Design and Evaluation of Jingzhiguanxin Monolithic Osmotic Pump Tablet

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A monolithic osmotic pump tablet (MOPT) of Traditional Chinese Medicine Compound Recipe (TCMCR) was successfully prepared and active components of Jingzhiguanxin prescription which has been widely used in China and Japan was selected as model drug. Analysis methods of maker compound *in vitro* of danshensu, paeoniflorin and safflor yellow A were built, and different methods were compared by f_2 factors. The results showed that there were fine correlation among them. Finally UV method of safflor yellew A was chosen to determine the release of the drugs, which was fast, convenient, met the need of determination and could represent other methods. During the research, single factor influence selection was studied emphatically. It showed that there were significant influence between different varieties and quantity of osmotic promoting agents, different kind of retardants, different varieties and quantity of PEG (polyethylene glycol) and membrane weight. However, no significant influence existed between different quantity of retardants and SDS, different membrane orifices and methods of dissolution. Based on the single factor influence selection, an optimal formulation was decided, and three maker compounds of Jingzhiguanxin MOPT could isochronous release and at the same time they had good zero order release characteristics to 8 h. Paeoniflorin release *in vivo* was estimated by deconvolution, the results shown that there were a good *in-vitro in-vivo* correlation (r=0.9571).

Key words monolithic osmotic pump tablet; Jingzhiguanxi prescription; f_2 factor; *in-vitro in-vivo* correlation; paeoniflorin

Traditional Chinese Medicine (TCM) has a very long history. And there is a large quantity of Traditional Chinese Medicine Compound Recipes (TCMCR) that have their clear and definite curative effects. However, most preparations of TCM hesitate around the normal dosage form, while those with higher efficiency, longer-acting time and lower dosage are rare. With the development of modern excipients, use of modern technique and advanced technology of preparation, various good prerequisites are supplied for sustained-release and controlled-release dosage forms of TCM.[1—3)]

Osmotic Pump Controlled Release Preparation is a new drug delivery system with eternally drug delivery rate as characteristic and with the osmotic pressure difference between the inside and the outside of the semipermeable membrane as drug delivery power.[4,5)] Till now, it is one of the most ideal preparations in controlled-releasing effect and has achieved the world advanced level. And it has many advantages, such as reducing risk of side effect, improving patients' compliance, *in vivo* predictability of release rate based on *in vitro* data, *etc*.

Jingzhiguanxin (JZGX) prescription is an effective TCMCR, with improving blood circle and clearing up silt in the body, composed of "danshen" (*Radix Salviae Miltiorrhizae*), "chishao" (*Radix Paeoniae Rubrae*), "chuanxiong" (*Rhizoma Chuan-xiong*), "honghua" (*Flos Carthami*) and "jiangxiang" (*Lignum Dalbergiae Odorafera*). The main action of JZGX prescription is to cure the disease of coronary disease and angina arising from the silts of blood in the hearts. It can also protect the cardiac muscle.

JZGX tablets embodied in Ch.P (2005)[6)] have several disadvantages, such as larger dosage (6 to 8 tablets each time and 3 times a day), unenlightened technology of preparation, no quantificational target, *etc*. After being made into osmotic pump tablet, it is more glabrous and easily swallowed. Furthermore, its release is better than common tablets and provides small drug concentration fluctuation in blood and small toxicity through controlled-releasing.[7)]

Most Chinese medicines are administered orally as decoctions or tablets in clinics, in which the composite formulae will isochronous release and produce a synergistic effect or antagonistic action. So it is not enough that only one maker compound is studied. In this paper, three maker compounds, which represent three main drugs made of JZGX prescription, were studied. It is expected that three maker compound could isochronous release and produced a synergistic effect or antagonistic action.

Experimental

Materials The drug from JZGX prescription was made by our laboratory colleagues. Healthy beagle dogs (9.3±1.0 kg) were supplied from the Lab Animal Center of Shenyang Pharmaceutical University. Polyethylenegly400 (PEG400), Polyethylenegly1500 (PEG1500), Polyethylenegly4000 (PEG4000), Polyethylenegly6000 (PEG6000) and acetic acid were obtained from Shenyang Reagent Factory. Tragacanth were obtained from Shanghai Chemical Reagent Stocking and Purvey Station. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Shandong Yuwangshiye Co. Ltd. other reagents were of analysis grade.

Foundations of Analysis Methods In this prescription danshensu, paeoniflorin and safflower yellow A were chosen to be the target components. Their chromatographic conditions were as follows:

Table 1. Chromatographic Condition of Each Target Component

Target component	Mobile phase	Wavelength
Danshensu	Methanol : 0.1% acetic acid (20 : 80, v/v)	273 nm
Paeoniflorin	Acetonitrile : 0.1% phosphate (20 : 80, v/v)	230 nm
Safflower yellow A	Methanol : acetonitrile : 0.7% phosphate (35 : 5 : 65, v/v)	402 nm

The HPLC system consisted of a pump (LC-10ATVP HPLC pump, Shimadzu, Japan), an UV detector (SPD-10AVP Spectrometer, Shimadzu, Japan). Diamonsil C18 (Diamonsil C18 200 mm×4.6 mm, 5 μm, Dikma, Beijing) was chosen as column while 30 °C as column temperature in all the methods above. At the same time, UV method of safflower yellow A was also found and it could be detected at the wavelength of 402 nm. Under the

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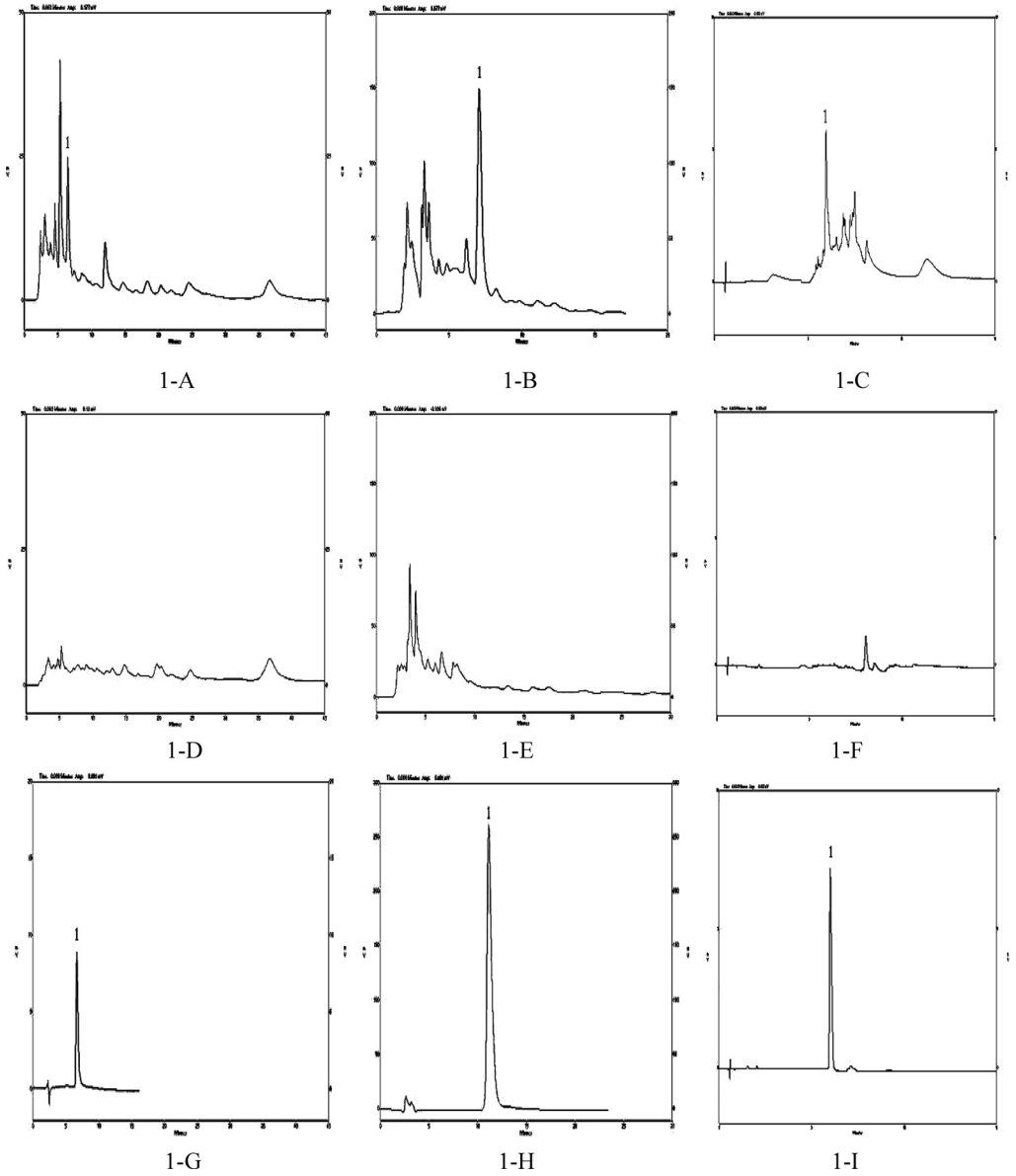

Fig. 1. The HPLC Chromatograms of Danshensu, Paeoniflorin and Safflower Yellow A in JZGX MOPT, and Their Negative Control and Standard Samples

1-A: danshensu in JZGX MOPT; 1-B: paeoniflorin in JZGX MOPT; 1-C: safflower yellow A in JZGX MOPT; 1-D: negative control of danshen; 1-E: negative control of chishao; 1-F: negative control of honghua; 1-G: danshensu standard; 1-H: paeoniflorin standard; 1-I: safflower yellow A standard.

conditions described above, the HPLC chromatograms of danshensu, paeoniflorin and safflower yellow A in JZGX MOPT, and their negative control and standard samples were shown in Fig. 1. The retention times of danshensu, paeoniflorin and safflower yellow A were approximately 6.72, 7.09 and 6.30 min, respectively. No interfering peaks were observed within the time frame during which danshensu, paeoniflorin and safflower yellow A were eluted.

Release behaviors of target components with different analysis methods were shown in Fig. 2.

The similar factor (f_2) was used to evaluate the release between different drugs and different methods. f_2 is calculated by the following equation:

$$f_2 = 50 \cdot \log\{[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \cdot 100\}$$

where R_t and T_t represent the average percentage drug released from the test tablets and reference tablets at the t time point; n is the number of time points tested. Two curves are thought to be statistically similar if the f_2 value is above 50. The results showed that there was fine correlation among them. As it was fast, convenient and could meet the need of determination, UV method of safflower yellow A was adopted to determine the release of the target component.

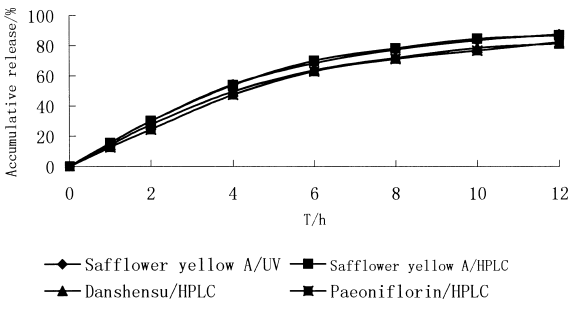

Fig. 2. Release Curves of Different Methods and Drugs

Preparation of Core Tablet Core tablets containing JZGX prescription (302.5 mg/tablet) were prepared according to the following composition: lactose, NaCl, tragacanth, sodium dodecyl sulfate (SDS) and magnesium stearate. Blend drug and excipients. Prepare a wet granulation with binder. Dry and screen through an 18-mesh screen. Add the magnesium stearate, blend, and compress the particles into tablets.

Coating The core tablets were coated by using a pan coater and 3%

(w/v) of cellulose diacetate (CA) in acetone containing known level of PEG was used as coating solution. Coating condition were as follows: diameter of pan coater, 20 cm; rotating rate, 40 r/min; obliquity, 30°; temperature, 35 °C; spray rate, 7 ml/min; spray pressure, 6—8 atm. The coating level was determined from the weight gain of each tablet. After coating, the tablets were dried overnight at 40 °C to remove residual solvent. Two orifices with diameter of 0.8 mm for drug release were drilled on both side surfaces of the coated tablet mechanically. Then the osmotic pump tablets were made.

The Calibration of Safflower Yellow A/UV To determine the concentration of samples, A calibration curve was constructed based on the analysis of various concentrations of safflower yellow A in release solutions by UV. The calibration curve for safflower yellow A was linear over the concentration range of 10.1—80.8 $\mu g \cdot ml^{-1}$. The regression equation was found to be: $C=101.69A+0.7585$ ($r^2=0.9999$), where C is the concentration of safflower yellow A and A is the absorbance.

***In Vitro* Drug Release Test** Drug releases from the different formulations were determined using P. R. China Pharmacopoeia Dissolution Apparatus with 200 ml of distilled water containing 0.5% SDS as dissolution medium. The temperature of the dissolution medium was maintained at 37 °C and the rotation speed of the paddle was adjusted to 50 r/min. 5 ml samples were withdrawn at 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h and replaced with medium of equal volume. The concentration of drug released from the osmotic pump tablets was measured with an ultraviolet spectrophotometer (UV-9100, Beijing Ruili Analysis Instrument Company, China) at 402 nm.

In Vivo* Studies** A total of 6 dogs were used for this study, which were distributed into 2 groups with 3 dogs in each group. Animals were kept in an environmentally controlled breeding room for 1 week before the start of the experiments. They were fed standard laboratory chow with water and fasted overnight before the experiments. In the first trail period, each dog was given a single dose of 3025 mg (at doses containing 200 mg paeoniflorin) tablets of either formulation (tablet or MOPT) in a randomized fashion with 200 ml water. The beagles were fed at 4 h post-dosing and water was available ad libitum since 2 h post-dosing. Approximately 4 ml blood samples were drawn into heparinized tubes through an indwelling cannula before 0 h and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 24 h after administration of reference tablets. Blood samples were drawn before 0 h and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 16, 24, 36 h after administration of test tablets. The blood samples were centrifuged at 1600 ***g for 10 min, plasma was separated and kept frozen at −20 °C. After a period of 7 d, the study was repeated in the same manner to complete crossover design. The resulting plasma 0.5 ml was then mixed with 1.0 ml acetonitrile and 80 μl internal standard (pentoxifylline 50 $ng \cdot ml^{-1}$). The denatured protein precipitate was separated by centrifugation at 1600 ***g*** for 10 min. The supernatant was mixed with 5 ml ether by ultrasonic vortex for 1 min. Then the mixture was centrifuged at 1600 ***g*** for 10 min. The ether layer was discarded to get rid of the non-polar interfering impurities. The water layer was evaporated to dryness below 40 °C in vacuum and then dissolved in 50 μl of mobile. A 20 μl volume of this sample solution was injected into HPLC for analysis.

***In Vivo* Data Analysis** All data were subsequently processed by the computer program WINNONLIN (SCI, Lexington, KY, U.S.A.) The non-compartmental pharmacokinetic parameters of half-life ($t_{1/2}$), mean residence time (*MRT*), area under the plasma concentration–time curve (*AUC*) were calculated based on the moment theory.

Results and Discussion

Influence of Retardants Fix the quantity of drug and other excipients in the formulation and the technique of coating. Tragacanth, hydroxypropyl methyl cellulose (HPMC) K100M and HPMC K4M were selected as the retardants, and the influence on release of different retardants was examined. The results were shown in Fig. 3.

From the above it was seen that there was some influence on release between different retardants. The release of HPMC K100M and HPMC K4M were fine after 4 h, but their were too fast at 0—4 h; The release of tragacanth was good at 0—8 h, although it was slower after 8 h, which could fast by regulating the amount of excipients, so tragacanth was chosen to be the retardant.

Influence of Osmotic Promoting Agents Fix the quantity of drug and other excipients in the formulation and the technique of coating. Lactose (60 mg) and NaCl (60 mg) were selected as the osmotic promoting agents, and the influence on release of different osmotic promoting agents was examined. The results were shown in Fig. 4.

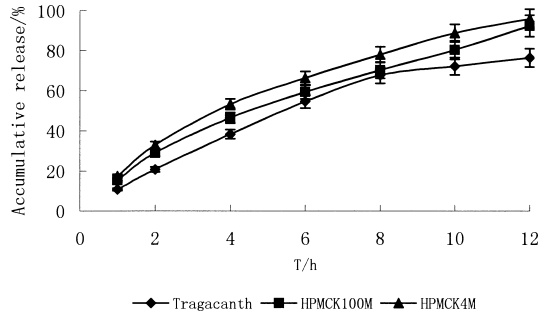

Fig. 3. Influence on Release of Different Retardants

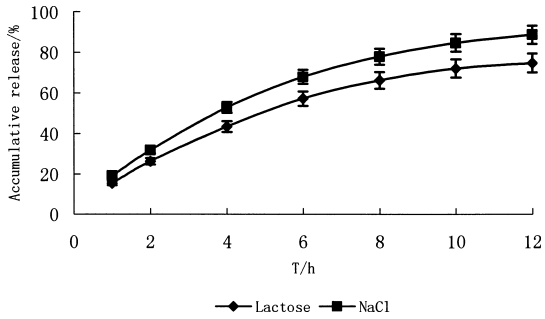

Fig. 4. Influence on Release of Different Osmotic Promoting Agents

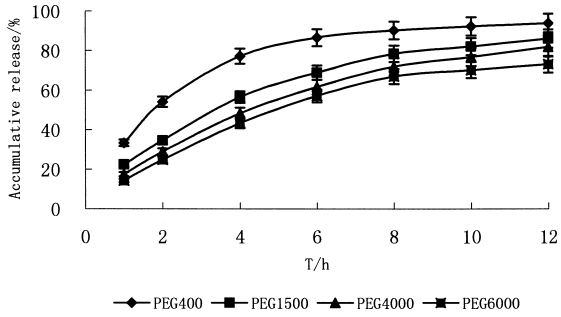

Fig. 5. Influence on Release of Different PEG

Significant influence was observed of different osmotic promoting agents. The drug in this formulation had poor condensability. Although the osmotic promoting effect of lactose was no better than that of NaCl, it could improve the condensability. From the above, lactose and NaCl were both selected as the osmotic promoting agent.

Influence of Different PEG Fix the formulation of core tablets, various PEG were adopted to be plasticizer with controlling of the same weight gain. Influence of PEG on release was investigated. The results could be seen in Fig. 5.

Figure 5 showed that different PEG had a marked influence on drug release. The release of PEG400 and PEG1500 were too fast at 0—4 h, the release of PEG6000 was slower after 8 h, so PEG-4000 was selected.

Influence of Amount of PEG-4000 To study the influence of amount of PEG-4000 on the drug release profiles, the CA membranes were plasticized with 10%, 20% and 30% of

PEG-4000. Figure 6 showed that increase in PEG-4000 level led to an increase of drug release rate.

Since PEG-4000 was a hydrophilic plasticizer, the more PEG-4000 incorporated into the CA membrane, the more void space formed after leaching, and as a result, the higher the permeability of membrane and the release rate obtained. However, high percentage of PEG-4000 in CA would make the membrane fragile. So finally, the amount of PEG-4000 in the formulation was 20%.

Influence of Membrane Thickness To evaluate the role of membrane thickness on the release rate of drug, the same core tablets were coated with three different thickness membrane (with the membrane gain of 4%, 6% and 8%). Influence of thickness membrane on release was investigated. The results could be seen in Fig. 7.

Figure 7 showed that different thickness membrane had a marked influence on drug release. The release of 4% and 6% were too fast at 0—4 h, the release of 8% was attached to 80% at 12 h and the linearity of release was fine, so 8% was selected at last.

Optimal Formulation Based on the results obtained, the optimal formulation was as following: each core tablet was made up of 302.5 mg drug, 60 mg lactose, 60 mg NaCl, 30 mg tragacanth, 9 mg SDS and 4 mg magnesium stearate; membrane weight gain was 8%; percent of PEG-4000 to CA membrane was 20%; orifice size was 0.8 mm. The different marker compound release profile of the optimal formulation was shown Fig. 8.

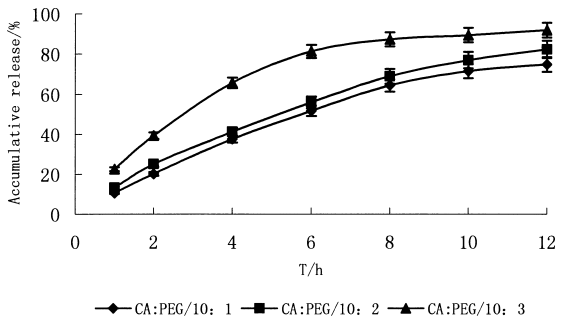

Fig. 6. Influence on Release of Different Quantities of PEG4000

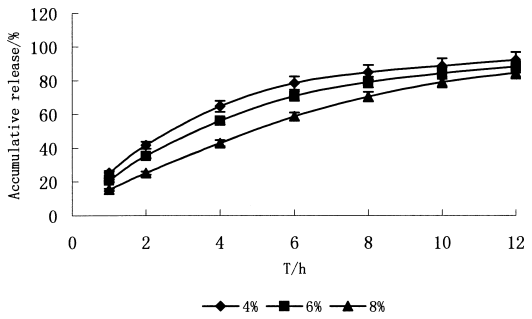

Fig. 7. Influence on Release of Different Membrane Thickness

***In-Vitro in-Vivo* Correlation (IVIVC)** Paeoniflorin, one of the bioactive components in *Radix Paeoniae Rubrae*, has been reported to exhibit many pharmacological effects.[8—13] After the calibration curve for the determination of paeoniflorin in dog plasma ($y=0.013x-0.0574$, $r^2=0.9964$) was prepared over a linear range of 5—250 ng·ml^{-1} and the mean recovery of 78.25% was obtained according to the method of plasma operation at item 2.7, IVIVC was developed. The plasma concentration *vs.* time profile of paeoniflorin in beagle dogs is shown in Fig. 9. It was found that C_{max}, t_{max}, $t_{1/2}$ and $AUC_{0—\infty}$ were 94.36 ng/ml, 280.00 min, 276.60 min and 42266.87 ng·min/ml, respectively. A IVIVC of JZGX MOPT was investigated using deconvolution method.[14,15] A good linear regression relationship was observed at Table 2. The correlation coefficient r is equal to 0.9571, which indicates that there is a good IVIVC for JZGX MOPT.

Discussion Since there are many effective compounds in TCMCR, the release profile of one compound could not represent that of other compounds. In this paper, three compounds (danshensu; paeoniflorin; safflor yellow A) were chosen as maker compounds, which are the effective compounds

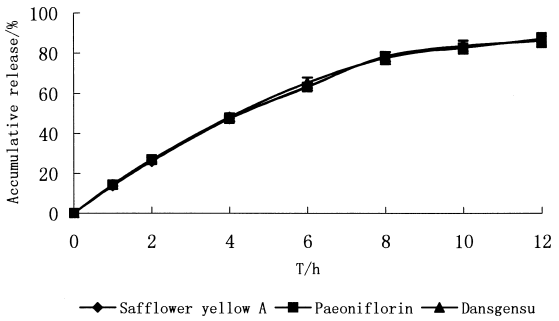

Fig. 8. The Release Profile of the Optimal Formulation

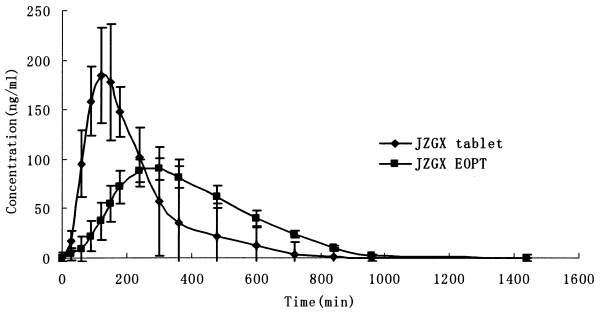

Fig. 9. Plasma Concentration–Time of Paeoniflorin in Beagle Dogs after Oral Administration of Two Formulations (Each Dose Containing 200 mg Paeoniflorin)

Each point represent the mean±S.D. ($n=6$).

Table 2. The IVIVC Results of Paeoniflorin after Oral Administration JZGX MOPT

T/h	1	2	4	6	8	10	12
Release of MOPT/% (Y)	14.29	26.78	47.29	63.19	78.47	82.72	87.31
AUC of tablet/ng h/ml	33.20	149.79	297.08	126.23	56.82	33.53	15.44
Paeoniflorin conc. of MOPT/ng/ml	9.42	37.43	88.71	81.55	61.88	40.04	24.17
Input function (R)	0.28	0.15	0.82	0.97	1.04	1.32	1.51
Correlative equation			$Y=54.556R+9.6182$ $r=0.9571$				

of three main drugs of Jingzhiguanxin prescription. It is more reasonable using three maker compounds than anyone compound.

Tragacanth, which was selected as the retardants in osmotic pump tablet firstly, has very good effect of retardarce. It could slower release of drug and made release profile to meet zero order process.

Conclusion

In this paper, Analysis methods of maker compound *in vitro* of danshensu, paeoniflorin and safflor yellow A were built, and UV method of safflor yellew A was chosen to determine the release of the drugs. Single factor influence selection was studied. The optimal formulation of JZGX MOPT was found based on the single factor influence selection, which was able to isochronous release danshensu, paeoniflorin and safflor yellow A at a rate of approximately zero order up to 8 h in 0.5% SDS solution. The study of this paper on pharmacokinetics indicates that there is a good IVIVC (r=0.9571) for paeoniflorin of JZGX MOPT.

References

1) Xiao X. H., Huang L. Q., Ma X. J., *J. Chinese Meteria Medica*, **28**, 282—286 (2003).
2) Fang W. X., *J. Chinese Meteria Medica*, **27**, 721—724 (2002).
3) Tang H. M., Li R., *Chinese J. Experimental Traditional Medical Formulae*, **8**, 56—57 (2002).
4) Theeuwes F., *J. Pharm. Sci.*, **64**, 1987—1991 (1975).
5) Theeuwes F., *Drug Dev. Ind. Pharm.*, **9**, 1331—1357 (1983).
6) "The Pharmacopoeia of the People's Republic of China," 1st ed., Vol. 1, Chemical Industry Press, Beijing, 2005, pp. 653—654.
7) Li X. D., Pan W. S., Nie S. F., Wu L. J., *J. Controlled Release*, **96**, 359—367 (2004).
8) Dezaki K., Kimura I., Miyahara K., Kimura M., *Jpn. J. Pharmacol.*, **69**, 281—284 (1995).
9) He J. X., Akao T., Tani T., *J. Pharm. Pharmacol.*, **55**, 313—321 (2003).
10) Kimura Masaa., Kimura I., Kimura Masay., *Jpn. J. Pharmacol.*, **39**, 387—390 (1985).
11) Kimura M., Kimura I., Muroi M., Nakamura T., Shibata S., *Jpn. J. Pharmacol.*, **41**, 263—265 (1986).
12) Kimura M., Kimura I., Nojima H., *Jpn. J. Pharmacol.*, **37**, 395—399 (1985).
13) Kimura M., Kimura I., Takahashi K., *Planta Med.*, **45**, K15—19 (1982).
14) Negrin C. M., Delgado A., Llabres M., Evora C., *Biomaterials*, **22**, 563—570 (2001).
15) Grundya J. S., Andersonb K. E., Rogersb J. A., Fosterb R. T., *J. Controlled Release*, **48**, 9—17 (1997).