LC/MS/MS method capable of simultaneous determination of the nine components was developed and validated (Wang et al., 2009). To compare the content of the bioactive compounds in the SWT products, samples from each SWT products were prepared as follows.

For the reference product, CU-SWT, 100 mg of SWT powder was accurately weighed and mixed with 9.5 mL of extraction solvent (50% methanol). The mixture was shaken for 5 min, and then sonicated for 30 min. After cooling to room temperature, the mixture was made up to a final volume of 10 mL with 50% methanol. For CU-

SWHJ, which is in liquid form, the sample was diluted 100 times and filtered before assay. For the commercial SWT products, the samples were extracted with reference to their preparation methods described in their product information brochure. Briefly, for SWT granule products, 100 mg SWT granules extracted with 10 mL of water in 60 °C water bath for 30 min. For SWT powder products, 100 mg of powder were mixed and then extracted with 10 mL of 50% methanol under sonication for 30 min. For SWT liquid products, they were diluted with 50% methanol. All extracted samples were filtered through a 0.45 μm nylon filter before injection into LC/MS/MS for analyses.

2.2.3. Dissolution and metabolism studies

The dissolution tests for all SWT products were carried out according to the USP dissolution test using a rotating paddle apparatus (Erweka DT80, Heusenstamm, Germany) (USP, 2008). About 4 g of different SWT products were added to the initial dissolution medium containing 900 mL hydrochloric acid solution (pH 2.0) with paddle stirred at 100 rpm. After 120 min, the medium pH was adjusted to 7.0 by sodium hydroxide solution. At 0, 10, 20, 30, 60, 90, 120, 150, 180, 240 min, a 1 mL sample was withdrawn, followed by replacing with the same volume of pre-warmed blank dissolution medium. Each experiment was carried out in triplicate. The collected sample was immediately centrifuged at 13,200 rpm for 5 min and the supernatant was analyzed using the developed LC/MS/MS method described in Section 3.2. The similarity factor (f_2, see Eq. (1)) was utilized to compare the dissolution profile of different SWT products. The dissolution profiles will be considered similar when f_2 value is between 50 and 100.

$$f_2 = 50 \times \log_{10}\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

Based on chemical structures and literature data, the potential metabolic reactions of the SWT bio-active components in the gut included hydrolysis (for PF, FA and GA) and intracellular glucuronidation, sulfation and methylation. Thus, reactions in Caco-2 lysate as well as human intestinal tissue homogenized mixture (HTH) were conducted. Reactions in rat intestinal tissue homogenized mixture (RTH) were also performed for comparison.

Caco-2 cells (passage number 37–42) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 100 unit/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C. Cells were then seeded onto the Transwell® inserts (Corning Costar Co., NY) coated with a collagen layer in 6-well plate at a density of about 3×10^5 cells/well and cultured for 21 days (Zhang et al., 2004). The medium was changed every other day prior to the permeation studies. Transepithelial Electrical Resistance (TEER) was used to monitor the integrity of the monolayer. The Caco-2 cell monolayers were collected after 21 days of culture. The cells were lysed in cold Tris buffer (50 mM, pH 7.4) followed by 15 min sonication in ice-cold water bath.

For RTH studies, two rats were sacrificed under anesthesia. The whole small intestine was then removed and washed with the ice cold Tris buffer (50 mM, pH 7.4). The tissue was cut into small pieces and mixed with 1 volume of cold Tris buffer. Afterwards, the mixture was homogenized and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was used for protein quantification and incubation for the metabolic reactions. The HTH was prepared similar to RTH as described above. The protein contents of the Caco-2 lysate, HTH and RTH were determined by Bio-Rad protein assay kit (Hercules, CA, US).

In vitro intestinal metabolism experiment was performed by co-incubation of individual active component, or SWT extract, or mixture of active components (similar to the amount present in the SWT extract) at 37 °C with Caco-2 lysate, RTH or HTH. The tested compounds, SWT extract or the mixture at the final concentrations of 10 μg/mL were preincubated with 1 mg/mL protein in reaction buffer (100 mM Tris, 0.08 mM $MgCl_2$ and 0.25 μg/mL alamethicin) for 5 min at 37 °C. The cofactors such as uridine 5′-diphosphoglucuronic acid (UDPGA), (adenosine 3′-phosphate 5′-phosphosulfate) PAPS or S-(5′-adenosyl)-L-methionine (SAM) were added for the glucuronidation, sulfation and methylation of the test compounds, respectively. The incubation was terminated by adding 200 μL of acetonitrile. After centrifugation, the supernatant was collected and stored at −80 °C until analysis by LC–MS (selected ion monitoring, SIM mode).

2.2.4. Identification of absorbable markers

Using *in vitro* Caco-2 cell monolayer model, the relevant markers (those with good permeability) were identified from the following preparations: (a) individual components, (b) mixture of these components at equivalent concentrations to that existed in each SWT product (determined according to quantitative analysis of SWT product described above), and (c) the extract of each SWT products.

The Caco-2 monolayer cells were prepared using the same method as describe above. All transport studies were conducted at a TEER values above 600 Ω cm^2 (after subtracting the background value of the transwell) (Zhang et al., 2004).

The permeability studies were performed in transport buffer containing phosphate buffer saline (PBS) (pH 7.4) with supplement of calcium (0.9 mM) and potassium chloride (0.9 mM). The Caco-2 cells monolayer was rinsed twice and pre-incubated with the transport buffer at 37 °C for 15 min. Pre-warmed 1.5 mL of transport buffer containing the individual test compound or mixture or SWT extract was loaded to the apical, i.e. donor chamber. At the same time, 2.6 mL drug-free transport buffer solution was loaded to the basal side (receiver chamber). Aliquots of 0.3 mL samples were withdrawn from the receiver chamber at 10, 20, 30, 60 and 90 min. After each sampling, the same volume of blank transport buffer was replaced. The integrity of the monolayer was checked by measuring the TEER at the beginning and end of the experiment.

To estimate the intracellular content of the nine components, the Caco-2 cells were collected after the permeability test and rinsed with 50 mL ice-cold saline three times. The cells were then lysed with 5 mL methanol by sonication for 15 min. All samples collected for the permeability study and intracellular content determination were centrifuged for 10 min at 12,000 rpm. The supernatant was removed and stored at −80 °C until analysis.

The apparent permeability coefficient (P_{app}, cm/s) was calculated as Eq. (2).

$$P_{app} = \frac{(dC/dt) \times V}{A \times C_0} \quad (2)$$

where "dC/dt" is the change of the drug concentration in the receiver chambers over time, "V" is the volume of the solution in the receiver chambers (cm^3); "A" represents the membrane surface area (4.7 cm^2); "C_0" is the initial or loading concentration in the donor chamber.

2.2.5. Stability tests

Stability test was preformed for the five soluble and potentially absorbable components (GA, SA, PF, FA and Lig) using two representative products, CU-SWT and CU-SWHJ. These products were initially stored in three plastic bottles containing desiccant and then stored at 4 °C, 25 °C and 40 °C and protected from light. Samples were then taken for analysis at 1, 2 and 3 months storage time (duration designated within the period of the grant proposal).

Table 1
Concentrations of the nine compounds found in CU-SWT, CU-SWHJ, commercial SWT and CU-single herb products.

	Concentration (mg/g) ± SD (n = 3)						
	GA	SA	PF	FA	Lig	Bu	CXQ, PO, Cat
(a) Products in solid dosage form							
CU-SWT	0.987 ± 0.089	0.075 ± 0.002	8.651 ± 0.240	0.525 ± 0.015	1.127 ± 0.224	N.D.	N.D.
SWT1	0.131 ± 0.009	0.044 ± 0.002	0.054 ± 0.004	0.020 ± 0.002	0.037 ± 0.002	N.D.	N.D.
SWT2	0.506 ± 0.070	0.059 ± 0.001	1.930 ± 0.143	0.154 ± 0.041	0.034 ± 0.003	N.D.	N.D.
SWT3	2.527 ± 0.043	0.045 ± 0.001	16.59 ± 1.14	0.383 ± 0.013	0.377 ± 0.032	N.D.	N.D.
	Concentration (μg/mL) ± SD (n = 3)						
	GA	SA	PF	FA	Lig	Bu	CXQ, PO, Cat
(b) Products in liquid dosage form							
CU-SWHJ	307.9 ± 13.9	26.5 ± 0.9	2794 ± 225	173.3 ± 10.5	33.2 ± 1.0	N.D.	N.D.
SWHJ	32.7 ± 1.5	2.07 ± 0.04	5795 ± 366	1.62 ± 0.47	1.02 ± 0.04	0.42 ± 0.03	N.D.

Remark: N.D. = not detectable.

The SWT product containers were left at room temperature for 1 h before samples were obtained for analysis. The contents of the identified components were analyzed by the LC/MS/MS method described previously.

2.3. Data analysis

One-way ANOVA was utilized to compare the contents of the active components in CU-SWT and CU-SWHJ at baseline and 1, 2, 3 month samples. The P_{app} values of individual compound present in different products were also compared. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Active components of SWT from data mining (literature search)

A total of nine compounds were identified based on extensive literature search for the 4 herbs that composed the SWT products, namely ferulic acid (FA), senkyunolide A (SA), ligustilide (Lig), butylphthalide (Bu), paeoniflorin (PF), gallic acid (GA), paeonol (PO), catalpol (Cat) and ligustrazine (CXQ).

3.2. Quantitative analysis of the identified active components in each SWT product

Of all six SWT products investigated, only five out of the nine components, namely GA, FA, PF, Lig and SA, were present in quantifiable amount, while Bu could be only detected in one product (SWHJ). All others including PO, CXQ, and Cat were not detectable, most likely due to poor solubility or low content. Large variations in the contents among the six SWT products were observed (see Table 1). Three factors that might have contributed to the variations include the source of the raw herbs, the different preparation method, and differences in formulations (Wang et al., 2009). Lig and SA were poor water soluble compounds which could not be extracted effectively by decoction with water. In addition, Lig was very unstable at room temperature or under the light (Cui et al., 2006; Yi et al., 2007).

3.3. Determination of in vitro dissolution and metabolism

The dissolution profiles of identified active components (GA, FA, Lig, PF, and SA) from the three commercial solid dosage form SWT are shown in Fig. 2. Based on the f_2 value, no dissolution profiles were similar among these SWT products of the five compounds (see Table 2). Lower percent dissolutions for FA, SA and Lig in CU-SWT in comparison to the marketed SWT products (SWT1 and SWT2, see Fig. 2) were observed. There was a substantial reduction in dissolution of GA after 120 min at pH 7.0 which indicated that the GA might not be stable when it passes from stomach to small intestine due to a change of pH from about 2 to 7.

Unexpectedly, Lig could not be detected in SWT2 in the dissolution study despite of similar content observed as shown in Table 1. This could be due to differences of other components in SWT1 compared to SWT2 formulation since formulation differences are known to alter bioavailability of certain substances.

No gastro-intestinal hydrolysis of PF, FA or GA was found. No sulfation, glucuronidation and methylation were observed except for FA. The sulfation and glucuronidation of FA however occurred only with pure compound FA (Caco-2, Caco-2 lysate, RTH and HTH studies) but not when FA was present in a mixture (as extract or compound mixture). Such discrepancy between the pure compound and mixture suggested that the gastrointestinal metabolism of FA may be inhibited by other co-occurring components. These data appear to indicate that the extract from the whole TCM formula (containing multiple components) could yield different results from that of the pure individual components and should be utilized when performing BAPK studies.

3.4. Identification of absorbable marker(s)

Among the five quantifiable components, PF and GA were poorly permeable. The poor permeability of these 2 compounds may be related to low dissolution (GA) and hydrophilicity (PF, see below).

The P_{app} values of the remaining three components (both as individual pure compound and SWT extracts) are presented in Fig. 3. The P_{app} values of FA from CU-SWT extracts were similar between individual components or mixtures. However, the P_{app} values of SA and Lig in CU-SWT extracts were 74% and 80% higher than those from individual components, respectively. These results suggested a potential enhancement in absorption/transport of SA and Lig by those co-occurring components in CU-SWT extract (not present in standard mixture) during the transport process across the Caco-2 monolayer. In other SWT products, the P_{app} values of SA and Lig

Table 2
Similarity factors (f_2) among SWT products of SWT1, SWT2, and CU-SWT.

	SWT1 *vs* SWT2	SWT2 *vs* CU-SWT	SWT1 *vs* CU-SWT
FA	32.2	25.5	24.1
PF	33.9	32.6	32.2
Lig	N.A.	N.A.	7.5
SA	7.0	18.4	21.6
GA	44.7	24.9	32.0

N.A.: not applicable due to undetectable compounds.

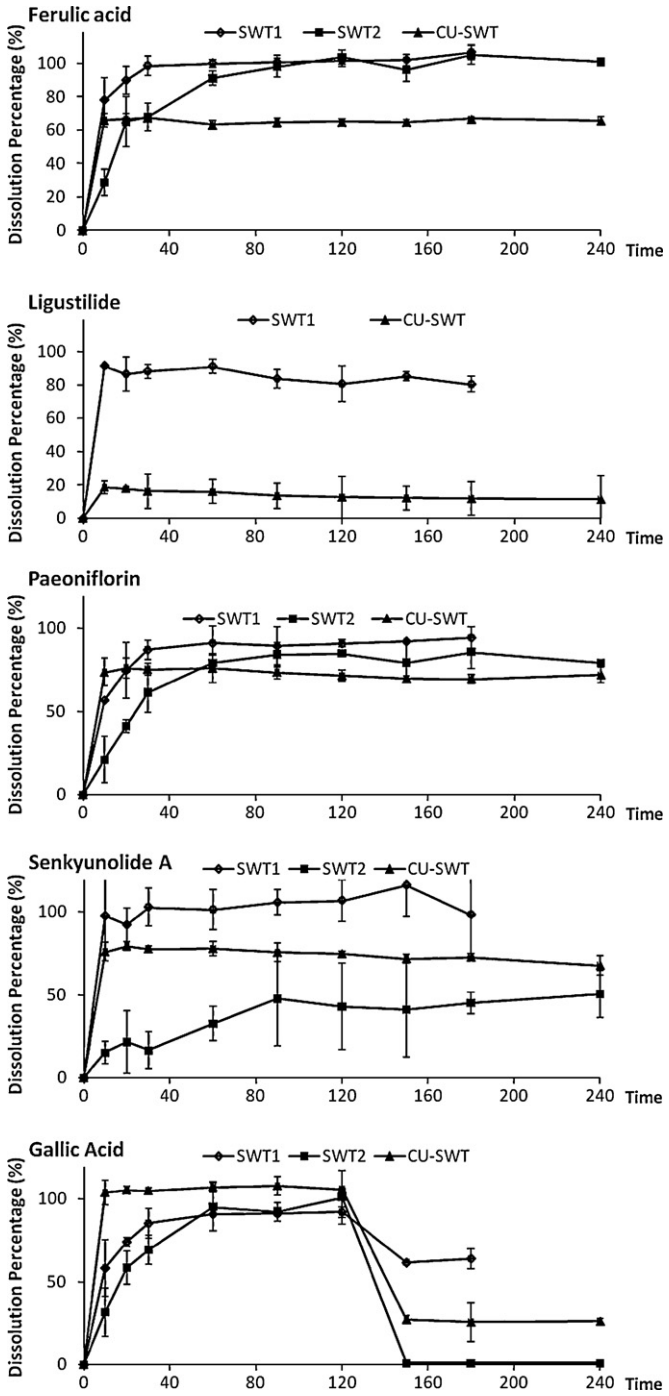

Fig. 2. Dissolution profiles of three solid dosage products of SWT (SWT1, SWT2 and CU-SWT).

were not significantly different whether they are present in mixture or individual pure form.

3.5. Stability results and relevant marker for SWT

The five soluble and quantifiable components in CU-SWT and CU-SWHJ, namely GA, SA, PF, FA and Lig, when tested for their storage stabilities at 4, 25 and 40 °C, all were found to be relatively stable at both low and room temperatures over a 3 months period. Under high temperature (40 °C) storage for 3-month, SA, FA and Lig were found to be degraded significantly for CU-SWHJ, but not for CU-SWT (see Figs. 4 and 5).

4. Discussion

The present study demonstrated that a proposed *in vitro* BAPK method for identifying potential relevant stability markers for TCM products is feasible and can be successfully accomplished for SWT products. The results of the SWT *in vitro* BAPK permeability studies are generally consistent with that observed in other *in vivo* studies. The poorly permeable components (PF, Cat, GA) identified in our study are consistent with their reported low oral bioavailability or low plasma concentration observed in animals or human subjects (Wang et al., 2008; Lu et al., 2009; Konishi et al., 2005). Those with good permeability (FA, Lig, and SA) observed in our study also corresponded to their good absorption reported in *in vivo* studies (Li and Bi, 2003; Yan et al., 2008). Based on the current findings, PF, the SWT marker recommended by CP 2005 (The State Pharmacopoeia Commission of P.R. China, 2005), was found to be poorly permeable. PF is a glucuronide conjugate with highly water soluble which may be responsible for the low permeability and bioavailability (Class 3 according to the biopharmaceutics classification systems). In addition, the *in vitro* metabolism study showed that it cannot be hydrolyzed to its aglycone which may be more permeable and also bioactive. Thus PF is not suitable to be used as a relevant marker for SWT. FA, Lig, or SA either alone or in combination would be preferred since they are capable to be absorbed leading to *in vivo* bio-activity. Since Lig was reported not stable (Cui et al., 2006; Yi et al., 2007), a stabilizer was utilized in CU-SWT and was stored under condition of protection from light. According to the BAPK results and stability data, FA, Lig, and SA were found to be both permeable and stable over 3 months at room temperature. Thus, these three compounds can be potentially designated as relevant marker(s) suitable for QC of SWT products.

When comparing different SWT products, the contents of the three "relevant" markers were found to vary significantly, especially for FA and Lig. The Lig could not even be detected in SWT2. The differences of other components in these SWT products may be one of the reasons. However this needs to be further confirmed. In addition, the dissolution profiles of these products are quite different according to the f_2 calculation results, which could attribute to the different physicochemical properties of the drug and/or the formulation factors. The results suggested that these SWT products may exhibit different pharmacokinetic process *in vivo* (Wang et al., 2007). Such product-to-product variation or batch-to-batch variation for the same product (as we have observed in the manufacturing of our reference SWT products) could result from selection of herbs and manufacturing process. Thus, the identification of the relevant markers could provide a step towards quality improvement of TCM products. In the future, QC of TCM can be based on a designated range of variation for content and uniformity of the "relevant" markers, just like the "standards" designated by USP, for the conventional drugs.

Although the present study demonstrated the feasibility of our BAPK method for natural products including CM or TCM with known active components (e.g. from prior study reported in the literature), the applicability of this approach may be extended to those products in which the active components are unknown. Under such condition, a general qualitative approach could be utilized (with the relative ratios of the potential "relevant" markers (i.e. permeable markers assumed to be active) obtained from the sequential BAPK steps and using a "generic" LC–MS/MS assay capable of separating the different water soluble components for the extract).

The data generated from our BAPK method (*in vitro* technique of dissolution, metabolism, and permeation) may not represent the true results from *in vivo* conditions. Nevertheless, these data should allow *in vivo* confirmation of potential relevant markers to be more feasible (e.g. quantitative determination of specific potential mark-

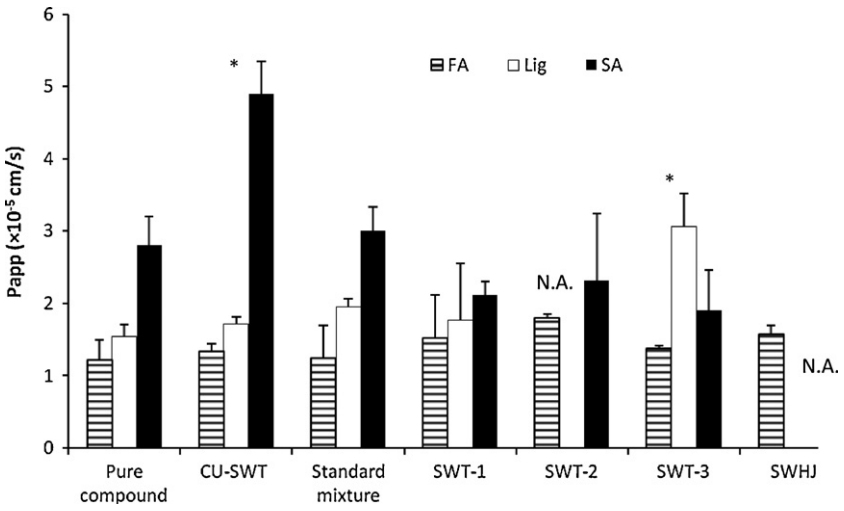

Fig. 3. Comparison of P_{app} values of ferulic acid, Z-ligustilide and senkyunolide A obtained from pure compound form *vs* that from their standard mixture/SWT product forms in Caco-2 monolayer model. $^{*}p < 0.05$ (one-way ANOVA); N.A.: not applicable due to low content detected.

ers from plasma samples). While the relevant markers identified by the BAPK method may not represent the overall activity of SWT, these identified markers are expected to relate to some *in vivo* activity, if not the entire activity of the product.

The BAPK approach as demonstrated from our pilot work is relatively labor intensive. However, high throughput systems for dissolution, permeability or *in vitro* metabolism determinations have already been utilized in large pharmaceutical companies. By using such a high throughput system, it is possible that in the future relevant markers for TCM products can be identified rapidly, and then followed by subsequent *in vivo* specific confirmation as desired.

While the technique for individual steps of our BAPK method is not new, the use of such techniques in a sequential manner as described in our study for use in identifying potential relevant markers for purpose of quality control of CM has not been systematically investigated. The present study demonstrated that this BAPK approach is feasible for SWT. Such *in vitro* method may prove to be useful and practical in identifying relevant marker(s) for quality control of other TCM products in the future, especially if a high throughput system is utilized concurrently.

As a conclusion, the present BAPK method appears to be a feasible approach in identifying potential relevant markers for SWT, a well known TCM formula for the treatment of woman's disease.

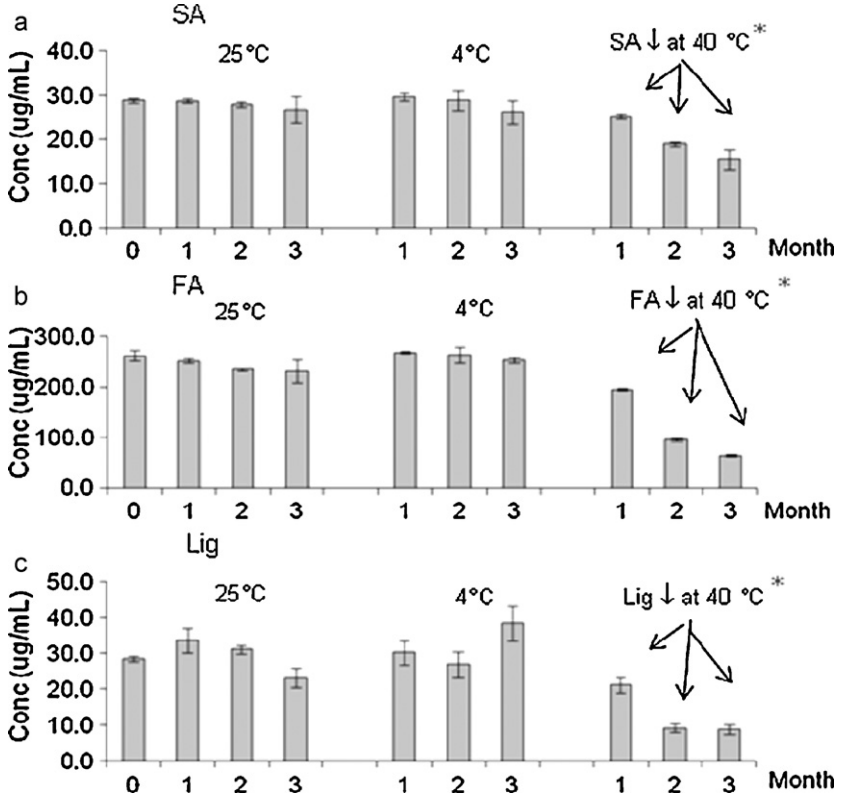

Fig. 4. Stability results of CU-SWT (liquid dosage form of SWT). $^{*}p < 0.05$ (one-way ANOVA).

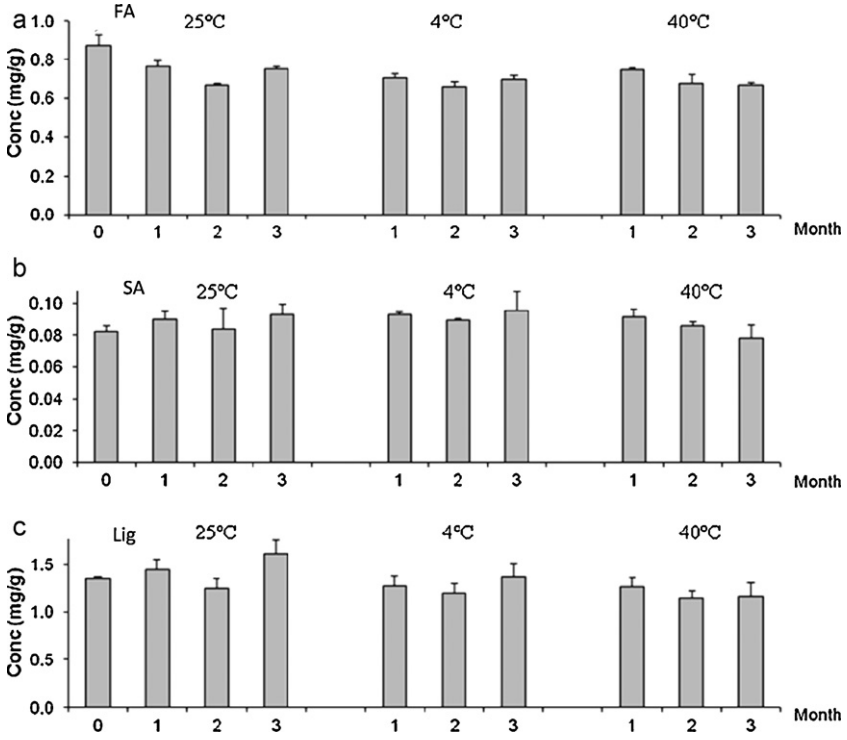

Fig. 5. Stability results of CU-SWT (solid dosage form of SWT).

Such *in vitro* method may prove to be useful and practical for identifying relevant marker(s) of other TCM products for QC, especially if high throughput system can be set up in the future.

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