Quickly quantifying the dissolution fingerprints of compound Danshen dropping pill by HPLC

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Abstract: To firstly accurately overall determine the all components of Compound Danshen Dropping Pills (CDDP) and assess how about the authentic quality is for each bath of samples through a quantified fingerprint that only can qualitatively identified which kind of a herbal or Traditional Chinese Medicine may be. The systematically quantified fingerprint method (SQFM) was employed to identify that the qualities of S4 and S6 were belong to best (grade 1), those of S3, S5, S7, S8 and S9 belong to better (grade 2), those of S2 and S10 belong to good (grade 3), and only S1 falling to fine (grade 4) where those were all with 19 fingerprint peaks. On the basis, the important HPLC dissolution fingerprints (DFPs) of CDDPs were meticulously established to investigate the dynamic process in water, 0.1 mol/L hydrogen chloride (HCl) and 0.5% sodium dodecyl sulphate (SDS), respectively, in which SQFM executed the excellently quantified analyses for the DFPs. During the evaluation, the eighth time points of DFPs were selected as the standards for all dissolving to calculate how high are the macro quantitative similarity (P_m) of DFPs for the other different time points. Finally in terms of Weibull function, $\ln\ln [1/(1–P_m)]=–0.5951x^2+3.8146x–4.5195$ ($r=0.9974$), $\ln\ln [1/(1–P_m)]=–0.5555x^2+3.5814x–4.6126$ ($r=0.9972$) were acquired in water and in 0.1 mol/L HCl solution respectively, but without in the SDS solution. In order to specially monitor the dissolution dynamic changes of the important fingerprints to guide the preparation technology and improve their bioavailability, We investigated the four strong peaks of CDDP–DFP and calculated out the fitting equations as follows, $\ln\ln [1/(1–A_i/A_{max})]=y$, the first one (No.6, Protocatechualdehyde), $y=–0.5603x^2+3.4516x–3.8974$ ($r=0.9988$); the second one (No.19, Salvianolic acid B), $y=–1.7127x^2 +9.6655x–11.947$ ($r=0.9897$); the third one (No.4, Danshensu), $y=–0.4239x^2+3.0436x–3.6276$ ($r=0.9985$); the forth one (No.17, unkown), $y=–0.5019x^2+3.3706x–4.1053$ ($r=0.9979$), which indicated Salvianolic acid B with a prominent discrepancy from others. The quantified HPLC fingerprint can perfectly both quantitatively reflected the authentic quality of CDDPs and accurately overall quantitatively measure the dissolution dynamic varieties *in vitro* to provide the important information for bioavailability, in which water is proven to be the best dissolution medium for CDDP whose $T_{50}=4.1$ min (released 50%) and $T_{90}=8.0$ min (released 90%).

Key Words: CDDP; SQFM; dissolution fingerprints; macro qualitative similarity (S_m); macro quantitative similarity (P_m)

Introduction

The right way of quality control of Herbal drug (HD) and Traditional Chinese Medicine (TCM) will be make use of all their constituents quantified simultaneously by chromatographic fingerprints, for which our research group have been finishing a series of theories and techniques and also computer software for scientific evaluation (1-3). The investigatement of all component dissolution is one of the

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Table 1 The practical quality grade assessed by SQFM

Para.	I	II	III	IV	V	VI	VII	VIII
$S_m \geq$	0.95	0.90	0.85	0.80	0.70	0.60	0.50	$S_m < 0.5$
$P_m/\% =$	95-105	90-110	80-120	75-125	70-130	60-140	50-150	0-∞
$\alpha \leq$	0.05	0.10	0.15	0.20	0.30	0.40	0.50	>0.50
Grade	1	2	3	4	5	6	7	8
Quality	best	better	good	fine	moderate	common	defective	inferior

most important characteristics for a HD or TCM, for which directly affects the drug absorption and bioavailability (4,5). According to the related instruction principle, most of TCM needn't do the dissolution test in its quality control but detect the disintegration time (6-11), for which may make TCM quality control blind and nonscientific in some extents. Compound Danshen Dropping Pills (CDDP) is composed of *Radix Salviae Miltiorrhiae*, *Radix Notoginseng* and *Broneolum Syntheticum* (12), which is the most important modern TCM with a nearly 4 billion yuans production per year. Today, CDDP is most widely used as an OTC medicine for treating, preventing before the cardiovascular and cerebrovascular disease and also for the emergency medical treatment (13-16). Since 1997, CDDP came into Food and Drug Administration (FDA) and enter the II, III stage of IND as the first TCM. The sales of CDDPs accounted more than 3 billion yuans per year in nearly ten years and ranked the first in TCM market (17-20). It is very needed further studying the new quality control method from the important indicatrix of CDDP to reflect the dissolution process for providing the previous information of its absorption *in vivo*, though there was chromatographic fingerprint to qualitatively examine CDDP consistency in fingerprint distribution (21) that have not been the best adequate quality control method.

The paper presented here expounded on employing the SQFM to quantitatively identify both quality and the dissolution process changes *in vitro* tests, in order to ensure CDDP stability and reproducibility in quality, in which the HPLC-FPs and DFPs *in vitro* were simultaneously established to possibly comprehensively analyze HDs or TCMs as the most ideal quality control method.

Applying the SQFM to assess CDDP-FPs (22,23)

It was a very effective way to control the TCM by quantified fingerprint technology because of TCM being the complicated science characteristics. In order to control the whole quality

for CDDPs, SQFM performed the analyses of CDDPs from the macro qualitative similarity(MQLS) S_m that could give a full view of quantity and distribution ratio for TCM-CFP, seen Eq.[1], the macro quantitative similarity (MQTS) P_m which could make both the big and small fingerprint peaks isobar, described in Eq.[2], where x_i and y_i are each peak area in sample and reference respectively, and the variation coefficient of α see Eq. [3] which was able to control the range limits of the fingerprint peak leveling differences between SFP and RFP. The multivrate fingerprints simultaneously identified rule had been listed in *Table 1*, which was obviously proven a good way in many a practical CFP analysis.

$$S_m = \frac{1}{2}(S_F + S_F') = \frac{1}{2}\left(\frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2}\sqrt{\sum_{i=1}^{n} y_i^2}} + \frac{\sum_{i=1}^{n} \frac{x_i}{y_i}}{\sqrt{n\sum_{i=1}^{n}(\frac{x_i}{y_i})^2}}\right) \quad [1]$$

$$P_m = \frac{1}{2}(C + P) = \frac{1}{2}\left(\frac{\sum_{i=1}^{n} x_i y_i}{\sum_{i=1}^{n} y_i^2} + \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} S_F\right) \times 100\% \quad [2]$$

$$\alpha = \left|1 - \frac{P}{C}\right| \quad [3]$$

Experimental section

Instruments

The analysis was performed on an Agilent 1100 series LC system (Hewlett Packard, CA, USA) consisting of a diode array detector, a low-pressure quaternary pumps, an online degasser and an autosampler. All data acquired were preceded by ChemStation workstation (Agilent technology Inc.). KQ-50B ultrasonic bath (Kunshan ultrasonic instrument company limited), RE52 rotary evaporator (Yarong biochemistry instrument factory, Shanghai, China)
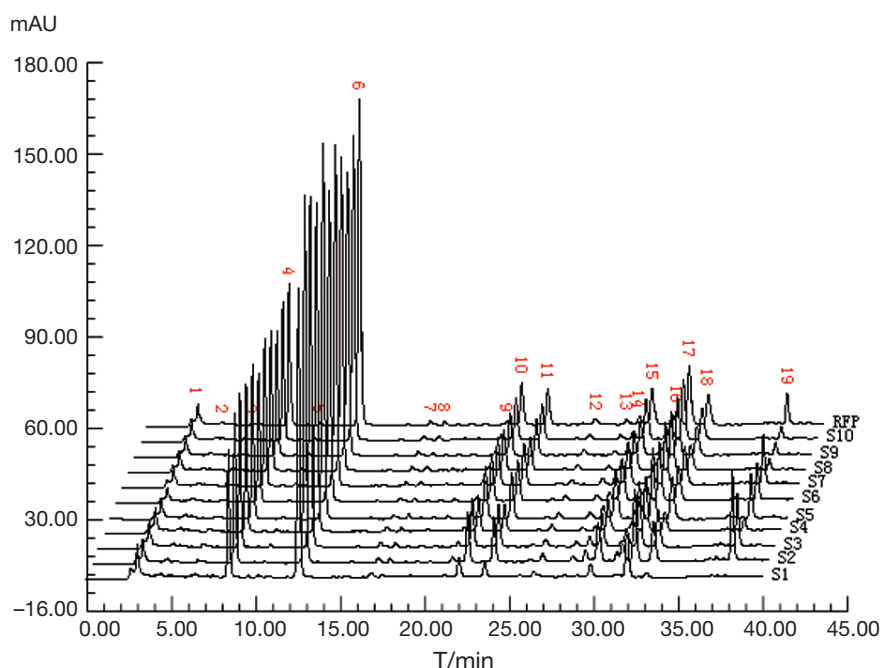
Figure 1 The HPLC fingerprints of 10 batches of CDDPs and their average reference fingerprint (RFP)

was used during the analysis.

Chemicals

Methanol (purchased from Yuwang industry limited company, Shandong, China) and glacial acetic acid (provided by Kemiou chemicals developing center, Tianjin, China) were both of HPLC grade. Water used for buffer solution was deionized and other chemicals were of analytical grade. PHA, DSS and SAB were all purchased from National Institute for the Pharmaceutical and Biological Products (Beijing, China, for quantified grade). CDDPs wrere from Tianjin Tasly Pharmaceutical Co.,Ltd (Tianjin, China).

Sample and standard preparation

Sample solutions

10 pills were accurately weighed and extracted with 50 mL 50% (*V/V*) ethanol for 1 hour. Then the filtrate was concentrated to approximately 10 mL under reduced pressure in a rotary evaporator. Finally, 50% (*V/V*) methanol was added to make the volume of 25 mL.

Standard solutions

PHA of 5.0 mg was accurately weighed and 50% (*V/V*)

methanol was added to make 25 mL exactly to serve as the standard solution after shaking. DSS and SAB solutions were prepared in the same way.

Chromatographic condition

Chromatographic separation was carried out on a CenturySIL C18 BDS column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of 1% acetic acid in water (A) and methanol with 1% acetic acid (B) using a gradient program of 10-32% B in 0-15 mins; 32-45% B in 15-30 mins; 45-80% B in 30-50 mins. The flow rate was 1.0 mL/min. Detection wavelength was set at 290 nm to record the chromatograms. The column temperature was kept at (30±0.15) ℃. An aliquot of 10 μL sample solution was loaded for analyses.

Results and discussion

Method suitability tests

The DSS, PHA, SAB standard solutions and sample solution were injected for 10 μL respectively and recorded the chromatograms. By comparing the retention times of peaks and ultraviolet spectrograms in the sample and standard chromatograms, we drew the conclusion that the peak of 8.64 mins was from DSS (peak 4), 12.90 mins was from PHA (peak 6) and the peak of 37.52 mins was from

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Table 2 The genius quality results of 10 batches of CDDPs quantified by SQFM

Para.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	RFP
S_m	0.921	0.985	0.991	0.994	0.973	0.992	0.991	0.991	0.972	0.99	1.0
P_m	75.2	110.8	94.4	95.5	96.5	98.7	93.6	106.5	109.4	115.6	100
α	0.172	0.054	0.052	0.018	0.076	0.01	0.007	0.023	0.057	0.051	0
Grade	4	3	2	1	2	1	2	2	2	3	1
Quality	fine	good	better	best	better	best	better	better	better	good	best

S_m, macro qualitative similarity; P_m, macro quantitative similarity; α, coefficient of variation

SAB (peak 19). The above chromatograms were shown in *Figure 1*. For the good separation with the neighboring peaks and its adequate retention time, the peak of PHA was selected as the reference peak.

Injection precision test

The sample solution was injected six times successively and recorded the chromatograms. The RSDs of the relative retention times of all fingerprint peaks were below 1.0% and the RSDs of relative peak areas were all below 3.0% when PHA was selected as the reference, which indicated the method with a good injection precision.

Stability of the solution

The sample solution was injected after preparing for 0, 2, 4, 8 and 12 hrs, and recorded the chromatograms respectively. The RSDs of the relative retention times of all fingerprint peaks were less than 2.0%, and the relative peak areas were less than 3.0%, in which PHA was selected as the reference, indicating that the sample solution was stable within 12 hours.

Method reproducibility

Six replicates of CDDP were prepared according to the procedure described in section "Sample and Standard Preparation" to inject and record the chromatograms. The RSDs of the relative retention time of all peaks were below 1.0%, and that of the relative peak area was within 3.0%, which showed that the method reproducibility was satisfactory.

The establishment and evaluation of CDDP-FPs

The sample solutions of 10 batches of CDDPs were prepared according to the procedure described above, loaded into the HPLC system and recorded the chromatograms, seen *Figure 1*. By comparing the ten CDDP-FPs, we marked 19 co-possessing peaks, in which the appearance ratio of each common peak was only needed more than 70%. The RFP was set up in terms of calculating the mean of the 10 batch of CDDP fingerprint signals, for which severed as the norm to evaluate their quality results listed in *Table 2*. In regard to the 10 batches of CDDPs, the qualities of S4 and S6 were belong to best (grade 1), those of S3, S5, S7, S8 and S9 belong to better (grade 2), those of S2 and S10 belong to good (grade 3), and only S1 falling to fine (grade 4) with lower contents of all 19 peak components and a larger variance coefficient. Hence the complicated TCM sample were comprehensively briefly quantified to give the authentic evaluation results, in which SQFM was demonstrated to be the best way for identifying herbal drugs and TCM quality.

The development of DFPs

In dissolution test, a Chp cup method was used at a rotational speed of 100 rpm and at a temperature of 37.0±0.5 ℃. Dissolution media (100 mL) were respectively the water, 0.1 mol/L hydrogen chloride (HCl) and 0.5% sodium dodecyl sulphate (SDS) which were de-gassed. Accurately weighed 20 pills of CDDP were subsequently added to each of the dissolution cups. At 2, 5, 9, 14, 20, 30, 45, and 60 min, samples were pipetted and replaced with 2 mL volume of dissolution media. The solution was immediately filtered through 0.45 μm Millipore filter and then determined by HPLC to record the chromatograms. Being low concentration of sample, the results showed that there were 1, 1, 2 peaks were missing in 0.1 mol/L hydrogen chloride (HCl), 0.5% sodium dodecyl sulphate (SDS) and water, respectively, seen *Figure 2*.

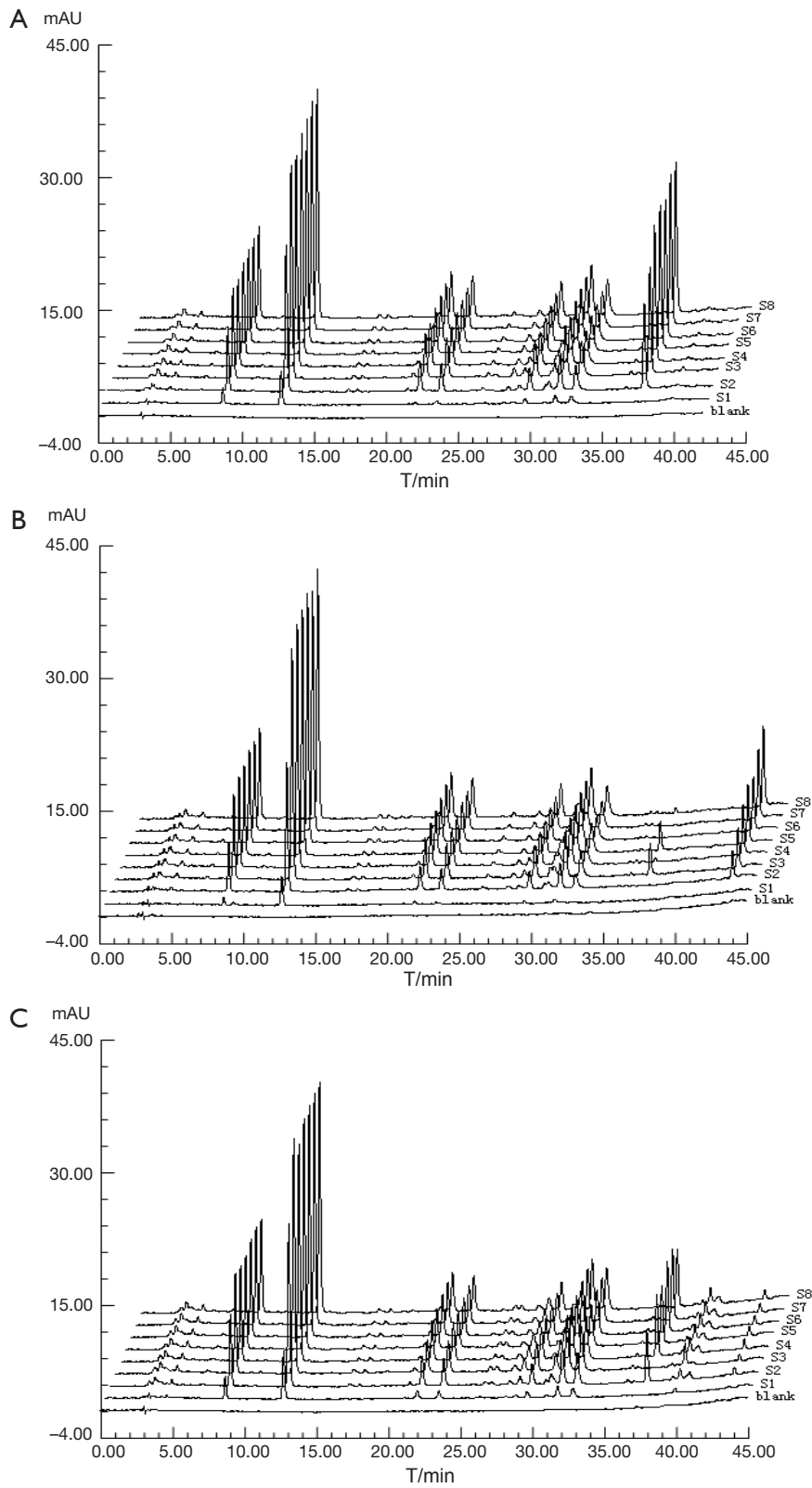In this study, S_m and P_m were chosen to evaluate the

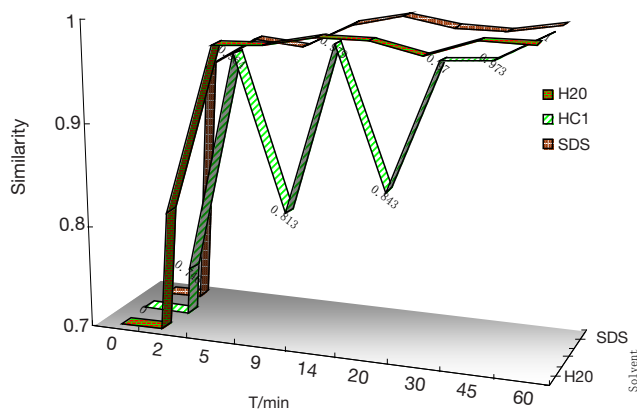**Figure 2** The DFPs of samples in A. water, B. 0.1 mol/L HCl and C. 0.5%SDS media for one hour

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Figure 3 The S_m changing diagrams of S10 dynamically dissolution fingerprints in three kinds of dissolution media

Figure 4 The P_m changing diagrams of S10 dynamically dissolution fingerprints in three kinds of dissolution media

DFPs and judge the dissolution stability both from the entirety and the mainly important indicatrix. All the chromatograms integrated of CDDP in three different dissolution media were loaded into the 4.0 edition software:the digitized evaluation system of the supper information characteristcs for TCM-FPs invented by Guoxiang SUN, etc., in which we took the chromatogram at final time point for the referential fingerprint dissolved thoroughly. The fingerprint quantity evaluation results were seen in *Figure 3* to display that CDDP was dispersed very slowly in 0.1 mol/L HCl, perhaps happening to degradation of some components from the S_m in 9 and 20 mins points. Comparing the sum of peak area of three different dissolution media, water finished the quick dissolution rate, 0.5% SDS solution was secondary and that was the last in 0.1 mol/L HCl according to the P_m of the three different dissolution media, plotted in *Figure 4*.

Fitting rule of P_m in CDDP dissolution process

The integrated releasing behavior of CDDP could further be investigated by constructing the fitting equations. According to Weibull function, dissolution equations were fitted by lnln $[1/(1-P_m)]$ via ln$t$ to draw the results as fellows, in water: lnln $[1/(1-P_m)]=-0.5951x^2+3.8146x-4.5195$, $r=0.9974$; In 0.1 mol/L HCl solution: lnln $[1/(1-P_m)]=-0.5555x^2+3.5814x-4.6126$, $r=0.9972$; and the fitting equation in 0.5% SDS solution was not shown, because there were up and down changes leading to a discordant tendency. Finally, water was chosen as the optimal dissolution media for its well dissolution stability
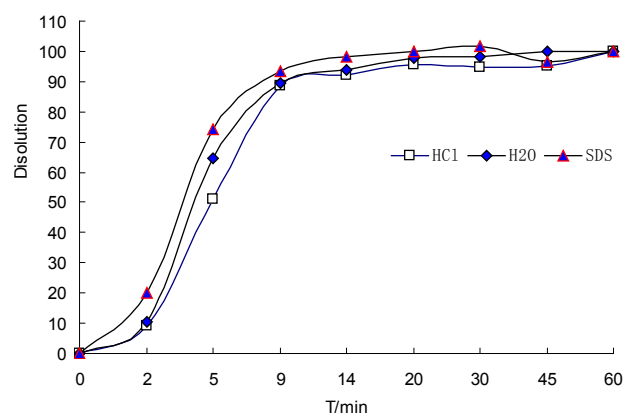
and high dissolution efficiency, in which we could get the accumulation dissolution rate of CDDP at any time point according to the fitting equation. The dissolution parameters were followed: $T_{50}=4.02$ mins (released 50%), $T_d=4.80$ mins (released 63.2%), $T_{90}=7.968$ mins (released 90%), so the overall ability of CDDP in water should dissolved more than 50% in 4.1 minutes and more than 90% in 8.0 minutes.

Investigating the variation of the four strong dissolution fingerprint peaks of CDDPs

The therapeutic outcome of TCM was due to the coherent effects of chemical constituents at equal pace (9,10). All the components certainly took the same important roles in TCM therapeutic process, so the dissolution rule of important chemical constituents was studied separately in order to guide the preparation technology and improve their bioavaibility. We investigated the four strong peaks of CDDP-DFP and constructed corresponding fitting equation. The relative stripping curves of the four strong peaks were shown in *Figure 3* and fitting equations of four strong peaks were as follow, if let lnln $[1/(1-A_i/A_{max})]=y$, the first strong peak (No.6 peak, PHA for 12.9 mins), $y=-0.5603x^2+3.4516x-3.8974$, $r=0.9988$; the second strong peak (No.19 peak, SAB for 37.5 mins), $y=-1.7127x^2+9.6655x-11.947$, $r=0.9897$; the third strong peak (No.4 peak, DSS for 8.6 mins), $y=-0.4239x^2+3.0436x-3.6276$, $r=0.9985$; the forth strong peak (No.17 peak, unkown), $y=-0.5019x^2+3.3706x-4.1053$, $r=0.9979$. The fitting equations of four strong peaks

were gained for specially monitoring the dissolution dynamic changes of the 4 important fingerprint peaks that had the same dissolution tendency on whole except SAB the second strong peak.

Conclusions

Comparing with the western medicine, compound recipe of TCM for medication not only had its own special feature, but also made chemical component complicated and the indicatrix composition uncertain that limited the study and research of quality control for TCM. In this paper, basing on the CDDP-HPLC-FPs, we separately studied the vitro dynamic process of CDDP and the four strong peaks of DFP in water for finding a better way to wholly control CDDP quality and monitor the dissolution state for each fingerprint component. The DFP can better reflect the holo-dissolution characteristics through the fitted equation, for which served as novel method to measure the dissolution dynamic process to make sure the concentration of CDDP *in vitro* or *in vivo* under control all time. Furthermore, we constructed four fitting equations for the four strong peaks and observed what difference existed in their dissolution process. DFPs could provide how stable and fast were for the most important fingerprints and supply much important information about their dissolution rule. In a word, we put forth a good way to evaluate the HD or TCM quality both from the wholly quantitative analyses and the quantitative DFPs that can post the best adequate way for controlling the quality of HD or TCM.

Acknowledgements

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