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Quality Control Studies on ZuoJin Sustained-Release Tablets

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ABSTRACT

ZuoJin sustained-release tablets (ZJSRT), which consist of *Rhizoma coptidis–Evodia rutaecarpa* powder (6:1, g/g), have been widely used for a long time as a treatment of gastrointestinal disorders in China. Because ZJSRT is a kind of Chinese medicine prescription (CMP), it comprises many components, which makes the quality control studies more difficult. The main aim of this paper was to evaluate the release of four alkaloids using kinetic models and to assess the dissolution behaviors of ZJSRT using total quantum statistical moment (TQSM). The release data generated during in vitro release studies were fitted to zero-order, first-order, Higuchi, Hill, Weibull, and Michaelis–Menten models. The dissolution behavior data were obtained by calculating the TQSM parameters. The release kinetics of ZJSRT from most formulations follow a classical Fickian diffusion mechanism but do not follow an empirical model. The data from the TQSM parameters demonstrate that all components in ZJSRT released at nearly the same ratio and the same time. Therefore, the study should contribute to the quality control for CMP such as ZJSRT.

KEYWORDS: Dissolution; Chinese medicine; release kinetics; ZJSRT.

INTRODUCTION

Traditional Chinese medicine has been used in China for several millennia and has played a role in the prevention and treatment of diseases, especially for complicated and chronic diseases such as SARS and AIDS (*1*, *2*). CMP are composed of several herbs that usually contain hundreds or thousands of compounds. Therefore, it is a challenge to find the proper ways to control quality and to evaluate release action in dissolution testing (*3*). Some researchers only used information from one or two compounds, called markers, to construct release profiles. Others investigated Chinese medicine sustained-release preparations by the bioassay method (*4*). However, only focusing on two or three compounds may not properly describe or evaluate the release profiles for CMP.

Chromatographic fingerprint analysis (*5*) has been accepted by the World Health Organization as a strategy for the assessment of herbal medicines. High-performance liquid chromatography (HPLC) fingerprints can uniquely identify and evaluate the authenticity of herbal samples. With the recent advances in fingerprint data acquisition and analysis software, the qualitative and quantitative analyses of dissolution compounds in CMP herbs are easier to carry out in vitro.

The HPLC fingerprints were looked upon as elution curves that consist of many independent normal distribution curves, whose information was calculated by TQSM. The formulas are listed as follows (*6*).

1. AUC_T: total zero moment, area under the curve of total quantum

$$AUC_T = C_T \sum_{j=1}^{m} k_{r,j}\, k_{c,j} = \sum_{j=1}^{m} A_j \qquad (0 < \lambda < t)$$

where C_T is the total content of all the ingredients, $K_{r,j}$ is response coefficient, $K_{c,j}$ is the constituent radio, A_j is the area for peak *j*, and λ is retention time.

2. $MCRT_T$ ($\overline{\lambda}_T$): total first moment, mean chromatographic retention time of total quantum

$$\overline{\lambda}_T = \frac{\sum_{j=1}^{m} \int_0^t \lambda R_j \, d\lambda}{\sum_{j=1}^{m} \int_0^t R_j \, d\lambda} = \frac{\sum_{j=1}^{m} A_j \lambda_j}{\sum_{j=1}^{m} A_j} \qquad (0 < \lambda < t)$$

where R_j is one of Gauss curve for peak *j* in the chromatographic fingerprint.

3. $VCRT_T$ ($\overline{\sigma}_\tau^2$): total *second* moment, variance of chromatographic retention time of total quantum

$$\overline{\sigma}_T^2 = \frac{\sum_{j=1}^{m} \int_0^t (\lambda - \overline{\lambda}_T)^2 R_j \, d\lambda}{\sum_{j=1}^{m} \int_0^t R_j \, d\lambda} = \frac{\sum_{j=1}^{m} A_j (\sigma_j^2 + \lambda_j^2)}{\sum_{j=1}^{m} A_j} - \overline{\lambda}_T^2 \qquad (0 < \lambda < t)$$

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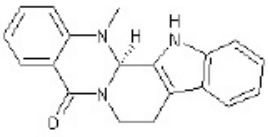
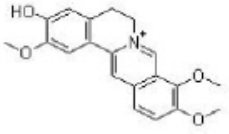
where σ^2_j is the variance for peak *j*.

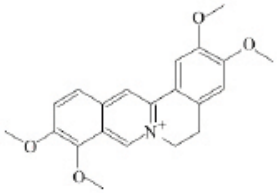
ZuoJin prescription is widely used to purge intense heat, soothe the liver, warm the stomach, as an anti-nociceptive (*7*, *8*), and for gastrointestinal disorders in the clinical practice of CMP (*9*). Evodiamine, jatrorrhizine, palmatine, and berberine hydrochloride are regarded as the most important pharmacologically active constituents, whose chemical structures are shown in Figure 1.



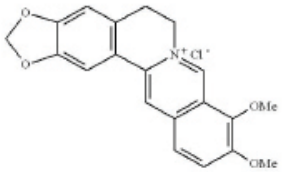
Evodiamine ($C_{19}H_{17}N_3O$, 303.36)



Jatrorrhizine ($C_{20}H_{20}NO_4$, 465.28)



Palmatine ($C_{21}H_{22}NO_4$, 352.40)



Berberine hydrochloride ($C_{20}H_{18}ClNO_4$, 371.81)

In this investigation, we first quantified the percent release of the four alkaloids and then fit zero-order, first-order, or Higuchi model equations. Simultaneously, CFA and RCH were carried out to fit the models. Moreover, the TQSM parameters were calculated to trace the dissolution behaviors.

MATERIALS AND METHODS

Materials and Instruments

Analytical grade sodium dodecyl sulfonate (SDS), hydrochloric acid, phosphoric acid, and HPLC-grade methanol and acetonitrile were obtained from Shanghai SSS Reagent Co., Ltd. (Shanghai, China). Water was redistilled, and 0.1% aqueous hydrochloric acid was used as a dissolution medium. The four reference standards evodiamine (110802-200504), jatrorrhizine (110733-200505), palmatine (110732-200506), and berberine hydrochloride (110713-200609) were purchased from National institutes for Food and Drug Control (China). ZJSRT was manufactured by the Pharmacy Department of Hunan University of Traditional Chinese Medicine (China).

All dissolution tests were performed on a ZRS-8G apparatus (Tianda Tianfa Technology Co., Ltd., Tianjin, China), which is a manual-sampling dissolution bath with six vessels to allow dissolution testing of six tablets simultaneously. The amount dissolved was determined using a Waters 2695 HPLC equipped with a Breeze chromatography workstation and a Waters 2487 Dual Absorbance Detector (Waters, Milford, MA, USA). Drugs and reagents were weighed on a Metler Toledo AG 214 balance (Metler, Greinfensee, Switzerland).

Dissolution Study

Dissolution profiles were obtained from the release information of evodiamine, jatrorrhizine, palmatine, berberine hydrochloride, CFA, and RCH.

All dissolution tests were conducted according to the *ChP* (*10*) appendix XD, the apparatus first-determination method. Dissolution media (0.1 N HCl, pH 1.2) were filtered and deaerated with a 0.45-µm nylon filter before use. A volume of 1000 mL of medium was used in each vessel, agitated at a rotation speed of 100 rpm, and the bath temperature was set at 37 ± 0.5 °C. Five-milliliter samples were drawn at 1, 2, 4, 6, 8, 10, and 12 h and replenished with 5 mL of fresh isothermal dissolution medium. Samples drawn were immediately centrifuged at 6000 *g* and filtered, after which the supernatant was evaporated to dryness at 37 °C under a stream of nitrogen. The residue was dissolved in 1.0 mL methanol, and then the samples were analyzed by HPLC. Parallel trials were performed in triplicate ($n = 3$), and the mean values were used for data analysis (*11*, *12*).

Methods of Analysis and Validation

HPLC was carried out on an Apollo C_{18} column (4.6 mm × 250 mm, 5.0 µm, Grace, Deerfield, IL, USA) at 40 °C with a flow rate of 1.0 mL/min and an injection volume of 20 µL. The wavelength was 345 nm. The mobile phase was a mixture of 0.5% SDS–water containing 0.1% phosphoric acid (mobile phase A) and 0.5% SDS–60% aqueous acetonitrile containing 0.1% phosphoric acid (mobile phase B). The mobile phase gradient program was carried out as follows: 0–20 min, 80% A; 20–30 min, 75% A; 30–40min, 65% A; 40–100 min, 20% A (*13*). A short method validation program was performed for HPLC to determine the percent dissolved. Linearity, accuracy, precision, stability, recovery, and specificity were assessed.

Data Analysis

Linear equations were constructed by a least-squares linear regression analysis using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The concentrations of test samples were calculated from the linear regression equations. The release data generated during in vitro release studies were fitted to zero-order, first-order, and Higuchi models by EXCEL. The TQSM parameters of CFA and RCH were calculated as the formula previously reported.

RESULTS AND DISCUSSION

Method Validation

The release data were measured by HPLC with UV. The chromatogram shows good separation of the four alkaloid peaks from other peaks. The theoretical plates were all greater than 3000, and the retention times of evodiamine, jatrorrhizine, palmatine, and berberine hydrochloride were 64.2, 69.5, 79.7, and 84.5 min, respectively (Figure 2). The four alkaloid standard calibration curves exhibited linearity with coefficients (*r*) greater than 0.993. The accuracy (recovery) of spiked solutions was between 97.64% and 103.35%,

Table 1. Precision, Recovery, and Stability of the Analytes in ZJSRT (n = 6)

Analyte	Spiked conc. (μg/mL)	Precision (%RSD)		Recovery (%) (n = 6)	Stability (%RSD for 24 h)
		Intra-run	inter-run		
evodiamine	200	1.98	1.94	101.91	1.24
jatrorrhizine	150	1.35	1.89	97.64	1.38
palmatine	300	1.82	2.01	99.18	1.49
berberine hydrochloride	100	1.90	1.78	103.35	1.51

intra- and inter-precision (RSD, relative standard deviation, n = 6) were less than 1.98% and 2.01%, respectively, and stability evaluation (RSD, n = 6, 1–48 h) was less than 1.51% (Table 1), which is appropriate for assessment of analytes in the dissolution study.

In Vitro Dissolution Study

The vitro dissolution data of the four alkaloids, CFA, and RCH (calculated with AUC_T) are presented in Table 2 and Figure 3. The release of evodiamine, jatrorrhizine, palmatine, and berberine hydrochloride from ZJSRT was slow in the dissolution medium initially with approximately 17.90%, 18.63%, 17.95%, and 12.63% released, respectively. After 2 h, the release was rapid; with ZJSRT dissolving to nearly 100% in 14 h. Maximum releases of the drug were 96.38%, 97.25%, 98.26%, and 98.69%, respectively. The maximum for CFA was 99.15% and 99.32% for RCH.

Release data were subjected to zero-order, first-order, Higuchi, Hill, Weibull, or Michaelis–Menten models to establish the release mechanism and kinetics of drug release from ZJSRT (Table 3). The coefficients infer that the release kinetics of ZJSRT from most formulations follow a classical Fickian diffusion mechanism but not an empirical model. We can also see that the four alkaloids in ZJSRT may follow different dissolution kinetics from the others, and their dissolution behavior can be controlled using the formulations. However, obviously every ingredient release model could not demonstrate the release behavior of ZJSRT fully, but CMP efficacy theory demands that we focus on the constituent release behaviors as a whole, so we studied the dissolving behaviors of ZJSRT using TQSM. In this paper, CFA and RCH were designed to be studied integrally, and the results are listed in Tables 2–5 and Figures 3b and 4.

The results show that the parameters of TQSM can describe the ZJSRT release behaviors. The stable number of peaks for CFA (four) illustrate that CFA had a synchronous release behavior, but the increasing number of peaks for RCH after 4 h indicates that many constituents released at different rates. The increasing value of zero moment means that all components released gradually, before the original 2 h. The release velocity was slow, then rapid, and ZJSRT dissolved to nearly 100% within 14 h; that the values of the first moment and second moment were stable between some interval infers that all ingredients in ZJSRT had dissolved at the same time and nearly the same ratio (Figure 4).

Table 2. Accumulative Percent Release at Different Time Points

Time (h)	Constituent (% mean ± SD, n = 3)					
	evodiamine	jatrorrhizine	palmatine	berberine hydrochloride	CFA	RCH
1	13.61±0.51	13.93±0.61	14.75±0.72	11.26 ± 0.55	12.37 ± 0.45	12.53 ± 0.34
2	17.90 ± 0.72	18.63 ± 0.63	17.95 ± 0.74	12.63 ± 0.90	14.59 ± 0.89	14.94 ± 0.99
4	38.82 ± 1.15	37.17 ± 1.18	38.46 ± 1.23	28.69 ± 1.13	31.99 ± 1.12	34.02 ± 1.12
6	56.13 ± 1.23	53.60 ± 1.22	55.25 ± 2.31	43.46 ± 1.25	47.45 ± 1.23	50.22 ± 1.25
8	68.55 ± 1.94	70.00 ± 2.21	73.08 ± 2.44	62.87 ± 2.21	66.00 ± 2.21	68.14 ± 1.39
10	81.84 ± 2.21	80.81 ± 2.42	82.82 ± 2.36	79.32 ± 2.38	80.29 ± 3.12	81.97 ± 2.01
12	83.50 ± 2.45	85.28 ± 3.78	86.30 ± 2.42	95.69 ± 2.41	92.18 ± 2.25	91.49 ± 3.08
14	96.38 ± 3.25	97.25 ± 2.25	98.26 ± 2.58	98.69 ± 2.52	99.15 ± 0.84	99.32 ± 0.12

Table 3. Results of Model Fitting

Model	Formulation					
	evodiamine	jatrorrhizine	palmatine	berberine hydrochloride	CFA	RCH
Zero-order	$Y = M_t/M_\infty = Bt+A$					
B	0.0649	0.0659	0.0667	0.0746	0.0720	0.0715
A	0.1087	0.1013	0.1081	0.0090	0.0418	0.0560
r	0.9836	0.9882	0.9838	0.9931	0.9945	0.9925
first-order	$Y = \ln M_t/M_\infty = Bt+A$					
B	0.1457	0.1459	0.1454	0.1772	0.1654	0.1629
A	-1.7915	-1.7920	-1.7671	-2.1485	-2.0063	-1.9633
r	0.9280	0.9380	0.9333	0.9583	0.9526	0.9447
Higuchi	$Y = M_t/M_\infty = Bt_{1/2}+A$					
B	0.3154	0.3189	0.3238	0.3547	0.3444	0.3437
A	-0.2197	-0.2289	-0.2284	-0.3486	-0.3085	-0.2960
r	0.9942	0.9945	0.9927	0.9816	0.9888	0.9915
Hill	$Y = \ln[M_t/(M_\infty - M_t)] = Blgt+A$					
B	1.7455	1.7944	1.8929	2.2191	2.1582	2.1893
A	-1.0711	-1.0897	-1.1080	-1.3878	-1.3114	-1.3023
r	0.9422	0.9286	0.9142	0.8924	0.8829	0.8813
Weibull	$Y = \ln(1-M_t/M_\infty) = B\,t+A$					
B	-0.2141	-0.2296	-0.2540	-0.3061	-0.3052	-0.3133
A	0.3195	0.3915	0.4649	0.7800	0.7573	0.7669
r	0.9496	0.9395	0.9256	0.9263	0.9063	0.9002
Michaelis–Menten	$Y = \Delta[t/(M_t/M_\infty)] = B\,(M_\infty/M_t)+A$					
B	-0.7671	-0.8457	-0.8046	-0.1556	-0.3933	-0.4015
A	13.656	13.840	13.458	13.600	13.560	13.252
r	0.7810	0.8588	0.7744	0.2458	0.5941	0.5962

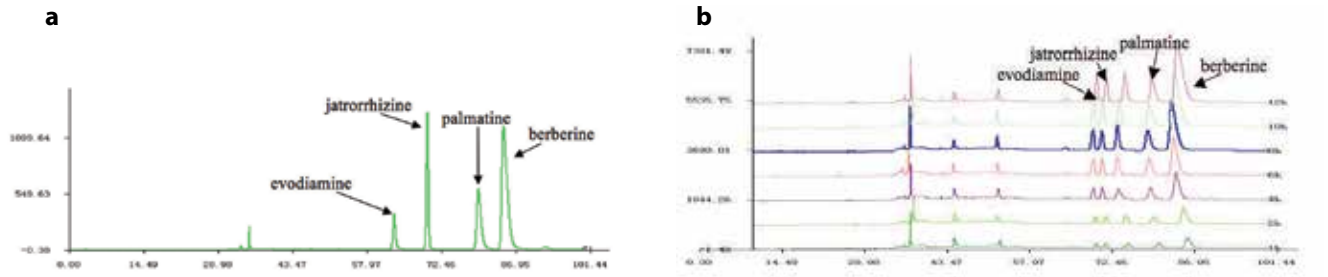


Figure 2. HPLC dissolution fingerprints for (a) four-mixture standard and (b) ZJSRT at 2, 4, 6, 8, 10, and 12 h.

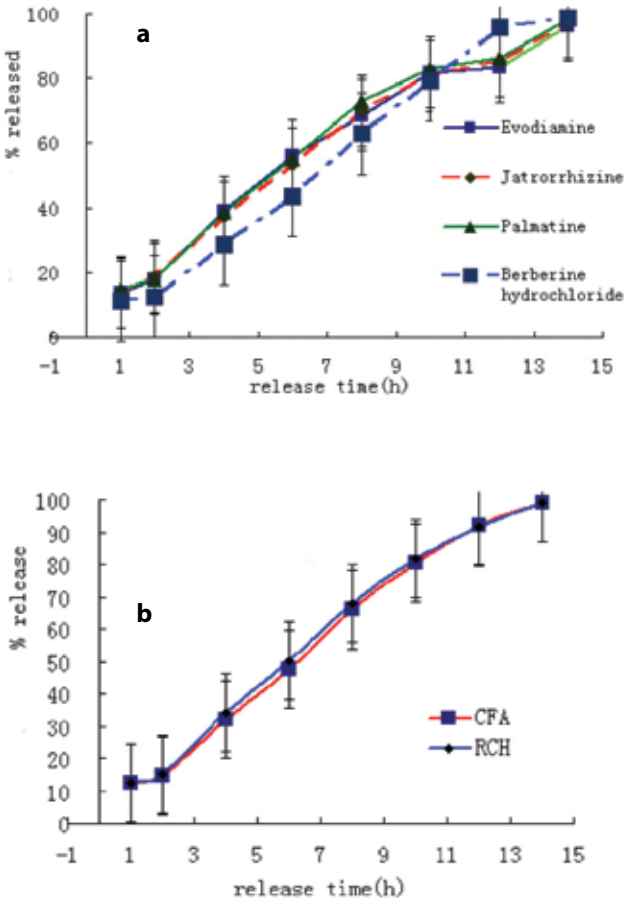


Figure 3. Cumulative percentage of constituents released (mean ± SD, n = 3) for (a) four alkaloids; (b) multicomponents.

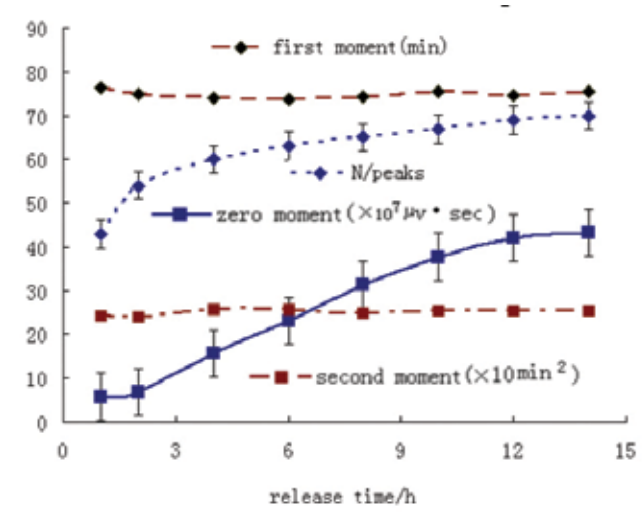


Figure 4. TQSM parameters of RCH fingerprints from 1 to 14 h of ZJSRT.

Table 4. TQSM Parameters of CFA from 1 to 14 h of ZJSRT

Sampling time	Parameters			
(h)	Number of peaks	AUCT (μv·sec)	(min)	(min2)
1	4	4.027 × 107	81.78	25.89
2	4	4.752 × 107	80.36	25.21
4	4	1.042 × 108	79.96	26.08
6	4	1.545 × 108	79.74	25.18
8	4	2.149 × 108	79.78	26.79
10	4	2.614 × 108	80.21	26.35
12	4	3.001 × 108	80.97	25.95
14	4	3.084 × 108	81.01	25.88
RSD (%)		59.30	0.89	2.07

Table 5. TQSM Parameters of RCH from 1 to 14 h of ZJSRT

Sampling time	Parameters			
(h)	Number of peaks	AUCT (μv·sec)	(min)	(min2)
1	43	5.765 × 107	76.34	242.7
2	54	6.877 × 107	74.80	240.0
4	60	1.566 × 108	73.92	257.1
6	63	2.311 × 108	73.71	256.9
8	65	3.136 × 108	73.99	249.0
10	67	3.773 × 108	74.31	255.5
12	69	4.211 × 108	75.38	253.3
14	70	4.320 × 108	75.39	253.3
RSD (%)	14.75	58.98	1.23	2.59

CONCLUSIONS

The present investigation shows that the four alkaloids, CFA, and RCH in ZJSRT follow different kinetic models and the dissolution behavior of ZJSRT could be used to trace the multicomponent system such as ZJSRT and other CMP in vitro. Moreover, TQSM (*14*) may be a better method to evaluate and assess the dissolution behaviors of CMP, a multicomponent system, when considering a multicomponent system integrally.

This paper provides support for investigating the in vitro dissolution kinetics of ZJSRT, which is desirable for research on the quality control of CMP.

ACKNOWLEDGMENTS

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