Int J Clin Exp Med 2017;10(7):11254-11266
www.ijcem.com /ISSN:1940-5901/IJCEM0054404

Original Article

Screening the effective constituent of perilla compound prescription on anti-motion sickness in mice model

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Received April 1, 2017; Accepted May 18, 2017; Epub July 15, 2017; Published July 30, 2017

Abstract: Motion sickness will not be life-threatening, but it will bring a lot of inconvenience for daily life, also reducing combat effectiveness of military troops or affecting the health and work efficiency of astronauts. In the article, a more effective mice motion sickness model which selected kaolin consumption, food intake and halo response index as evaluation indexes was firstly established. Then n-butanol extract of perilla compound prescription (including medicinal and homologous plants named perilla, zingiber, pericarpium citri reticulatae and mint) was defied as effective chemical part to improve mice's evaluation indexes of motion sickness mentioned above, at the same time reducing their AVP levels and increasing their CORT levels to alleviate the mice's symptoms of motion sickness. What's more, the anti-motion sickness activity of n-butanol constituent was attributed to four multi-component substances (water, 25% ethanol, 65% ethanol and 95% ethanol elution parts). After that, MPLC, silica gel column chromatography, TLC and HPLC-MS were also used to analyze the main compounds of the n-butanol effective constituent. Three compounds of n-butanol extract were identified, one of them is Pachyaximine A, which has potential cholinolytic function related with motion sickness. But, the n-butanol extract can only play a short-term effect on motion sickness. In order to offer a long-term effect, a kind of sustained-release membrane was made. Its transdermal absorption behavior conformed to the Higuchi equation, which was Qn = 94.46t1/2+170.42. And its accumulated transmittance η could reach 78.97% after 24 h, indicating a good sustained-release effect to prolong effective time of preventing motion sickness in mice.

Keywords: Perilla compound prescription, motion sickness, effective constituent, N-butanol extract, sustained-release membrane

Introduction

The task comes from manned space pre-research project. Tail-suspended rats or cells were handled with microgravity, according to the specific symptoms of motion sickness to make a targeted screening for effective parts of the Traditional Chinese medicine compound preparation [1].

Motion sickness was firstly proposed by Irwin which is organism's neurological disorders caused by abnormal movements [2], in which the space motion has the highest incidence sickness about 50%, while seasickness, airsickness and carsickness can respectively reach 25-30%, 10% and 4% [3-5]. The patient will produce dizziness, upper abdominal discomfort, nausea, vomiting, cold sweat, pale and other vestibular response. With the continuous development of space industry, all the countries have begun to attach importance to the prevention and treatment of motion sickness. Related subjects involve neuroscience, clinical medicine, military medicine and other fields.

For these years, the motion sickness was mainly cured by drug therapy and acclimatization training [6]. But, neural drugs scopolamine, o-tolylhenazine, ephedrine and amphetamines often have a mass of side effects, such as lethargy, memory loss, blurred vision and other adverse reactions [7, 8]. Therefore, to develop new anti-motion sickness preparations with significant therapeutic efficacy and low side effects is very important [9]. Medicinal and homologous plants named Perilla, Zingiber,

pericarpium citri reticulatae and mint all have extensive resources, easy access, low prices and fewer side effects. Traditional Chinese medicine argued that Perilla has the role of preventing stomach from vomiting, but the reports on it can be counted on one's finger, there is no report about the Perilla which have the function of treating motion sickness or releasing the related symptoms after Wang JZ proved that Perilla has the role of vomiting and promoting gastrointestinal movement. In contrast, the study of Zingiber has became more mature all over the world, Yang et al and Huang et al were found that diphenyl heptane compounds in Zingiber possess vomiting efficacy and antibacterial activity [10, 11]. European countries also had already included Zingiber in pharmacopoeia and widely used in the treatment of motion sickness [12]. At the same time, hesperidin, citrinin, limonin and alkaloids contained in Pericarpium citri reticulatae were testified to possess the role of anti-inflammatory and gastrointestinal motility. Although the four kinds of single traditional Chinese medicine mentioned above were widely used in carsickness pills, not any anti-motion sickness studies so far, their compound preparations have not yet reported either [13-15]. If we can play their compound preparations' advantages of multi-component, multi-target, and multi-channel for treating motion sickness and make good use of their synergistic effect, multiplier effects can be achieved.

In addition to that, in order to overcome the shortcomings of traditional Chinese medicine machining, such as time-consuming process and preparation's rough, excess dark in color. Active chemical constituents was collected in this article by chemical methods to enhance the efficacy of preparations, which can provide a basis for the study of anti-motion sickness drugs and improving individuals' daily life, brought a great deal of convenience in the travel [16]. At the same time, promoting the development of the aerospace industry by protecting the health of astronauts, enhancing national maritime and aerial combat capability [17].

Materials and methods

Preparation of effective constituents

A total of 270 g of perilla compound prescription (perilla: Zingiber: pericarpium citri reticulatae:mint = 10:7:5:5) was reflux extracted with distilled water at 65°C for 5 h. The gotten solution was concentrated by evaporation and extracted with same volume of n-butanol (Kermel, Tianjin, China) three times. The supernatant was n-butanol extract and lower layer was water extract. After that, adding ten volumes of 95% ethanol (Kermel, Tianjin, China) into dried drug residue with solid/liquid ratio at 1:10, and then treating them by ultrasound for 4 h to get ethanol extract.

Animals

A total of 80 male Kun-ming mice were purchased from the Health and Anti-epidemic Service, Lanzhou, China. These mice were 6-8 weeks old, weighing 20.0±5 g, and maintained under conditions of controlled temperature (23±2°C), humidity (50±5%), and a 12 h light/dark cycle. Before being used for experiments, the mice were allowed at least 1 week to adapt to the environment. Mice were randomly divided into 12 groups (n = 5 per group) and 4 groups (n = 5 per group) respectively in the two experiments mentioned following. 1. Establishment of motion sickness model: These groups comprised of untreated control group, model set group, positive control group (0.83 mg/mL), n-butanol extract treatment group, water extract treatment group, ethanol extract treatment group, water elution of n-butanol extract treatment group, 25% ethanol elution of n-butanol extract treatment group; 65% ethanol elution of n-butanol extract treatment group and 95% ethanol elution of n-butanol extract treatment group. 2. Test of long-term prevention effects: These groups comprised of untreated control group, model set group, positive control group (0.83 mg/mL) and group of n-butanol extract treatment for long term.

The active compound samples to be tested were dissolved in normal saline (NS). Positive control group was tea diphenhydramine solution (Kermel, Tianjin, China). Each experimental treatment group was given corresponding constituents with crude drug 1.34 g/mL (Chinese Pharmacopoeia 2010) at the volume of 10 mL/kg. The mice in the control groups and model set groups were given NS daily on the basis of equal volume. All mice, except those from the untreated control group, were suffered vertigo stimulation. The procedures of the experiment

were strictly performed according to the generally accepted international rules and regulations.

Establishment and optimization of motion sickness model

The vertigo stimulation which in order to establish a simulated space motion sickness mode could be produced through the self-assembled halo stimulator, which came from Crampton and modified by the Institute of Extreme Environment and Protection in Harbin Institute of Technology. The instrument consists of two horizontal rotating arms and a frequency controller. Each rotating arm's length is 0.6 m, the arm could fix cage which can put 8 mice. Kaolin consumption, food intake and halo response index were selected as motion sickness indexes to make sure the maximal incidence of motion sickness in mice under the experimental condition [18, 19]. It was clockwise for l0 s with angular acceleration of 300 °/s2 to rotate until arriving peak speed 300 °/s. Then with the speed of angular acceleration of 300 °/s2 to decelerate for 10 s after uniform rotation for 35 s. At last, counterclockwise to repeat the above rotary motion mode with a 5 s interval. A cycle calendar was 2 min, recycling 20 cycles with time consuming for 40 min. According to the mouse biological habits, the experimental time was fixed at 19:00-22:00 every night. 30 min after oral administration with corresponding constituents for each mice group, the vertigo stimulation experiments were processed and repeated 3 times.

Study on the relationship between effective constituents and mice's blood hormones

Using EDTA-K2 blood collection (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to get the eyeball blood of each groups' mice, the blood was centrifuged for 20 min with 2000 rpm at 4°C. arginine vasopressin (AVP) and cortiso (CORT) enzyme immunoassay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were then used to produce pharmacodynamic experiment, according to the absorbance value (OD) with UV spectrophotometer (Shimadzu-UV-2450, Shanghai, China) at 450 nm wavelength, to calculate the AVP and CORT concentration in all samples [20].

Isolation and analysis of n-butanol extract

The n-butanol extract of perilla compound prescription were separated and purified by passing through an AB-8 macroporous adsorption resin column (Qingdao Haiyang Chemical Plant, China), at a flow rate of 5 mL/min and being stable for 12 h. After that, the mobile phase was eluted with a step gradient ethanol solvent system (distilled water, 25% ethanol, 65% ethanol and 95% ethanol). Eluted fractions were utilized to produce suspension with distilled water after being concentrated and dried. Then these suspensions were applied into mice model to process anti-athletic drug efficacy comparison. And the fraction with largest quality would be used in following experiments. Medium pressure liquid chromatographic (MPLC, Shimadzu Technologies, Japan) equipped with a quaternary gradient pump and a UV detector (column: Iso RediSep normal phase silica gel column, detection wavelength: 254 nm, flow rate: 40 mL/min, elution solvent consisted of A: chloroform, B: methanol with the following gradient program: 10 min, 90%A; 16 min, 80%A; 18 min, 10%A; 10 min, 0%A) was used to initially separate. And then, the eluent in the vicinity of the maximum UV absorption peak could be collected to be used in next experiment. Silica gel column chromatography (Qingdao Haiyang Chemical Plant, China) was used to handle with the eluent mentioned above, chloroform-methanol (1:4) would as the agent to track and detect the eluent with thin layer chromatography (TLC, Agilent Technologies, USA) after vacuum concentration. UV wavelengths were 254 nm and 365 nm, reagent was 5% sulfuric acid-ethanol solution, the same components could be combined and the spot with clearest color would be used in following analysis. Furthermore, the eluent of the spot could process UV spectrophotometer (Shimadzu-UV-2450, Shanghai, China) with 190-410 nm full scan and the UV absorption peak would as the HPLC detection wavelength to process high performance liquid chromatography tandem mass spectrometry (HPLC-MS, Finnigan Technologies, USA) analysis of n-butanol chemical sites. The HLPC-MS was developed using a reversed-phase C18 column (Agilent-TC, 250 mm × 4.6 mm, 5 μm i.d.) with the column temperature at 30°C. Sample injection quantity was 20 μL, the elution solvent consisted of 0.3% formic acid

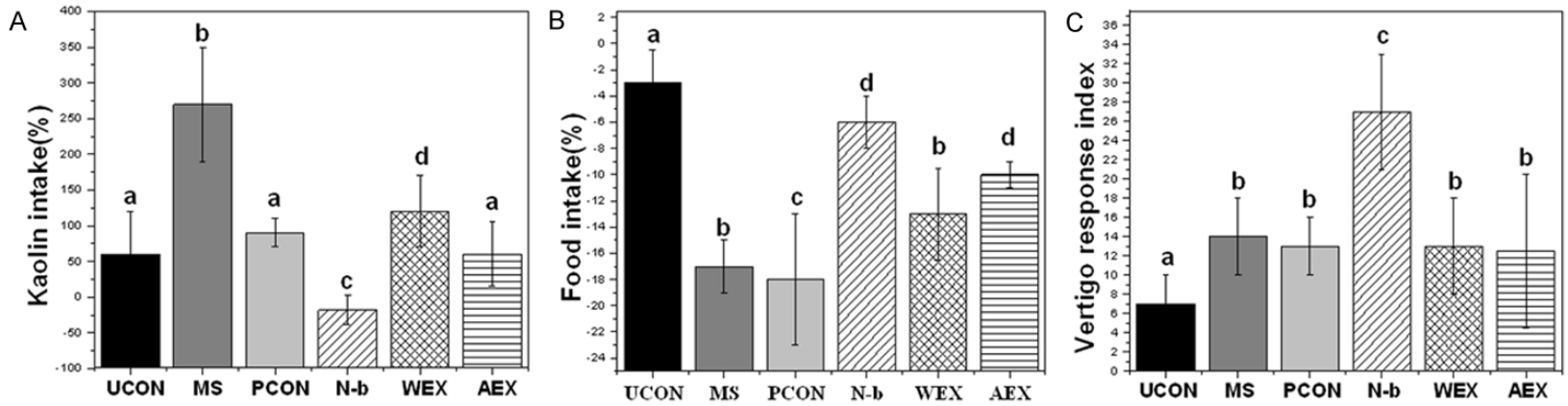


Figure 1. The effects of different constituents of perilla compound prescription for treating mice's motion sickness. Different letters (a-d) represent significant difference, $P<0.05$. A. Effects of various constituents on kaolin intake increment in mice model. B. Effects of various constituents on food intake increment in mice model. C. Effects of various constituents on vertigo response index in mice model. UCON = untreated control group; MS = model set; PCON = positive control group; N-b = N-butanol extract treatment group; WEX = water extract treatment group; AEX = alcohol extract treatment group.

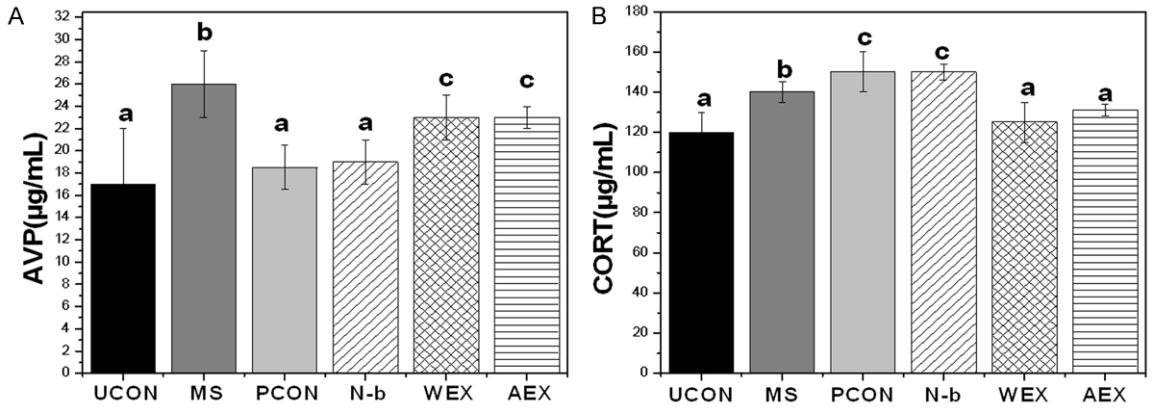


Figure 2. The pharmacodynamic tests of different constituents of perilla compound prescription. Different letters (a-d) represent significant difference, P<0.05. A. Effects of different constituents on AVP level in mice's blood. B. Effects of different constituents on CORT level in mice's blood. UCON = untreated control group; MS = model set; PCON = positive control group; N-b = N-butanol extract treatment group; WEX = water extract treatment group; AEX = alcohol extract treatment group.

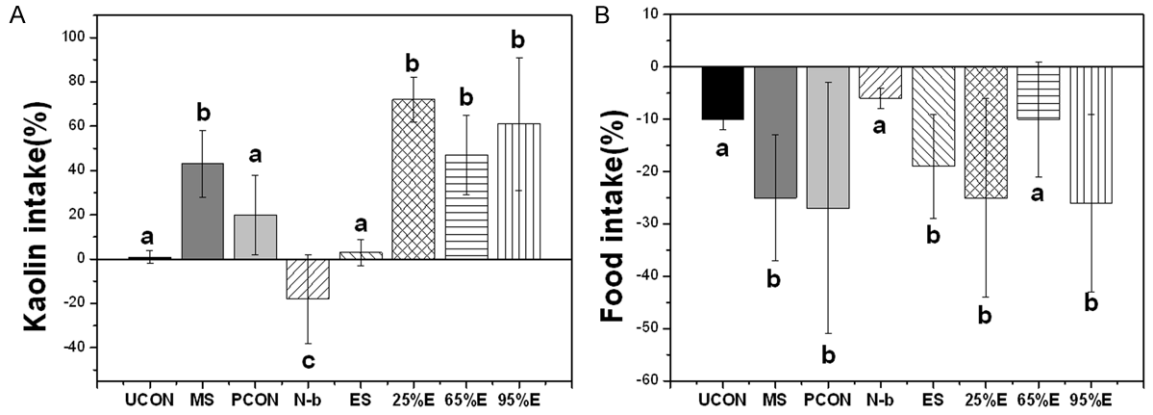


Figure 3. The effect of different elution components in n-butanol extract for treating mice's motion sickness. Different letters (a-d) represent significant difference, P<0.05. A. Effects of various elution components on kaolin intake increment in mice model. B. Effects of various elution components on food intake increment in mice model. UCON = untreated control group; MS = model set; PCON = positive control group; N-b = N-butanol extract treatment group; ES = water elution extract treatment group; 25%E = 25% ethanol elution extract treatment group; 65%E = 65% ethanol elution extract treatment group; 95%E = 95% ethanol elution extract treatment group.

(A):methanol (B) = 30:70, the flow rate was kept at 0.5 μL/min and mass spectrometry was in positive ion mode.

Long-term prevention effects for motion sickness

After oral administration with corresponding constituents for 10 days, the vertigo stimulation experiments were carried out at the following day 1, 3 and 5. Through the kaolin consumption and food intake to make sure the n-butanol extract's long-term prevention effects for motion sickness in different group mice.

Preparation of sustained-release membrane and transdermal absorption effect in vitro

Sustained-release membrane is an external transdermal patches, which can produce local or systemic effects [21]. Preparation of the membrane in this experiment referenced methods in the literature [22]. The matrix of sustained-release membrane was consisted with sodium carboxymethyl cellulose, sodium polyacrylate, polyvinyl alcohol, gelatin, glycerin, kaolin and water (0.5:1:3:2:18:4:50). After mixing the phase I and II, the 5% perilla compound prescription was put into the sustained-release

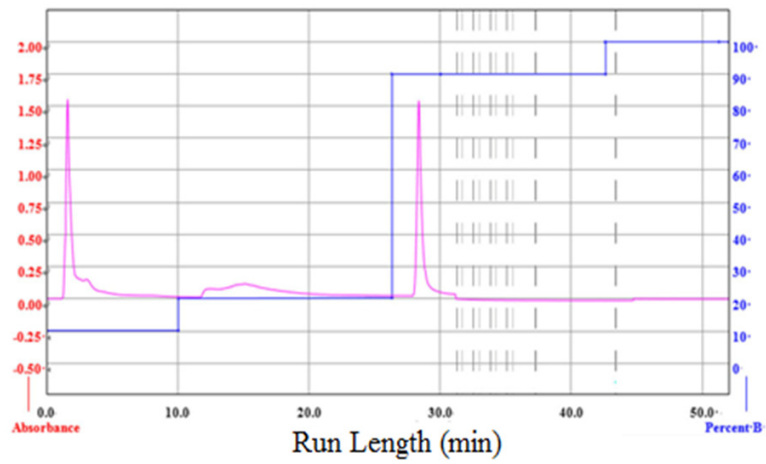


Figure 4. Medium pressure chromatography gradient elution.

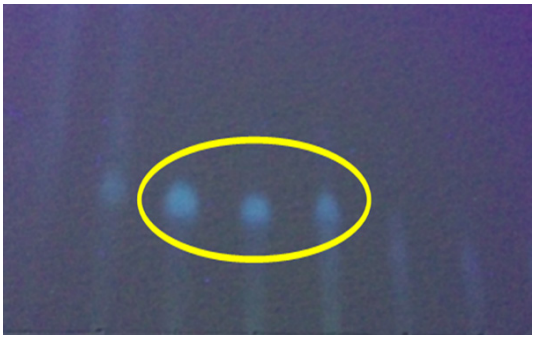


Figure 5. TLC analysis.

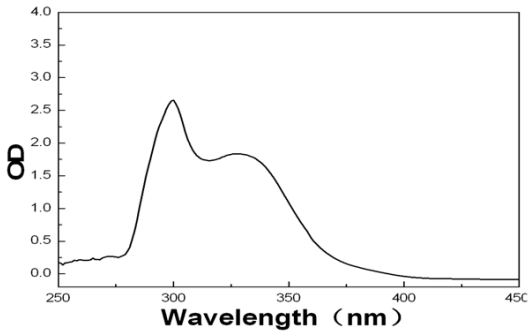


Figure 6. UV absorption spectra of ZY2.

membrane matrix. (Phase I: the polyvinyl alcohol dissolved with 90°C water, adding the amount of gelatin gel, until it natural swelled and then placed them in 60°C water to be dissolved. Phase II: polyacrylate, sodium carboxymethyl cellulose and kaolin were added into glycerin Sodium and then processed magnetic stir). The gotten materials were vacuum dried at 25°C for 1 min, and uniformly coated on non-woven fabric, the surface of the substrate was covered with a polyethylene as a protective film. And the Sustained-release membrane should through progress sensitivity test to testy its security [23].

Using optimized Franz diffusion cell administered transdermal experiments [24]. Making the sustained-release membrane close to the mice skin cuticles (Harbin Medical University, Harbin, China), and let them caught in the diffusion cell and cell cover. 1 mL receiving liquid was pipetted from the chute sampling port at different time points (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h), the reference cell was also set. The content of receiving liquid was detected by HPLC (Agilent Technologies, USA), which was developed using a reversed-phase C18 column (Agilent-TC, 250 mm × 4.6 mm, 5 μm i.d.), column temperature at 30°C, sample injection quantity was 20 μL, the elution solvent consisted of A: 0.1% formic acid, B: methanol with the following gradient program: 0-10 min, 90%A; 10-20 min, 85%A; 20-30 min, 80%A; 30-40 min, 75-90%A; 40-50 min, 90%A. The flow rate was kept at 1 ml/min, and the absorbance was measured at a wavelength of 230 nm. According to the formulas mentioned in literature to calculate related transdermal absorption parameters, which would reflect transdermal absorption effect in vitro [25].

Statistical analysis

All values were given as mean standard deviation. All results were analyzed by one-way analysis of variance (ANOVA) using Statistical Product and Service Solutions 19.0 software (SPSS19.0). A p-value <0.05 indicated that there was a significant difference.

Results and discussions

Model establishment and chemical constituents' effect for evaluation indexes

Motion sickness is autonomic dysfunction caused by vestibular stimulation, mainly per-

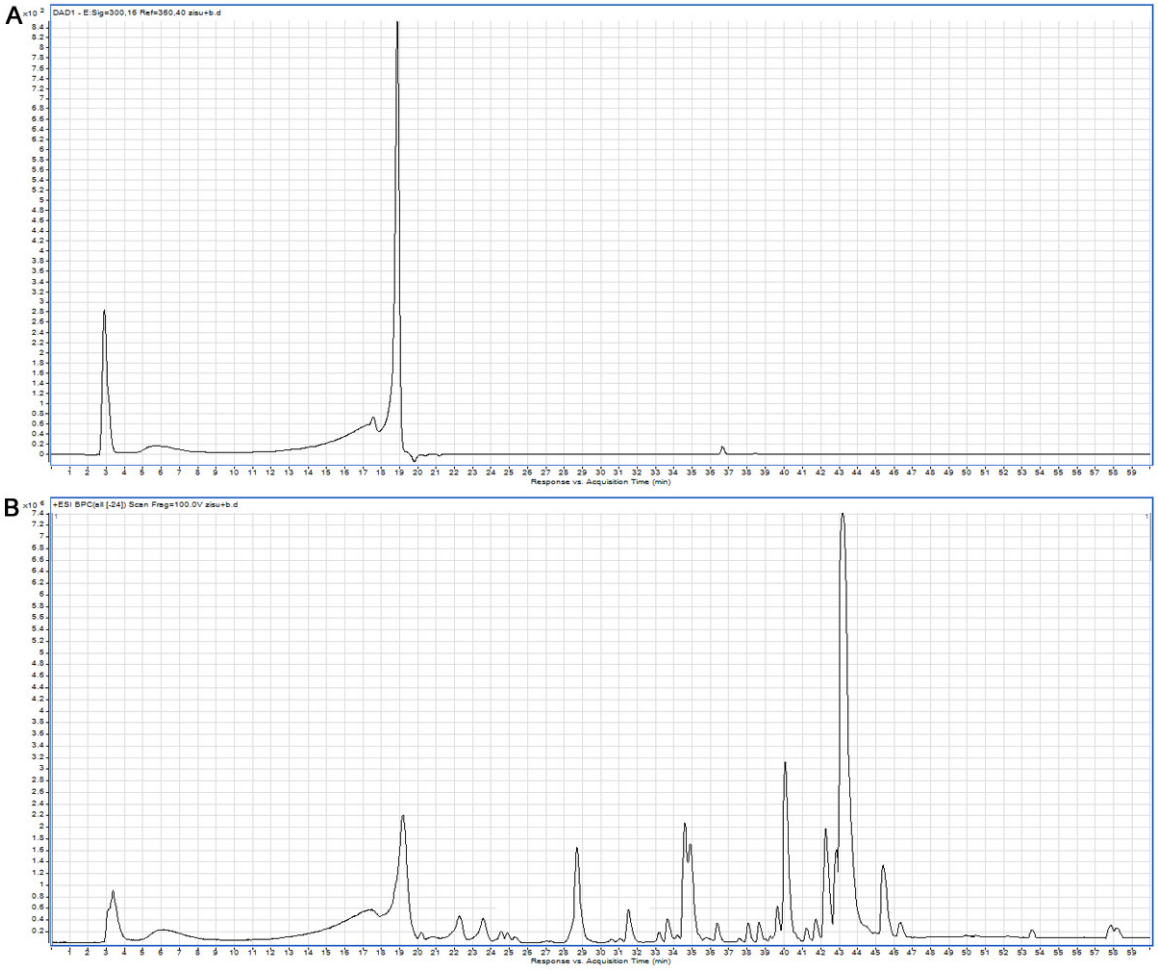


Figure 7. Analysis of ZY2 (A) Analysis results of ZY2 by HPLC. (B) Total ion mass spectrometry spectra of ZY2.

forming gastrointestinal dysfunction, such as nausea and vomiting. Mice are rodents and have no vomiting reflexes, but according to some reports, mice exhibit pica behavior, which corresponds to non-rodent animals' vomiting reactions [18]. So, based on kaolin consumption, which can be a reliable quantitative indicator to measure the degree of motion sickness. Some other scholars through the statistics of mice's defecation, shuddering and urination during the halo stimulation to make a calculation of halo reaction index, which can be evaluation index of motion sickness. But in this experiment, we found that the data of the halo reaction index were unstable, the individual difference was huge, and can not correspond with mice's pica behavior. Furthermore, many laboratories had used a variety of animal motion models, but the majority rotation modes were variable-speed rotation or linear reciprocating motion. In this experiment, by changing the rotation radius, acceleration, period and direction of the self-assembled halo stimulator, a more effective mouse motion sickness model was established. As shown in **Figure 1**, after a comprehensive investigation, the n-butanol extract group could be clearly defined as the effective constituent, which had a significantly effect on alleviating motion sickness of mice through reducing kaolin consumption and halo response index, also increasing food intake.

Pharmacodynamic effect for motion sickness

For the reason that arginine AVP levels will significantly rise in human's blood when suffered by vertigo stimulation, and the rise of CORT levels can protect their body from nausea and vomiting [26, 27]. From **Figure 2A**, we can find that the corresponding AVP concentration in the positive control group and the n-butanol

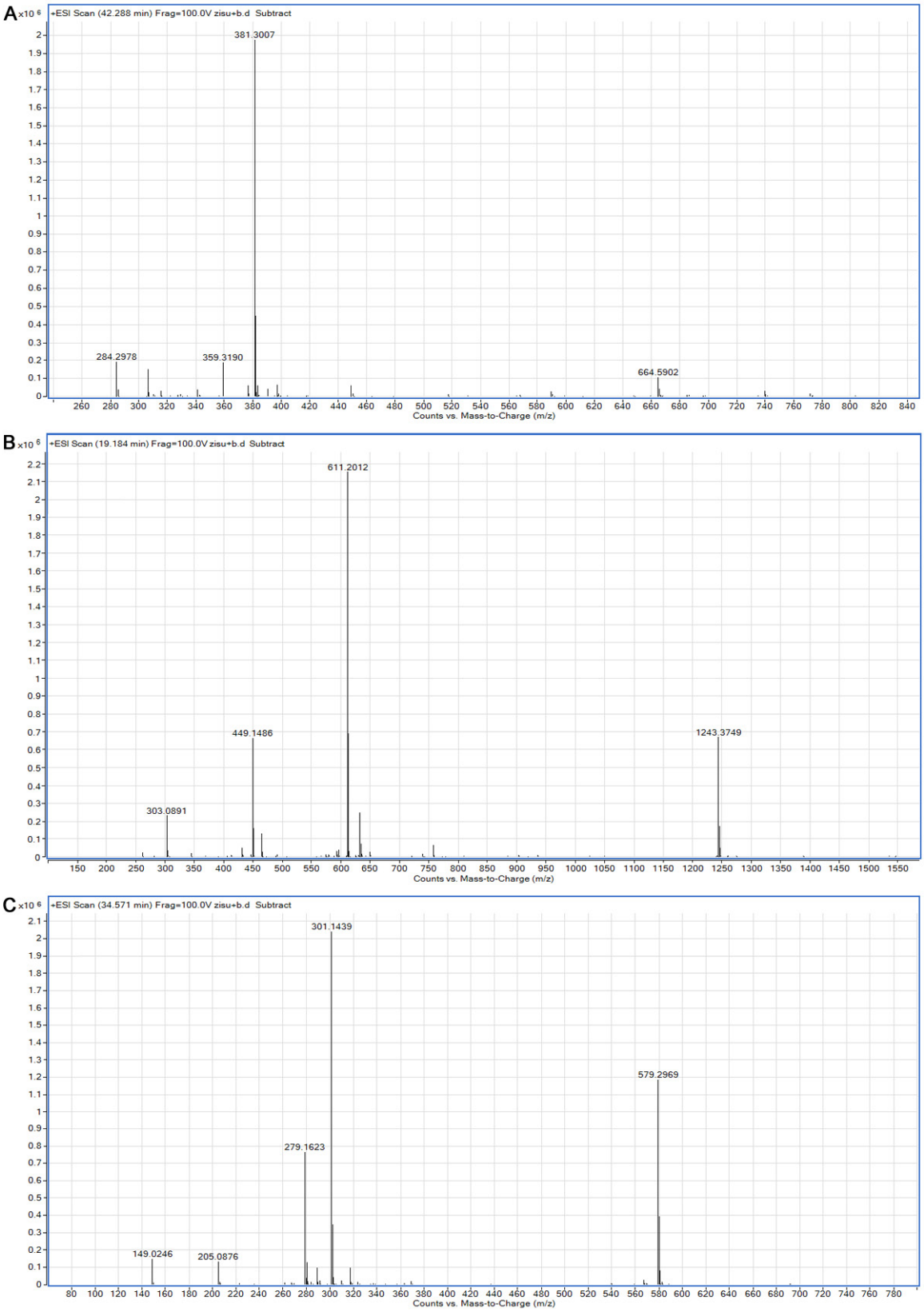
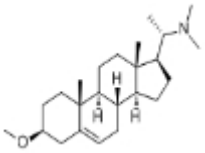
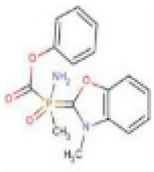
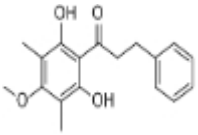
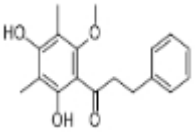
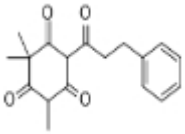


Figure 8. A. Positive ion mass spectrum (42.288 min); B. Positive ion mass spectrum (19.184 min); C. Positive ion mass spectrum (34.571 min).

Table 1. Molecular information of Pachyaximine A, Phosphonamidic acid and Myrigalone B, A, D

Formula	Name	Structure
$C_{24}H_{41}NO$	Pachyaximine A	
$C_{15}H_{15}N_{203}P$	Phosphonamidic acid	
$C_{18}H_{20}O_4$	Myrigalone B	
$C_{18}H_{20}O_4$	Myrigalone D	
$C_{18}H_{20}O_4$	Myrigalone A	

extract group, which had significant effect on motion sickness and significantly different from that of the model set, indicating changes in AVP levels can indirectly reflect the incidence of motion sickness in mice. There are also some studies reported that AVP can lead to gastric rhythm disorders and the production of unusually fast broadcast similar to motion sickness response [26]. The level of AVP in the blood of the people in the event of vomiting will be significantly increased and this experiment also found that mice produce motion sickness when AVP levels were significantly increased, which showed that the mice's response correspond to the human beings when they suffered motion disease [20, 28]. It can be speculated that the kaolin intake increment in mice can indirectly reflect vomiting symptoms of human beings when they suffered by motion sickness and the AVP levels can show the disease severity. As shown in **Figure 2A**, we found that the CORT level was higher in the n-butanol group, positive control group and the model set (because of stress adaptation in organism) than that in the untreated control group, suggesting that the level of CORT was indicative of the degree of adaptation for motion sickness. The higher the level of CORT, the less symptoms of motion sickness in mice, the more able to protect them from nausea and vomiting symptoms [27]. Therefore, we can conclude that n-butanol extract as an effective constituent can treat motion sickness by decreasing AVP levels and increasing CORT levels.

Chemical composition of n-butanol extract

It can be seen from **Figure 3**. that the water elution component of the n-butanol extract had a great effect on the behavior of the pica in mice, and the 65% ethanol elution component of n-butanol extract had a certain therapeutic effect on mice's loss of appetite, both of them had significant differencees with untreated control group. But, its comprehensive efficacy on motion sickness was lower than the overall effect of n-butanol site, indicating that the anti-motion sickness activity of n-butanol constituent was attributed to four multi-component substances (water, 25% ethanol, 25% ethanol and 95% ethanol elution parts). The mobile phase was eluted with 25% ethanol had largest quality (3.96±0.32 g of 10 g n-butanol extract) and it would be used in following experiments. The eluent could be collected and referred as ZY1 in the vicinity of maximum peak with MPLC as shown in **Figure 4**. Then the main ingredient of ZY1 was isolated by silica gel column chromatography and three spots were found with the value of 0.25 shown in **Figure 5**. The merger of the three spots was denoted as ZY2. Full wavelength scanning UV was processed to analyze ZY2 after being dissolved with methanol [29]. These results were shown in **Figure 6**. From scanning pattern, the constituents from ZY2 had a strong absorption at 300 nm. So 300 nm was used as the detection wavelength to analyze the chemical compounds by HPLC-MS. The results obtained by HPLC and total ion analysis were shown in **Figure 7**. According to the results by HPLC-MS, we defined the molecular formula by chemical compounds' molecular weight, abundance, spatial matching and the polarity of extractive. Some information were indicated as

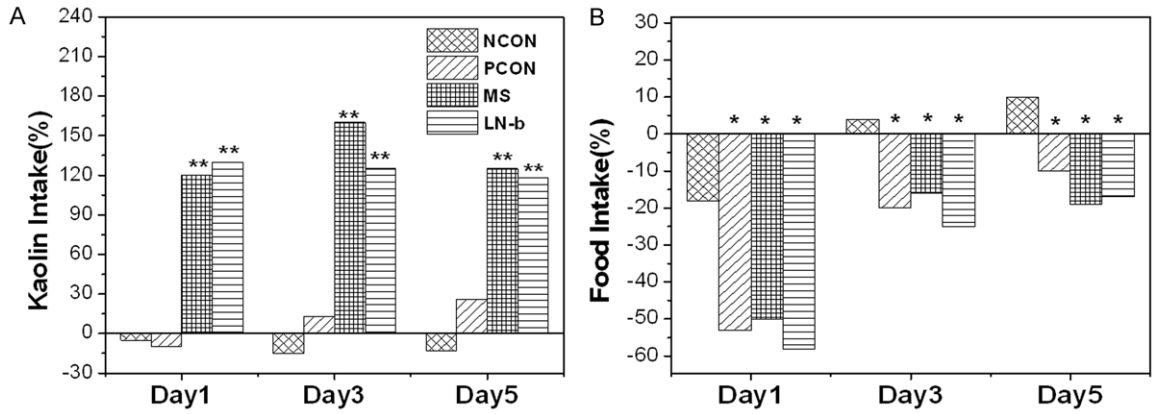


Figure 9. Effects of different mice group on motion sickness indexes when mice were lavaged with n-butanol for a long-term period. *P<0.05, **P<0.01 (compared with untreated control group). A. Effects on kaolin intake increment in mice model. B. Effects on food intake increment in mice model. UCON = untreated control group; MS = model set; PCON = positive control group; LN-b = the group of N-butanol extract treatment for a long term.

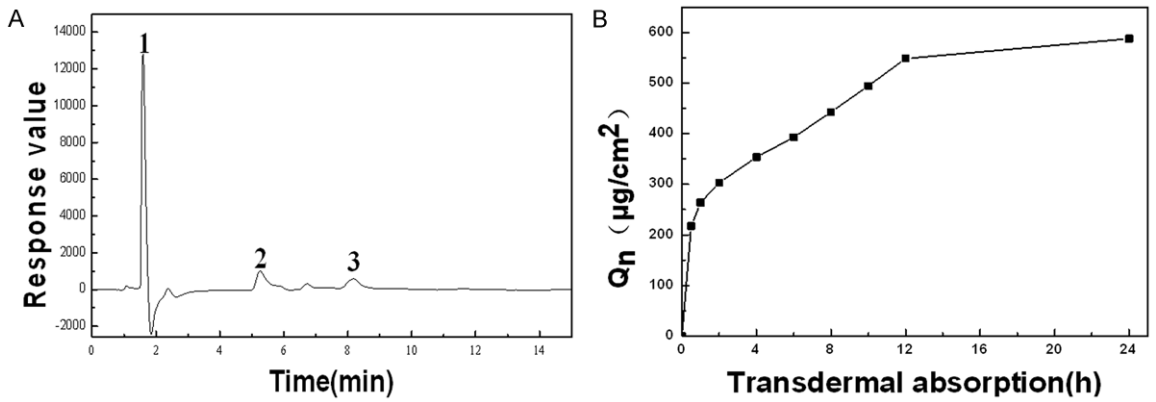


Figure 10. Percutaneous absorption experiments in vitro of sustained-release membrane. A. HPLC spectra of the reference cell. B. Percutaneous absorption curve in vitro of sustained-release membrane.

Table 2. List of cumulative permeation value Qn and accumulated transmittance η

Transdermal time (h)	Qn (μg/cm^2)	H (%)
0.5	217.45	29.21
1	263.81	35.44
2	303.18	40.72
4	353.74	47.52
6	393.06	52.80
8	442.71	59.47
10	494.57	66.43
12	548.34	73.65
24	587.92	78.97

shown in **Figure 8A**: From the ESI (+) spectra at 42.288 min, which could be clearly observed was that m/z 359.31 was [M+Na]+ ion signal, m/z 381.30 was [M+Na]+ ion signal. Through library comparison, the molecular formula was $C_{24}H_{41}NO$ and had a large polar material for the reason that the constituent was eluted by macroporous resin with 25% ethanol derived from n-butanol extract. It could only be found that Pachyaximine A (**Table 1**) was consistent with these analysis [30]. Qiu MH et al was firstly reported Pachyaximine A could be isolated from P. axiiiaris Franch, they also found out some isomerides of it, but after that, few scholars had reported these substances [31]. Pachyaximine A belongs to steroidal alkaloids. Steroid alkaloids are natural steroidal nitrogenous derivatives, also having the most complex type of structure in the alkaloids. Its bioactivity mainly reflected in antihypertensive, antitussive, anti-asthmatic, anticholinergic, cardiac

Table 3. Percutaneous absorption curve results of different models in vitro

Model	Absorption curve	R2
Zero-order release model	Qn = 19.76t+227.08	0.6875
First degree order release model	Qn = 99.33ln(t)+254.59	0.9428
Higuchi model	Qn = 94.46t1/2+170.42	0.9560

and so on [32, 33]. At present, the anticholinergic effects of steroid alkaloids have been reported at home and abroad, such as Lin et al had reported that the steroidal alkaloids extracted from Fritillaria had a strong anticholinergic effect. At the same time, The current treatment of motion disease is also a kind of anticholinergic drug named scopolamine [30]. Therefore, it was speculated that the mechanism of anti-motion disease of n-butanol extract was related to the anticholinergic effect of Pachyaximine A. From ESI (+) spectra at 19.184 min in **Figure 8B**, which can be used to define the chemical substance's molecular formula was $C_{15}H_{15}N_{203}P$ at m/z 303. The formula corresponds to Phosphonamidic acid (**Table 1**). Phosphonamidic acid also had anticholinergic effects, which could produce synergistic effect with Pachyaximine A to increase the efficacy of n-butanol extract on treating motion sickness [34, 35]. From the ESI (+) spectra at 34.571 min in **Figure 8C**, which can be used to define the chemical substance's molecular formula was $C_{18}H_{20}O_4$ at m/z 300. The formula corresponds to Myrigalone B (**Table 1**) with molecular weight of 300. There are some scholars had reported that Myrigalone B could be isolated from the extract of Camellia sinensis. It has a significant antioxidant activity and free radical scavenging activity [36-38]. This bioactivity could show a strong relationship with n-butanol extract's activity to treat motion sickness through inhibiting enzymatic lipid peroxidation in mice. Myrigalone B also has two isomerides named Myrigalone A and Myrigalone D, which show less activity on the antioxidant [37]. All these three chemical constituents can be determined by library comparison and polarity. The remaining molecular formula listed by Table S1 in appendix whose molecular matching-degree was more than 90%. From Table S1, No. 2, 3, 4, No. 6, 7, 8, 9, 10 and No. 11, 12, 13 are isomerides, which have the same mather nucleus, but different locations of hydroxyl group. These isomerides could be further identified after separation by C Nuclear Magnetic Resonance (CNMR) and H Nuclear Magnetic Resonance (HNMR).

Ability of transdermal absorption of sustained-release membrane

As **Figure 9** showed, mice after oral administration for 10 days with n-butanol at the day of 1, 3, 5. The increase of kaolin intake increment and the decrease of food intake increment still happened, which indicated n-butanol extract can only play a short-term efficacy for motion sickness. In order to prolong the effect of n-butanol extract, a kind of sustained-release membrane was introduced. Through orthogonal test, we acquired hydrophilic matrix material as the matrix of sustained-release membrane, which can reach a good release effect. At the same time, the sustained-release membrane's adhesion, formability and ointment content were consistent with international standards (Chinese Pharmacopoeia 2015). The phase diagram of reference cell solution was obtained by HPLC analysis as shown in **Figure 10A**, and other HPLC analysis of samples in different time were exhibited in Figure S1 in appendix. Cumulative permeation Qn and accumulated transmittance η could be calculated shown in **Figure 10B** and **Table 2** through the substance which No. 1 peak corresponded. In order to study the mechanism of sustained-release membrane, we adapted Qn-t as the research object and selected three commonly drug release mathematical models: zero-order release model, first degree order release model and Higuchi equation curve. By **Table 3** we found that Higuchi equation was compatible with the transdermal absorption behavior of sustained-release membrane of anti-motion sickness. It was Qn = 94.46t1/2+170.42, R2 = 0.9560. The sustained-release membrane's permeation rate was 94.46 μg/cm^2•h1/2, and the accumulated transmittance η could reach 78.97% after 24 h, which indicated a good release effect.

Conclusion

In this paper, we studied the effective constituents in compound perilla compound prescription, which provided reference for the refinement of traditional Chinese medicine, so as to reduce the dosage of drugs and strengthen the curative effect. We used a variety of purification analysis to explore the biological activities

of various chemical extracts of the perilla compound prescription and found that these constituents especially n-butanol extract can improve the biological indicators in the blood and increase appetite. We also identified that three compounds in n-butanol extract and found that one of them is steroidal alkaloids named Pachyaximine A with anticholinergic effect, which has a main role in anti-motion sickness. In addition, although n-butanol extract can improve motion sickness in mice significantly, the effect can only play a short-term effect. In order to improve the efficacy, a kind of membrane with slow-release effect was produced, its accumulated transmittance could reach 78.97% after 24 h. The method of producing sustained-release membrane can effectively reduce the sudden release phenomenon of many kinds of drugs. All the results not only would offer some data base and theory support in the aerospace and medical career on treating motion sickness, but also have a good application in our daily life, brought a great deal of convenience in the travel. At the same time, providing new method to overcome disadvantages in the progress of handling Chinese Traditional Medicine, such as time-wasting or preparation's undesirable character.

Acknowledgements

This research was supported by the Outstanding Academic Leaders Foundation of Harbin city (No. 2013RFXXJ042) and Planning Project for Space Application (01-1-08).

Disclosure of conflict of interest

None.

Authors' contribution

Weihong Lu conceived this study; Yingyu Zhou wrote the paper; Song Wei and Jiao Zhao conducted the experiments; Denis Baranenko and Yongzhi Li analyzed the data.

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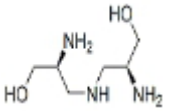
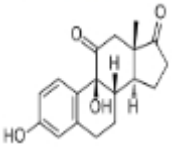
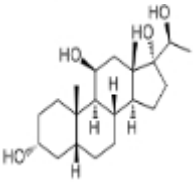
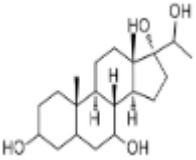
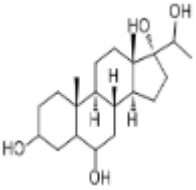
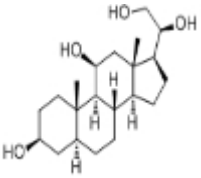
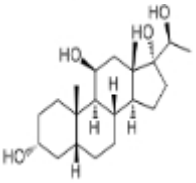
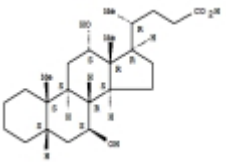
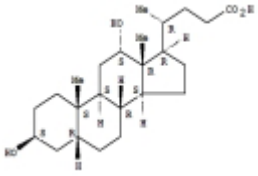
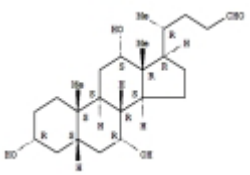
Table S1. The other analysis of HPLC-MS

No.	Molecular weight	Formula	Name	Structural formula
1	273.45	$C_{16}H_{35}N_2$	Anthraquinone alcohol derivative	
2	300	$C_{18}H_{20}O_4$	9β-Estra-1,3,5 (10)-triene-11,17-dione,3,9-dihydroxy- (7Cl,8Cl)	
3	353	$C_{21}H_{36}O_4$	5β-Pregnane-3α,11β,17α,20α-tetrol	
4	353	$C_{21}H_{36}O_4$	Pregnane-3,7,17,20-tetrol	
5	353	$C_{21}H_{36}O_4$	Pregnane-3,6,17,20-tetrol	
6	353	$C_{21}H_{36}O_4$	Pregnane-3,11,20,21-tetrol	
7	353	$C_{21}H_{36}O_4$	Pregnane-3,11,17,20-tetrol	
8	393	$C_{24}H_{40}O_4$	7β,12α-Dihydroxy-5β-cholan-24-oicacid;	
9	393	$C_{24}H_{40}O_4$	Cholan-24-oicacid,3,12-dihydroxy-,(3β,5β,12α)-(9Cl)	
10	393	$C_{24}H_{40}O_4$	Cholan-24-αl,3,7,12-trihydroxy-,3α,5β,7α,12α)-(9Cl)	

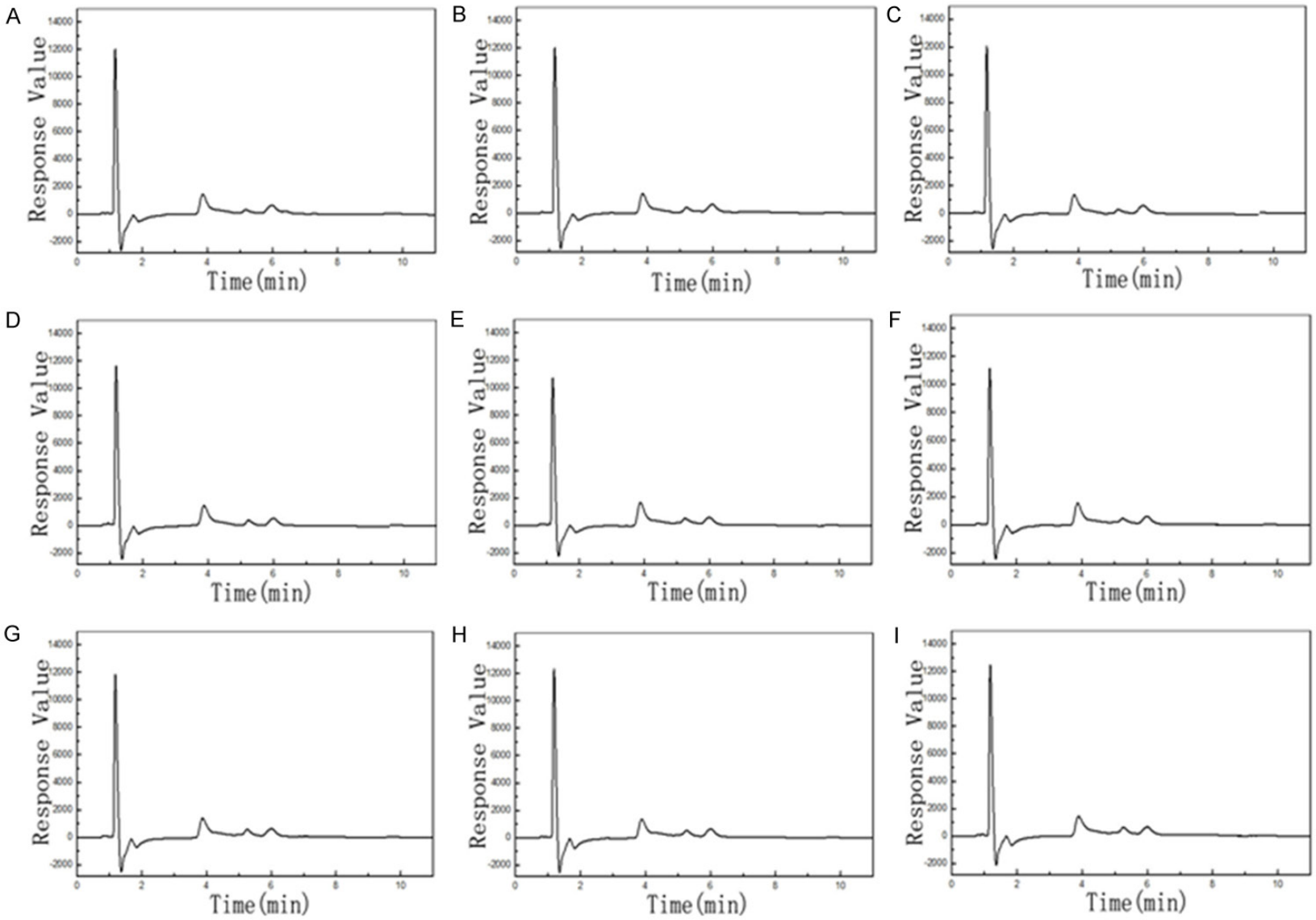


Figure S1. HPLC analysis of transdermal receiving fluid in different time (A) In 0.5 h (B) In 1 h (C) In 2 h (D) In 4 h (E) In 6 h (F) In 8 h (G) In 10 h (H) In 12 h (I) In 24 h.