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# Volatile components in Yinchenzhufu decoction and their pharmacokinetics after oral administration in rats†

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In China, Yinchenzhufu decoction (YCZFD) has been used to treat cholestatic liver disease in clinical practice for hundreds of years. Nonvolatile components in YCZFD, their composition, components absorbed in blood, and pharmacokinetic characteristics have been clarified. However, information about its volatile components is limited. The aim of the present study was to identify the components of the volatile oil (VO) of YCZFD, quantify the major volatile components in YCZFD, and reveal their pharmacokinetic characteristics. In YCZFD, 85 components representing 95.36% of the total oil composition were identified by gas chromatography-mass spectrometry. Next, 11 highly abundant components were quantified in YCZFD and YCZFD VO. Finally, a sensitive headspace solid-phase dynamic extraction-chromatography-quadruple mass spectrometry method for determining 8 volatile components in rat plasma was established and applied to compare the pharmacokinetics of YCZFD and YCZFD VO after oral administration in rats. These volatile components were rapidly absorbed and eliminated, and they presented highly different exposure levels. The area under the concentration–time curves of some volatile components in YCZFD was higher than that in YCZFD VO. The results showed that the water extract of YCZFD increased the exposure of volatile components. Our study provides valuable information for understanding the potential effective components of YCZFD.

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## 1. Introduction

Yinchenzhufu decoction (YCZFD) consists of *Artemisia capillaris* Thunb. (ACT), *Rhizoma Atractylodes Macrocephala* (RAM), *Radix Glycyrrhizae* (RG), *Radix Aconiti Lateralis Preparata* (RALP), *Rhizoma Zingiberis* (RZ), and *Cinnamomum cassia* Presl (CCP). It is a classic Chinese herbal prescription that has been used to treat cholestatic liver disease since the Qing Dynasty (18th century CE).<sup>1</sup> In China, YCZFD is still widely used to treat chronic liver failure with jaundice in modern clinical practice.<sup>2</sup> Experimental studies have shown that YCZFD decreases jaundice, improves liver function, and alleviates liver damage in animal models.<sup>3,4</sup> However, to date, the effective components associated with the effect of YCZFD against liver injury have not been very clear *in vivo*.

In our previous studies, nonvolatile components of YCZFD were quantified<sup>1</sup> and their pharmacokinetics were elucidated,<sup>5</sup> providing information about the potential effective components in YCZFD. However, information pertaining to the volatile components in YCZFD and their pharmacokinetics is limited. Volatile components extracted from plants are often mixed in volatile oil (VO), and they are characterized by various biological activities. The essential oil of *A. capillaris* exhibits anti-inflammatory effect.<sup>6</sup> Moreover, the essential oil and capillin from *A. capillaris* exhibit antibacterial activity.<sup>7,8</sup> The essential oil of *Rhizoma Zingiberis* shows antimicrobial,<sup>9</sup> anti-oxidant,<sup>10</sup> and anti-inflammatory activities.<sup>11</sup> The essential oils and atractylon from *Rhizoma Atractylodes Macrocephala* exert anti-inflammatory activity.<sup>12</sup> The essential oil of *C. cassia* shows antibacterial<sup>13,14</sup> and anti-inflammatory activities.<sup>15,16</sup> The activities of these volatile components may contribute to the effect of YCZFD against liver injury. Therefore, it is necessary to identify the volatile components in YCZFD and clarify their pharmacokinetic characteristics.

Therefore, in this study, we aimed to identify the volatile components in the VO of YCZFD, quantify the principal volatile components in YCZFD, elucidate their pharmacokinetic characteristics, and furtherly to explore the effect of water extract (WE) of YCZFD on the pharmacokinetics of volatile components. The findings of the present study would help better understand the potential effective constituents in YCZFD.

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## 2 Material and methods

### 2.1 Chemicals, reagents, and materials

$\alpha$ -Pinene,  $\beta$ -phellandrene, copaene, zingiberene, and curcumin were supplied by Shanghai ZZBio Co., Ltd (Shanghai, China). Camphene, eucalyptol, borneol, and naphthalene (internal standard, IS) were provided by Shanghai Yuanze Bio-Technology Co., Ltd (Shanghai, China). Caryophyllene and *trans*-cinnamaldehyde were purchased from the National Institutes for Food and Drug Control (Beijing, China). Atractylon was supplied by Nanjing Spring & Autumn Biological Engineering Co., Ltd (Nanjing, China). The chemical structures of these analytes are shown in Fig. 1. C7–C30 saturated alkanes and dichloromethane of GC grade were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Methyl *tert*-butyl ether was obtained from TCI (Shanghai) Development Co., Ltd (Shanghai, China). The crude herbs ACT, RAM, RG, and CCP were obtained from Shanghai Dehua Pharmaceutical Co., Ltd (Shanghai, China). RALP and RZ were purchased from Shanghai Kangqiao Chinese Medicine Tablet Co., Ltd (Shanghai, China). Sodium sulfate anhydrous was supplied by Sinopharm Chemical Reagent Co., Ltd.

### 2.2 Instrument

The gas chromatography-tandem mass spectrometry (GC-MS/MS) system consisted of an Agilent 7890A gas chromatograph combined with a 7000B triple-quadrupole mass spectrometry detector (Agilent, Palo Alto, USA).

### 2.3 Preparation of extracts

**2.3.1 VO of YCZFD.** Crude materials including ACT (900 g), RAM (1800 g), RALP (450 g), RZ (450 g), RG (900 g) and CCP (300 g), were immersed in a 10-fold amount of water for 2 h, and then

extracted *via* hydro-distillation process using a Clevenger-type apparatus for 6 h. VO was collected and dried with anhydrous sodium sulfate. VO at 0.12% (v/w) yield rate was stored in sealed dark vials at  $-80^{\circ}\text{C}$ .

**2.3.2 Water extract (WE) of YCZFD.** The crude materials (mentioned in Section 2.3.1) were prepared in accordance with the traditional preparation method of YCZFD.<sup>17</sup> Briefly, crude materials were immersed in water and boiled twice. After filtration, the two decoctions were mixed, concentrated with a rotary evaporator, and then freeze-dried to obtain the WE powder of YCZFD.

**2.3.3. YCZFD extract (VO + WE).** Before use, the WE powder was dissolved in water and VO was proportionally added, then both were mixed with ultrasound to obtain YCZFD extract suspension.

### 2.4 Identification of components in the VO of YCZFD

The GC-MS/MS system with an HP-5 MS 5% phenyl methyl siloxane column (30 m  $\times$  0.25 mm, 0.25  $\mu\text{m}$ ) and NIST11 library was used to identify volatile components in the VO of YCZFD. The carrier gas was helium applied at a velocity of 1.0 mL min<sup>-1</sup>, the oven temperature program was initial at 40  $^{\circ}\text{C}$ , then raised to 250  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C min}^{-1}$ , and finally held at 250  $^{\circ}\text{C}$  for 5 min. The injection was in split mode at the split ratio of 1 : 20 with the injection volume of 1  $\mu\text{L}$  and the injector temperature of 250  $^{\circ}\text{C}$ . A mass spectrometer was used in the electron impact (EI) mode, in which a filament attached to the source body emits electrons into the ionization chamber through the guidance of a magnetic field, with ionization potential set at 70 eV, ionization current at 150  $\mu\text{A}$ , and mass range at 50–500. The YCZFD VO/C7–C30 saturated alkanes were serially diluted with *n*-hexane and the supernatant was analyzed after centrifuging at  $4832 \times g$  for 10 min. According to Kovats method,<sup>18</sup> the linear retention index (RI) was

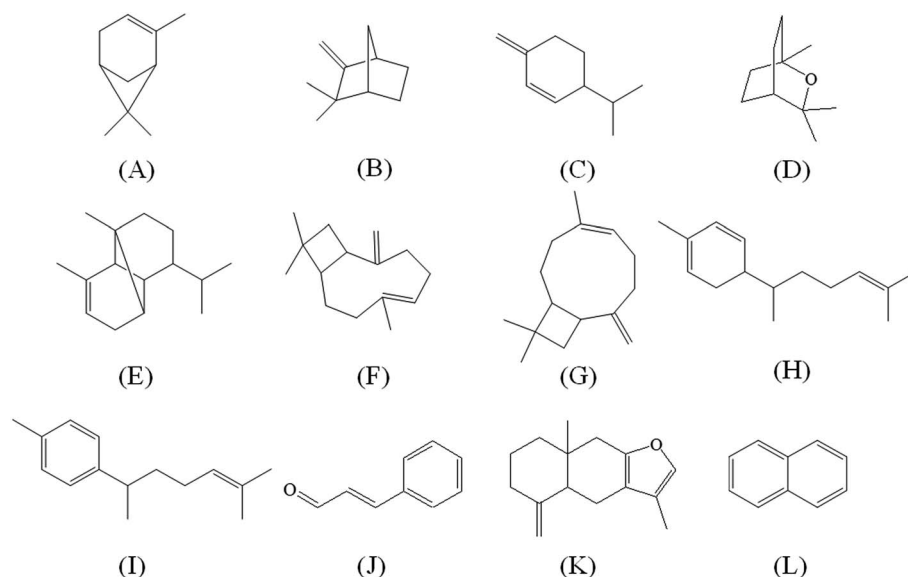


Fig. 1 Chemical structures of 11 volatile components and internal standards. (A)  $\alpha$ -Pinene, (B) camphene, (C)  $\beta$ -phellandrene, (D) eucalyptol, (E) copaene, (F) caryophyllene, (G) borneol, (H) zingiberene, (I) curcumin, (J) *trans*-cinnamaldehyde, (K) atractylon, (L) naphthalene.



calculated for all components in the YCZFD VO sample using the retention time (RT) of a homologous series of *n*-alkanes (C7–C30) injected in the same conditions as the reference. The components in the YCZFD VO were identified based on RI relative to that of *n*-alkanes, computer matching with those in the NIST11 library, and comparisons of the fragmentation pattern of the mass spectra with the data in the database. In addition, 11 standard compounds were used for comparison and final confirmation.

## 2.5 Quantification of 11 volatile components in YCZFD

**2.5.1 Experimental conditions.** The GC-MS/MS system was used for the quantitative analysis. The capillary column was CP9205 VF-WAXms (30 m × 0.25 mm, 0.25 μm). GC temperature program was initiated at 45 °C for 3 min, then raised to 130 °C at 2 °C min<sup>−1</sup> and to 250 °C at 13 °C min<sup>−1</sup>, and finally held at 250 °C for 5 min. The solvent delay was set at 4 min and the sample was injected in the split mode at the split ratio of 1 : 15 with the injection volume of 1 μL. Mass spectrometer was used in the EI source multiple reaction monitoring (MRM) mode with ionization potential set at 70 eV. The MRM parameters for the 11 volatile components and ISs are listed in Table 1. The temperature of the injector and ion source was 250 °C and 230 °C, respectively. The carrier gas was helium applied at a velocity of 1.0 mL min<sup>−1</sup>, and collision cell gases were helium and nitrogen at a velocity of 2.25 and 1.5 mL min<sup>−1</sup>, respectively.

**2.5.2 Method validation.** The developed GC-MS/MS method was validated for specificity, linearity, precision and accuracy, recovery, repeatability, and stability according to the US FDA guidelines (Draft Guidance for Industry on Analytical Procedures and Methods Validation, 2000) (details are presented in the ESI†).

**2.5.3 Sample determination.** YCZFD/YCZFD VO was serially diluted with methyl *tert*-butyl ether:dichloromethane (50 : 50, v/v) and centrifuged at 4832 × *g* for 10 min. Next, 90 μL of the supernatant was mixed with 10 μL of IS solution. One microliter of sample was injected into the GC-MS/MS system to determine the content of 11 volatile components.

## 2.6 Quantitation of the 8 volatile components of YCZFD in rat plasma

**2.6.1 Experimental conditions.** The solid-phase dynamic extraction (SPDE) device comprised a syringe, heater, gas station for aspiration of the desorption gas, and heatable flush station for preventing analyte carryover through flushing with argon, and they were assembled on a CTC Analytics GC PAL-autosampler. The replaceable SPDE cannulas were coated on the inner surface with a polydimethylsiloxane (PDMS) phase.<sup>18</sup>

The GC-MS/MS conditions, including capillary column, column temperature program, injector temperature, ion source temperature, carrier gas, collision cell gases, and MRM parameters, were the same as those in Section 2.5.1. The sample was injected in the splitless mode. The multiplier voltage was 1375 V.

**2.6.2 Headspace solid-phase dynamic extraction.** For extraction, 100 μL of plasma sample was spiked with 5 μL of IS solution and 10 μL of HCl (0.1 M), and then placed in a PTFE/silicone screw-cap glass vial. Next, 40 mg of sodium chloride was added, and the vial was sealed airtight and vortex-mixed for approximately 20 s. The vial was kept in a single point heater set at 50 °C with an autosampler during extraction.

The SPDE method has been previously reported;<sup>18</sup> here, it was partially modified as follows: the SPDE casing was inserted through the diaphragm to a depth of 20 mm, 40 times each time, and the volume of sampling headspace volume was 500 μL, and the extraction speed was maintained at 100 μL s<sup>−1</sup>. The sample was injected at 20 μL s<sup>−1</sup>. A blank run was appended to show the absence of carryover effects. To achieve the highest extraction efficiency, some parameters affecting the extraction rate were optimized, such as the type of SPDE sorbent coating, salting-out effect, concentration and volume of HCl added to the sample, number of extraction cycles, extraction temperature, preincubation time, desorption volume, and desorption flow speed.

**2.6.3 Preparation of the calibration standards and quality control (QC) samples.** The stock solutions of the 8 volatile analytes and IS (naphthalene) were prepared in methyl *tert*-butyl ether dichloromethane (50 : 50, v/v) and stored at −20 °C.

Table 1 Multiple reaction monitoring parameters for 11 volatile components and Internal Standard (IS)

| Components                   | Precursor ion | Product ion | Dwell time (ms) | CE (V) | RT (min) |
|------------------------------|---------------|-------------|-----------------|--------|----------|
| α-Pinene                     | 136           | 93          | 50              | 8      | 7.2      |
| Camphene                     | 136           | 93          | 50              | 8      | 8.6      |
| β-Phellandrene               | 136           | 93          | 50              | 8      | 15.1     |
| Eucalyptol                   | 154           | 139         | 50              | 2      | 14.9     |
| Copaene                      | 204           | 161         | 50              | 10     | 31.3     |
| Caryophyllene                | 189           | 105         | 50              | 22     | 37.2     |
| Borneol                      | 95            | 67          | 50              | 15     | 43.2     |
| Zingiberene                  | 204           | 119         | 50              | 8      | 44.5     |
| Curcumene                    | 202           | 132         | 50              | 10     | 46.8     |
| <i>trans</i> -Cinnamaldehyde | 131           | 77          | 50              | 30     | 51.1     |
| Attractylon                  | 216           | 108         | 50              | 20     | 52.0     |
| Naphthalene (IS)             | 128           | 102         | 50              | 30     | 44.7     |

Next, the 8 volatile analyte stock solutions were mixed and diluted with *tert*-butyl ether:dichloromethane (50 : 50, v/v) to prepare a mixed working solution. The mixed working solution was serially diluted with blank rat plasma to prepare standard and QC samples.

**2.6.4 Method validation.** The GC-MS/MS method of the 8 volatile components of YCZFD in rat plasma was validated for specificity, linearity, precision and accuracy, recovery, and stability according to the FDA guidelines for biological sample determination methods (Bioanalytical Method Validation Guidance for Industry, 2018) (details are presented in the ESI†).

**2.6.5 Pharmacokinetic study.** The animal experiments were approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (the approval number PZSHUTCM190628025). Sprague-Dawley rats (6 males and 6 females) weighing 210–260 g were randomly divided into the YCZFD and YCZFD VO groups. After fasting for 12 h, both group rats were separately intragastrically administered YCZFD extract of 4.80 g kg<sup>-1</sup> (equaling 24.0 g crude herbs per kg and containing camphene 1.17,  $\beta$ -phellandrene 2.81, eucalyptol 0.552, copaene 0.286, borneol 0.518, zingiberene 2.16, curcumen 0.593, and atractylon 11.0 mg kg<sup>-1</sup>) and YCZFD VO 29.2 mg kg<sup>-1</sup> (equaling 24.0 g crude herbs per kg and containing camphene 1.14,  $\beta$ -phellandrene 2.90, eucalyptol 0.542, copaene 0.281, borneol, 0.502, zingiberene 2.14, curcumen 0.569, and atractylon 11.1 mg kg<sup>-1</sup>). Thereafter, 220  $\mu$ L of blood was collected at 0, 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h post administration. The blood samples were immediately centrifuged to obtain plasma, and then stored at -80 °C until analysis.

The pharmacokinetic parameters of the volatile components were calculated using non-compartmental methods with Phoenix WinNonlin 6.1 (Pharsight, Mountain View, CA, USA) software. The observed value of  $C_{\max}$  was obtained from the observed data and the observed value of  $AUC_{0-t}$  was calculated using the trapezoidal rule.  $C_{\max}/\text{dose}$  and  $AUC_{0-t}/\text{dose}$  (the dose-normalized values) were calculated by dividing the observed values of  $C_{\max}$  and  $AUC_{0-t}$  by the dose in each extract. Data are expressed as mean  $\pm$  standard deviation (SD). The statistical differences between groups were determined using *t*-test, and *p* < 0.05 were considered statistically significant.

### 3. Results and discussion

#### 3.1 Identification of multiple volatile components in the VO of YCZFD

In order to improve the reliability of the qualitative analysis of volatile components, we used the automated mass spectral deconvolution and identification system for purification of the chromatographic peak; the relatively “pure” mass spectrogram was compared and matched with that in Wiley libraries (NIST11). Furthermore, values were confirmed by calculating linear retention indices (RI) of the test compounds to *n*-alkanes (C7–C30) under the same conditions as those of the reference. Furthermore, the RI was compared with values in the literature

to accurately identify similar compounds that matched NIST11 well. In addition, we also used as many commercially available standard compounds as possible for comparison and final confirmation. Based on these three approaches, the components in YCZFD VO were identified.

A typical chromatographic profile of the VO of YCZFD is presented in Fig. 2. The volatile components in the VO of YCZFD are listed in the order of their elution time in Table 2. Eighty-five volatile components were identified in the VO, which accounted for 95.4% of the total oil composition by normalization of whole chromatographic peak area. Sesquiterpene hydrocarbons (39.5%) and oxygenated sesquiterpene (30.5%) were the most abundant components. In this study, the composition of volatile components in VO of YCZFD was clarified for the first time.

#### 3.2. Quantitation of the 11 major components in YCZFD

**3.2.1 Method validation.** As shown in Table 2, YCZFD is a complex mixture of volatile components, and we selected 11 major components ( $\alpha$ -pinene, camphene,  $\beta$ -phellandrene, eucalyptol, copaene, caryophyllene, borneol, zingiberene, curcumen, *trans*-cinnamaldehyde, and atractylon) for quantification. These components are relatively more abundant, which the peak area is relatively large by normalization method of chromatographic peak area, and the standard reference substances are available.

The GC-MS/MS method established had good specificity. The calibration curves for all analytes showed good linearity ( $r^2 > 0.9962$ ) and the variation in intra- and inter-batch precisions for all analytes was less than 6.92%. The recovery rate varied from 92.1% to 105%. The corresponding RSDs did not exceed 11.1%. The repeatability (RSD < 3.87%) and stability (RSD < 9.65%) were also within the acceptable limits. The results indicated that the analytical method was sensitive, and reliable for the quantification of the 11 volatile constituents in YCZFD (details are presented in the ESI†).

**3.2.2 Sample determination.** The level of the 11 volatile components in five batches of YCZFD and YCZFD VO samples is summarized in Tables 3 and 4. The results showed that the levels of the components in the five sample batches were relatively stable. Among the 11 components, atractylon was present at the highest concentration, followed by  $\beta$ -phellandrene, zingiberene, and *trans*-cinnamaldehyde. There was no significant difference in the level of the 11 volatile components between YCZFD and YCZFD VO. The results also indicated that the established method can provide a basis for the comprehensive quality assessment of volatile components of YCZFD.

#### 3.3. Quantification of the 8 volatile components in YCZFD in rat plasma

##### 3.3.1 Method development

**3.3.1.1 Stability of zingiberene and curcumen.** Zingiberene and curcumen are susceptible to oxidation in rat plasma. To prevent oxidation, ascorbic acid at different concentrations were added as an antioxidant agent under dark conditions. The





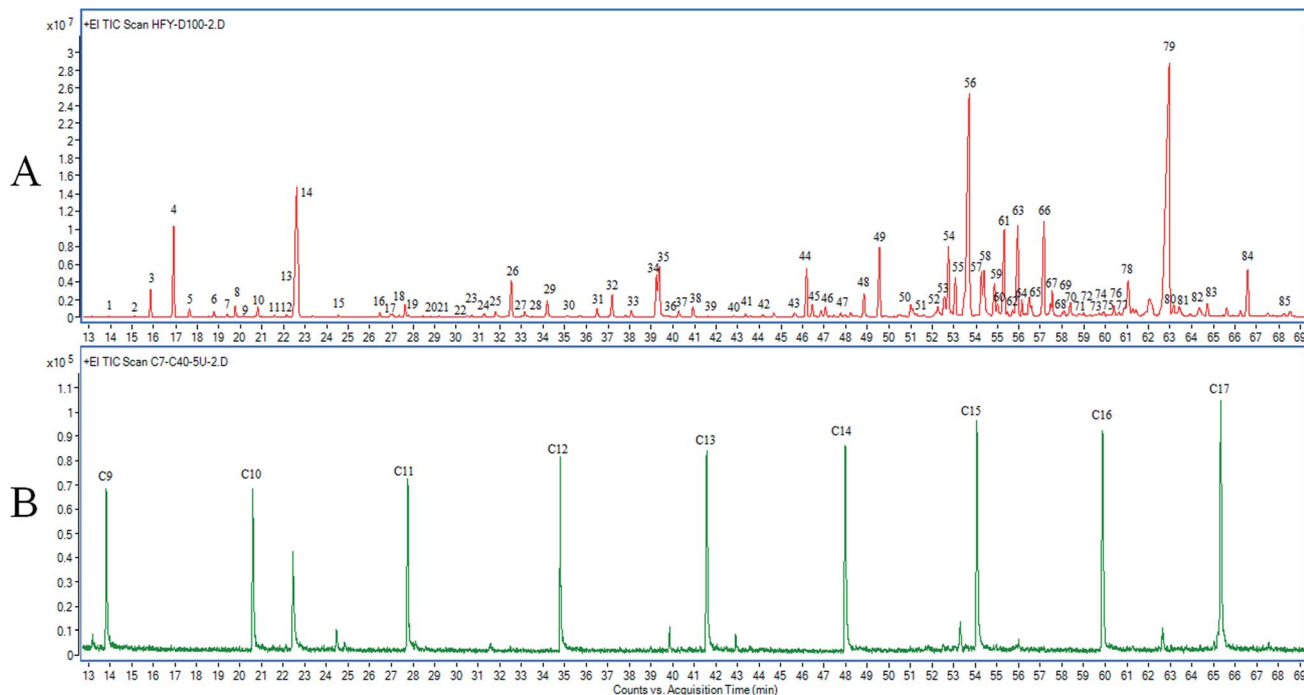


Fig. 2 Total ion compound (TIC) chromatogram of (A) the volatile oil of Yinchenzhufu decoction and; (B) *N*-alkanes containing 9 to 17 carbons.

Table 2 Chemical composition of the essential oil of Yinchenzhufu decoction<sup>a</sup>

| No. | Components                             | %    | RI   | RI lit. | Identif.  |
|-----|--|------|------|---------|-----------|
| 1   | 2-Heptanol                             | 0.03 | 901  | 886     | RI, MS    |
| 2   | Cyclene                                | 0.04 | 919  | 918     | RI, MS    |
| 3   | $\alpha$ -piene                        | 1.24 | 930  | 931     | RI, MS, S |
| 4   | Camphene                               | 2.97 | 946  | 943     | RI, MS, S |
| 5   | Benzaldehyde                           | 0.29 | 956  | 929     | RI, MS    |
| 6   | $\beta$ -Pinene                        | 0.17 | 973  | 970     | RI, MS    |
| 7   | Sulcatone                              | 0.10 | 982  | 960     | RI, MS    |
| 8   | $\beta$ -Myrcene                       | 0.34 | 988  | 981     | RI, MS    |
| 9   | Pseudolimonen                          | 0.01 | 1001 | 993     | RI, MS    |
| 10  | $\alpha$ -Phellandrene                 | 0.30 | 1003 | 1007    | RI, MS    |
| 11  | $\alpha$ -Terpinene                    | 0.06 | 1014 | 1017    | RI, MS    |
| 12  | $\beta$ -Cymene                        | 0.10 | 1022 | 1013    | RI, MS    |
| 13  | $\beta$ -Phellandrene                  | 7.19 | 1028 | 1030    | RI, MS, S |
| 14  | Eucalyptol                             | 2.56 | 1029 | 1023    | RI, MS, S |
| 15  | $\gamma$ -Terpinene                    | 0.08 | 1055 | 1053    | RI, MS    |
| 16  | $\alpha$ -Terpinolene                  | 0.15 | 1082 | 1080    | RI, MS    |
| 17  | 2-Nonanone                             | 0.08 | 1089 | 1074    | RI, MS    |
| 18  | 2,3-Epoxy-pinane                       | 0.06 | 1094 | 1095    | RI, MS    |
| 19  | $\beta$ -Linalool                      | 0.42 | 1098 | 1082    | RI, MS    |
| 20  | $\beta$ -Fenchol                       | 0.02 | 1115 | 1112    | RI, MS    |
| 21  | <i>cis</i> -p-Menth-2-en-1-ol          | 0.05 | 1121 | 1118    | RI, MS    |
| 22  | <i>trans</i> -p-Ment-2-en-1-ol         | 0.04 | 1139 | 1138    | RI, MS    |
| 23  | (-)-Camphor                            | 0.07 | 1142 | 1139    | RI, MS    |
| 24  | 2-Norbornanol                          | 0.04 | 1150 | 1142    | RI, MS    |
| 25  | Benzenepropanal                        | 0.19 | 1157 | 1123    | RI, MS    |
| 26  | endo-Borneol                           | 1.78 | 1168 | 1148    | RI, MS, S |
| 27  | Isomenthol                             | 0.02 | 1174 | 1174    | RI, MS    |
| 28  | (-)-4-Terpineol                        | 0.19 | 1177 | 1175    | RI, MS    |
| 29  | $\alpha$ -Terpineol                    | 0.60 | 1191 | 1172    | RI, MS    |
| 30  | <i>cis</i> -Piperitol                  | 0.07 | 1204 | 1190    | RI, MS    |
| 31  | ( <i>R</i> )-(+)- $\beta$ -Citronellol | 0.29 | 1225 | 1220    | RI, MS    |
| 32  | Citral                                 | 0.86 | 1235 | 1241    | RI, MS    |



Table 2 (Contd.)

| No. | Components  | %     | RI   | RI lit. | Identif.  |
|-----|---|-------|------|---------|-----------|
| 33  | Geraniol  | 0.25  | 1248 | 1238    | RI, MS    |
| 34  | $\alpha$ -Citral  | 1.23  | 1266 | 1250    | RI, MS    |
| 35  | <i>trans</i> -Cinnamaldehyde  | 2.63  | 1268 | 1243    | RI, MS, S |
| 36  | Phellandral   | 0.03  | 1273 | 1252    | RI, MS    |
| 37  | (–)-Bornyl acetate  | 0.22  | 1281 | 1273    | RI, MS    |
| 38  | 2-Undecanone  | 0.35  | 1291 | 1274    | RI, MS    |
| 39  | 2-Undecanol   | 0.03  | 1301 | 1294    | RI, MS    |
| 40  | Myrtenyl acetate  | 0.01  | 1319 | 1306    | RI, MS    |
| 41  | $\delta$ -Elemene   | 0.05  | 1332 | 1334    | RI, MS    |
| 42  | Cephreine   | 0.15  | 1348 | 1331    | RI, MS    |
| 43  | Ylangene  | 0.08  | 1363 | 1360    | RI, MS    |
| 44  | Copaene   | 2.26  | 1371 | 1376    | RI, MS, S |
| 45  | Berkheyaradulene  | 0.22  | 1382 | 1416    | RI, MS    |
| 46  | $\beta$ -Elemene  | 0.38  | 1385 | 1387    | RI, MS    |
| 47  | Cyperene  | 0.14  | 1397 | 1390    | RI, MS    |
| 48  | Caryophyllene   | 1.24  | 1414 | 1421    | RI, MS, S |
| 49  | $\gamma$ -Elemene   | 2.17  | 1426 | 1425    | RI, MS    |
| 50  | Humulene  | 0.53  | 1449 | 1454    | RI, MS    |
| 51  | $\alpha$ -Guaiene   | 0.08  | 1454 | 1440    | RI, MS    |
| 52  | Calarene  | 0.16  | 1468 | 1463    | RI, MS    |
| 53  | $\gamma$ -Murolene  | 0.92  | 1475 | 1471    | RI, M S   |
| 54  | Curcumene   | 2.86  | 1478 | 1472    | RI, MS, S |
| 55  | $\beta$ -Selinene   | 1.09  | 1483 | 1483    | RI, MS    |
| 56  | Zingiberene   | 13.96 | 1494 | 1492    | RI, MS, S |
| 57  | $\alpha$ -Farnesene   | 1.34  | 1503 | 1499    | RI, MS    |
| 58  | $\beta$ -Bisabolene   | 1.30  | 1505 | 1500    | RI, MS    |
| 59  | $\delta$ -Cadinene  | 1.20  | 1514 | 1514    | RI, MS    |
| 60  | Calamenene  | 0.42  | 1516 | 1517    | RI, MS    |
| 61  | $\beta$ -Sesquiphellandrene   | 2.68  | 1522 | 1516    | RI, MS    |
| 62  | Ledene  | 0.16  | 1524 | 1520    | RI, MS    |
| 63  | Selina-3,7(11)-diene  | 2.83  | 1532 | 1533    | RI, MS    |
| 64  | $\gamma$ -Selinene  | 0.67  | 1536 | 1531    | RI, MS    |
| 65  | $\alpha$ -Copaen-11-ol  | 0.74  | 1542 | 1541    | RI, MS    |
| 66  | Germacrene B  | 2.75  | 1553 | 1554    | RI, MS    |
| 67  | <i>trans</i> -Nerolidol   | 0.42  | 1558 | 1555    | RI, MS    |
| 68  | Caryophyllenyl alcohol  | 0.08  | 1568 | 1569    | RI, MS    |
| 69  | Spathulenol   | 0.19  | 1569 | 1569    | RI, MS    |
| 70  | Caryophyllene oxide   | 0.56  | 1574 | 1575    | RI, MS    |
| 71  | Carotol   | 0.21  | 1585 | 1594    | RI, MS    |
| 72  | Isoaromadendrene epoxide  | 0.02  | 1594 | 1590    | RI, MS    |
| 73  | Viridiflorol  | 0.06  | 1598 | 1594    | RI, MS    |
| 74  | Humulane-1,6-dien-3-ol  | 0.09  | 1601 | 1606    | RI, MS    |
| 75  | $\alpha$ -acorenol  | 0.30  | 1610 | 1598    | RI, MS    |
| 76  | $\gamma$ -eudesmol  | 0.15  | 1614 | 1626    | RI, MS    |
| 77  | $\alpha$ -eudesmol  | 0.23  | 1619 | 1637    | RI, MS    |
| 78  | (–)-Spathulenol   | 1.39  | 1622 | 1619    | RI, MS    |
| 79  | Atractylone   | 22.28 | 1656 | 1652    | RI, MS, S |
| 80  | Bulnesol  | 0.29  | 1660 | 1652    | RI, MS    |
| 81  | $\beta$ -bisabolol  | 0.62  | 1665 | 1619    | RI, MS    |
| 82  | $\alpha$ -Bisabolol   | 0.58  | 1681 | 1683    | RI, MS    |
| 83  | Eudesm-7(11)-en-4-ol  | 0.56  | 1689 | 1681    | RI, MS    |
| 84  | 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one                   | 1.63  | 1724 | 1730    | RI, MS    |
| 85  | 2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol | 0.15  | 1756 | 1732    | RI, MS    |
|     | Total   | 95.36 |      |         |           |
|     | Hydrocarbon monoterpenes  | 12.65 |      |         |           |
|     | Oxygenated monoterpenes   | 8.27  |      |         |           |
|     | Sesquiterpene hydrocarbon   | 39.51 |      |         |           |
|     | Oxygenated sesquiterpene  | 30.54 |      |         |           |
|     | Others  | 4.39  |      |         |           |

<sup>a</sup> RI: retention index; RI lit.: retention index of target compound in literature from database; MS: identify by comparing the mass spectrum fragments from the NIST11 database; S: identify by comparing with the standards.



Table 3 Contents of 11 volatile components in Yinchenzhufu decoction (mean  $\pm$  SD,  $n = 3$ )<sup>a</sup>

| Components                   | Content ( $\mu\text{g g}^{-1}$ ) |                  |                  |                  |                  |
|------------------------------|----------------------------------|------------------|------------------|------------------|------------------|
|                              | Batch no. 180611                 | Batch no. 180612 | Batch no. 180613 | Batch no. 180614 | Batch no. 180615 |
| $\alpha$ -Pinene             | 13.8 $\pm$ 0.4                   | 14.3 $\pm$ 0.3   | 13.5 $\pm$ 0.5   | 13.5 $\pm$ 0.4   | 13.3 $\pm$ 0.4   |
| Camphene                     | 48.6 $\pm$ 3.0                   | 47.7 $\pm$ 2.3   | 47.3 $\pm$ 1.0   | 49.4 $\pm$ 3.3   | 50.1 $\pm$ 2.3   |
| $\beta$ -Phellandrene        | 117 $\pm$ 6                      | 128 $\pm$ 5      | 121 $\pm$ 2      | 108 $\pm$ 2      | 129 $\pm$ 5      |
| Eucalyptol                   | 23.0 $\pm$ 0.5                   | 24.6 $\pm$ 1.2   | 23.4 $\pm$ 2.3   | 21.2 $\pm$ 1.8   | 23.7 $\pm$ 1.0   |
| Copaene                      | 11.9 $\pm$ 0.5                   | 12.8 $\pm$ 0.4   | 12.3 $\pm$ 1.0   | 11.9 $\pm$ 0.6   | 15.1 $\pm$ 0.4   |
| Caryophyllene                | 13.0 $\pm$ 0.4                   | 14.2 $\pm$ 0.4   | 13.9 $\pm$ 0.3   | 12.9 $\pm$ 0.2   | 15.2 $\pm$ 0.4   |
| Borneol                      | 21.6 $\pm$ 0.9                   | 23.9 $\pm$ 0.5   | 24.3 $\pm$ 1.4   | 22.8 $\pm$ 0.9   | 25.4 $\pm$ 0.7   |
| Zingiberene                  | 90.1 $\pm$ 2.9                   | 112 $\pm$ 1      | 109 $\pm$ 2      | 85.3 $\pm$ 1.3   | 106 $\pm$ 1      |
| Curcumene                    | 24.7 $\pm$ 1.0                   | 27.2 $\pm$ 0.7   | 26.3 $\pm$ 0.8   | 23.0 $\pm$ 1.2   | 26.8 $\pm$ 0.8   |
| <i>trans</i> -Cinnamaldehyde | 67.7 $\pm$ 2.1                   | 70.3 $\pm$ 3.3   | 72.2 $\pm$ 1.7   | 68.1 $\pm$ 3.3   | 71.6 $\pm$ 2.3   |
| Atractylon                   | 458 $\pm$ 18                     | 489 $\pm$ 18     | 434 $\pm$ 21     | 416 $\pm$ 17     | 504 $\pm$ 20     |

<sup>a</sup> “ $n = 3$ ” mean that three different samples were analyzed.

results showed that the addition of 5  $\mu\text{L}$  of 2  $\text{mg mL}^{-1}$  aqueous ascorbic acid solution to 100  $\mu\text{L}$  of plasma could maintain stable levels of zingiberene and curcumene under various storage and treatment conditions. To prevent the potential degradation in the sample collection stage, 10  $\mu\text{L}$  of 8  $\text{mg mL}^{-1}$  aqueous ascorbic acid solution was immediately added to 200  $\mu\text{L}$  of fresh blood samples, which were then placed on ice and immediately centrifuged to separate the plasma. In addition, all sample preparation procedures were performed under dark conditions.

**3.3.1.2 Optimization of the SPDE conditions.** The following types of coatings for solid phase dynamic microextraction were tested: the WAX coating (polyethylene glycol), CT-225 coating (25% cyanopropyl/25% phenylpolysiloxane/50% methylpolysiloxane), CT-1701 coating (14% cyanopropyl/86% dimethylpolysiloxane), CT-5 coating (15% diphenyl/95% dimethylpolysiloxane), PDMS coating (PDMS), and PDMS/AC coating (PDMS + 10% active charcoal). Our results showed that PDMS coating achieved the best extraction rate.

Because the salting-out effect usually results in an increase in recovery,<sup>19</sup> different amounts of NaCl were added to 100  $\mu\text{L}$  of

the spiked plasma standard to optimize recovery. The maximum salting-out effect was achieved with 40  $\text{mg}$  of NaCl per 100  $\mu\text{L}$  of plasma.

We also investigated the effect of pH on the extraction efficiency at five pH levels: initial plasma pH, two acidic pH values (achieved by adding 0.1 M HCl or 0.01 M HCl), and two basic pH values (achieved by adding 0.1 M NaOH or 0.01 M NaOH). As a result, the pH had no significant effect on the extraction efficiency of the other 8 components, and the acidic pH (by adding 0.1 M HCl) was used in the study.

According to the peak response, the number of extraction cycles of 50 was considered when the number of cycles ranged between 20 and 70 (Fig. 3A). The extraction temperature of 90  $^{\circ}\text{C}$  was chosen when the temperature ranged between 60 and 100  $^{\circ}\text{C}$  (Fig. 3B). The optimal preincubation time of 5 min was determined when preincubation time ranged between 5 and 20 min (Fig. 3C). The optimum desorption gas volume was considered to be 1 mL when desorption gas volume ranged between 0.25 and 1.00 mL (Fig. 3D), and the optimal desorption gas flow speed was 20  $\mu\text{L s}^{-1}$  when desorption gas flow speed ranged between 20 and 100  $\mu\text{L s}^{-1}$  (Fig. 3E).

Table 4 Contents of 11 volatile components in Yinchenzhufu decoction volatile oil (mean  $\pm$  SD,  $n = 3$ )<sup>a</sup>

| Components                   | Content ( $\mu\text{g g}^{-1}$ ) |                  |                  |                  |                  |
|------------------------------|----------------------------------|------------------|------------------|------------------|------------------|
|                              | Batch no. 180611                 | Batch no. 180612 | Batch no. 180613 | Batch no. 180614 | Batch no. 180615 |
| $\alpha$ -Pinene             | 14.3 $\pm$ 0.4                   | 14.8 $\pm$ 0.1   | 13.3 $\pm$ 0.4   | 13.7 $\pm$ 0.4   | 12.8 $\pm$ 0.3   |
| Camphene                     | 47.5 $\pm$ 1.3                   | 50.1 $\pm$ 0.4   | 49.1 $\pm$ 0.5   | 48.5 $\pm$ 3.1   | 48.9 $\pm$ 1.3   |
| $\beta$ -Phellandrene        | 121 $\pm$ 4                      | 131 $\pm$ 1      | 120 $\pm$ 2      | 104 $\pm$ 2      | 124 $\pm$ 4      |
| Eucalyptol                   | 22.6 $\pm$ 0.7                   | 24.2 $\pm$ 1.6   | 23.8 $\pm$ 0.5   | 22.2 $\pm$ 1.0   | 23.1 $\pm$ 0.4   |
| Copaene                      | 11.7 $\pm$ 0.5                   | 12.9 $\pm$ 0.3   | 12.0 $\pm$ 0.5   | 11.6 $\pm$ 0.2   | 14.7 $\pm$ 0.1   |
| Caryophyllene                | 13.4 $\pm$ 0.3                   | 13.8 $\pm$ 0.7   | 13.9 $\pm$ 0.3   | 12.6 $\pm$ 0.2   | 15.9 $\pm$ 0.9   |
| Borneol                      | 20.9 $\pm$ 0.7                   | 24.7 $\pm$ 0.4   | 23.5 $\pm$ 0.9   | 22.4 $\pm$ 0.5   | 26.0 $\pm$ 0.7   |
| Zingiberene                  | 89.1 $\pm$ 1.3                   | 114 $\pm$ 1      | 106 $\pm$ 1      | 90.9 $\pm$ 0.5   | 107 $\pm$ 1      |
| Curcumene                    | 23.7 $\pm$ 0.4                   | 28.6 $\pm$ 1.8   | 26.3 $\pm$ 1.7   | 23.5 $\pm$ 0.8   | 27.4 $\pm$ 1.0   |
| <i>trans</i> -Cinnamaldehyde | 69.7 $\pm$ 1.0                   | 72.2 $\pm$ 1.4   | 74.9 $\pm$ 2.7   | 70.3 $\pm$ 1.3   | 76.7 $\pm$ 0.4   |
| Atractylon                   | 463 $\pm$ 16                     | 504 $\pm$ 17     | 421 $\pm$ 10     | 438 $\pm$ 22     | 519 $\pm$ 9      |

<sup>a</sup> “ $n = 3$ ” mean that three different samples were analyzed.

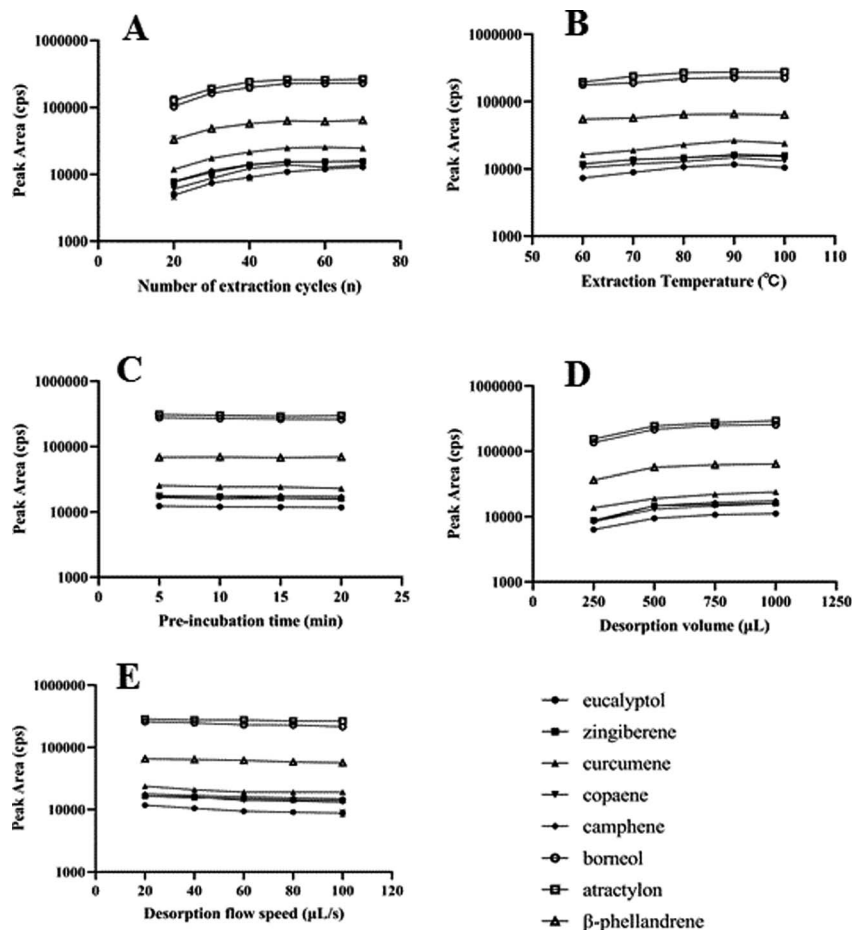


Fig. 3 Optimization of headspace solid-phase dynamic extraction conditions. (A) The number of extraction cycles; (B) extraction temperature; (C) pre-incubation time; (D) desorption volume; (E) desorption flow speed.

### 3.3.2 Method validation

**3.3.2.1 Specificity.** Typical MRM chromatograms are shown in Fig. 4. The chromatogram of the blank plasma sample showed no interference peak for both the analytes and IS, and the analytical method displayed good specificity.

**3.3.2.2 Linearity, accuracy, and precision.** The calibration curves of the analytes showed good linearity ( $1/x^2$  weighted regression model) in rat plasma (Table 5). The intra- and inter-batch precision and accuracy are summarized in Table 6. The RE% was within the acceptable criteria of  $\pm 15\%$  and the RSD% was less than 15%.

**3.3.2.3 Recovery.** The mean extraction recoveries of all analytes from rat plasma were 88.2–107% at three QC levels, and the RSD% was less than 15%.

**3.3.2.4 Sample stability.** All analytes in the rat plasma were stable at room temperature for 6 h, in an autosampler vial for 24 h, at  $-80^\circ\text{C}$  for 31 days, and after three freeze–thaw cycles (the RE% of all the analytes ranged from  $-10.7\%$  to  $4.11\%$  and the RSD% was within 11.4%) (Table 7).

The above results showed that the established quantitative method of 8 volatile components from YCZFD in rat plasma met the requirements for biological sample determination.

In the present study, the simultaneous quantitation of eight volatile components in a traditional Chinese medicine formula in plasma using the GC-MS/MS method was reported for the first time. Of these eight components, five components (camphene,  $\beta$ -phellandrene, copaene, zingiberene, and curcumene) had not previously been quantified in the plasma. In the methods reported in the literature, only a small number of components such as borneol,<sup>20</sup> atractylon,<sup>21</sup> and eucalyptol<sup>22</sup> were determined. Compared to the methods reported in the literature, this paper provided a sensitive and reliable method for the simultaneous determination of more volatile components in plasma with a simple, automatic SPDE.

**3.3.3 Pharmacokinetic study.** The developed method was successfully applied to quantify camphene,  $\beta$ -phellandrene, eucalyptol, copaene, borneol, zingiberene, curcumene, and atractylon in the plasma of rats after oral administration of YCZFD and YCZFD VO. The concentration–time curves of camphene,  $\beta$ -phellandrene, eucalyptol, copaene, zingiberene, curcumene, and atractylon are illustrated in Fig. 5. However, the plasma levels of borneol was below the lower limit of quantitation (LLOQ), and its concentration–time curves could not be drawn.





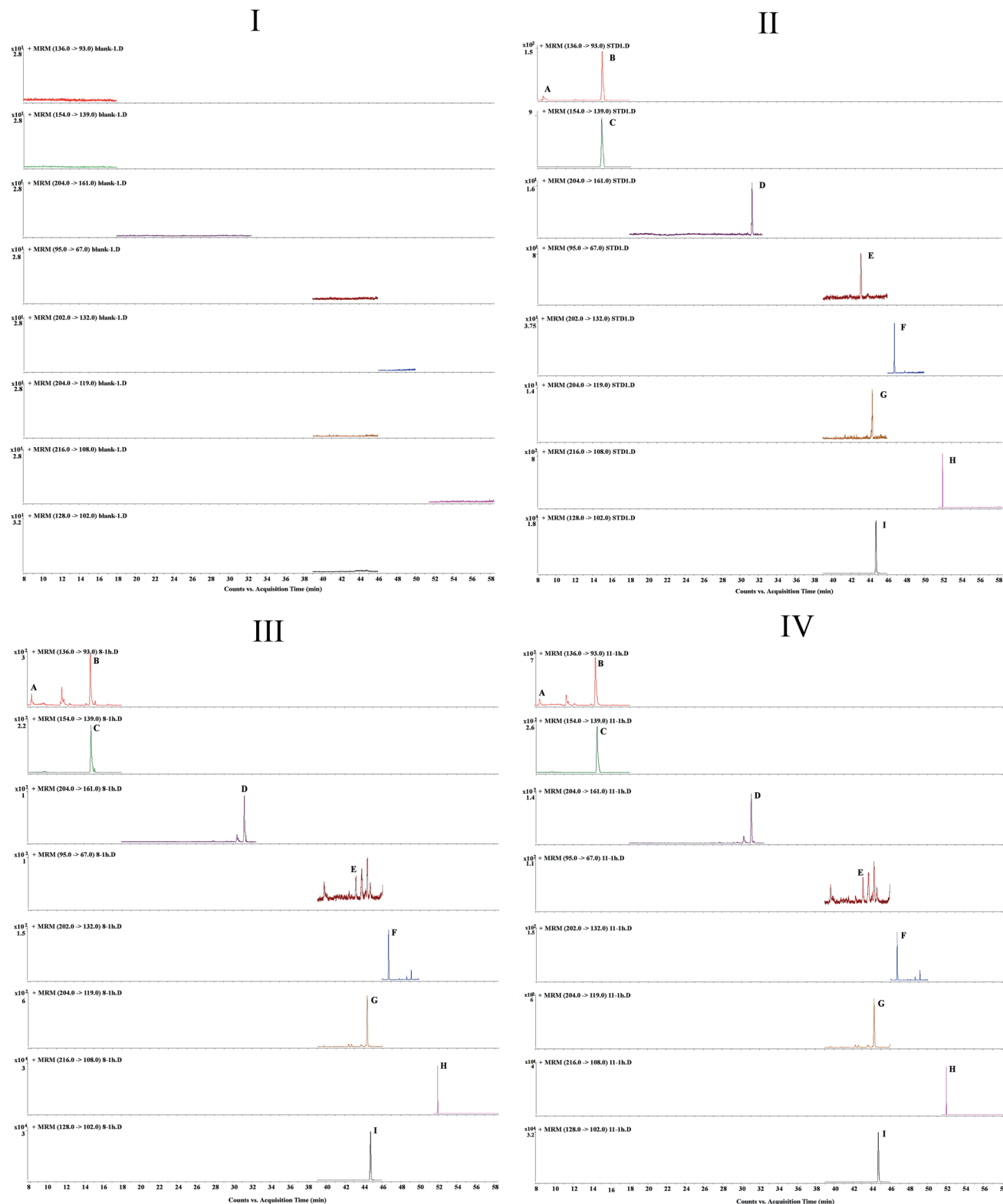


Fig. 4 Typical selected reaction monitoring chromatograms of 8 volatile components and their I.S. in rat plasma samples. (I) Blank rat plasma; (II) blank plasma spiked with reference components (LLOQ); (III) plasma sample 1 h after oral administration of Yinchenzhufu decoction volatile oil in rats; (IV) plasma sample 1 h after oral administration of Yinchenzhufu decoction in rats (A: camphene, B:  $\beta$ -phellandrene, C: eucalyptol, D: copaene, E: borneol, F: zingiberene, G: curcumene, H: atractylon, I: naphthalene).

Table 5 The calibration curves, linear ranges and LLOQs of 8 volatile components in rat plasma ( $n = 6$ )

| Components            | Calibration curve                             | $r^2$  | Linear range<br>(ng mL <sup>-1</sup> ) | LLOQ (ng mL <sup>-1</sup> ) |
|-----------------------|---|--------|--|-----------------------------|
| Camphene              | $Y = 0.000248 \times X + 1.52 \times 10^{-5}$ | 0.9913 | 1.00–500                               | 1.00                        |
| $\beta$ -Phellandrene | $Y = 0.00105 \times X + 4.83 \times 10^{-5}$  | 0.9940 | 1.00–500                               | 1.00                        |
| Eucalyptol            | $Y = 0.000171 \times X + 4.8 \times 10^{-6}$  | 0.9943 | 1.00–500                               | 1.00                        |
| Copaene               | $Y = 0.000195 \times X + 4.16 \times 10^{-7}$ | 0.9969 | 1.00–500                               | 1.00                        |
| Borneol               | $Y = 0.0074 \times X + 8.79 \times 10^{-5}$   | 0.9943 | 0.500–250                              | 0.500                       |
| Zingiberene           | $Y = 0.000225 \times X - 3E - 05$             | 0.9962 | 1.00–500                               | 1.00                        |
| Curcumene             | $Y = 0.000334 \times X - 2.4E - 06$           | 0.9945 | 1.00–500                               | 1.00                        |
| Atractylone           | $Y = 0.000485 \times X - 0.00057$             | 0.9922 | 8.00–4000                              | 8.00                        |

The results showed that after the oral administration of YCZFD and YCZFD VO, these volatile components had similar pharmacokinetic characteristics, such as rapid absorption ( $t_{\max} \leq 2$  h), especially camphene,  $\beta$ -phellandrene, and eucalyptol ( $t_{\max} < 0.5$  h), as well as rapid elimination (almost  $T_{1/2} \leq 4$  h). However, their exposure levels varied widely. The  $AUC_{0-t}$  of atractylon was the highest, the  $AUC_{0-t}$  of eucalyptol was approximately 13% that of atractylon, and the  $AUC_{0-t}$  of other components was less than 10% that of atractylon (Table 8). In the present study, atractylon and eucalyptol from YCZFD//YCZFD VO possessed a shorter  $T_{1/2}$  and a higher dose-normalized  $C_{\max}$  and  $AUC_{0-t}$  than that of atractylon from *Atractylodis* extract<sup>21</sup> and that of the eucalyptol monomer,<sup>23</sup> respectively. These results indicate that some coexisting components in YCZFD may promote absorption of both components, but speed up their elimination. This mechanism

needs to be studied further. After the administration of YCZFD, the exposure ( $AUC_{0-t}$ ) of most analytes was increased compared with that after YCZFD VO administration. In fact, the increase in exposure of camphene ( $p < 0.01$ ),  $\beta$ -phellandrene, and atractylon ( $p < 0.05$ ) was significant. In addition, compared with the YCZFD VO group, the YCZFD group showed short  $T_{\max}$  of curcumene; increased  $C_{\max}$  of camphene,  $\beta$ -phellandrene, copaene, and atractylon; and decreased  $t_{1/2}$  of  $\beta$ -phellandrene and copaene. The synergistic effect of traditional Chinese medicine formulae results from the interaction of multiple components in the formulae. The findings of this study showed that the WE of YCZFD increased the exposure of some volatile components, which may be beneficial to the overall effect of YCZFD.

In present study, the pharmacokinetic behaviors of multiple volatile components of YCZFD and the

Table 6 Intra- and inter-day variability for the assay of 8 volatile components in rat plasma

| Components            | Conc. (ng mL <sup>-1</sup> ) | Intra-day ( $n = 6$ )       |         | Inter-day ( $n = 18$ )      |         |
|-----------------------|------------------------------|-----------------------------|---------|-----------------------------|---------|
|                       |                              | Mean (ng mL <sup>-1</sup> ) | RSD (%) | Mean (ng mL <sup>-1</sup> ) | RSD (%) |
| Camphene              | 3.00                         | 3.17 $\pm$ 0.22             | 7.0     | 3.04 $\pm$ 0.22             | 7.3     |
|                       | 30.0                         | 30.5 $\pm$ 2.3              | 7.7     | 30.1 $\pm$ 2.2              | 7.4     |
|                       | 400                          | 377 $\pm$ 29                | 7.8     | 386 $\pm$ 27                | 6.9     |
| $\beta$ -Phellandrene | 3.00                         | 2.84 $\pm$ 0.08             | 2.9     | 2.91 $\pm$ 0.18             | 6.1     |
|                       | 30.0                         | 28.8 $\pm$ 2.2              | 7.5     | 29.5 $\pm$ 2.1              | 7.2     |
|                       | 400                          | 380 $\pm$ 28                | 7.5     | 380 $\pm$ 20                | 5.2     |
| Eucalyptol            | 3.00                         | 2.90 $\pm$ 0.18             | 6.3     | 2.94 $\pm$ 0.20             | 6.9     |
|                       | 30.0                         | 30.5 $\pm$ 2.3              | 7.6     | 29.9 $\pm$ 2.0              | 6.5     |
|                       | 400                          | 383 $\pm$ 15                | 4.0     | 388 $\pm$ 22                | 5.6     |
| Copaene               | 3.00                         | 3.23 $\pm$ 0.09             | 2.8     | 3.09 $\pm$ 0.21             | 6.8     |
|                       | 30.0                         | 28.9 $\pm$ 1.8              | 6.1     | 29.2 $\pm$ 1.6              | 5.5     |
|                       | 400                          | 404 $\pm$ 20                | 5.0     | 396 $\pm$ 21                | 5.3     |
| Borneol               | 1.50                         | 1.47 $\pm$ 0.13             | 9.2     | 1.51 $\pm$ 0.11             | 7.3     |
|                       | 15.0                         | 14.3 $\pm$ 1.2              | 8.4     | 14.6 $\pm$ 1.0              | 6.7     |
|                       | 200                          | 195 $\pm$ 17                | 8.7     | 199 $\pm$ 14                | 6.9     |
| Zingiberene           | 3.00                         | 2.86 $\pm$ 0.21             | 7.5     | 2.92 $\pm$ 0.21             | 7.1     |
|                       | 30.0                         | 29.2 $\pm$ 1.5              | 5.2     | 30.2 $\pm$ 1.7              | 5.7     |
|                       | 400                          | 386 $\pm$ 27                | 7.1     | 389 $\pm$ 27                | 6.9     |
| Curcumene             | 3.00                         | 3.14 $\pm$ 0.28             | 8.8     | 2.97 $\pm$ 0.25             | 8.6     |
|                       | 30.0                         | 29.5 $\pm$ 2.0              | 6.6     | 30.3 $\pm$ 2.1              | 6.8     |
|                       | 400                          | 423 $\pm$ 29                | 6.9     | 404 $\pm$ 30                | 7.3     |
| Atractylone           | 24.0                         | 25.2 $\pm$ 1.6              | 6.3     | 24.6 $\pm$ 1.6              | 6.5     |
|                       | 240                          | 228 $\pm$ 18                | 8.1     | 238 $\pm$ 16                | 6.7     |
|                       | 3200                         | 3110 $\pm$ 240              | 7.8     | 3080 $\pm$ 170              | 5.6     |



Table 7 Stability of 8 volatile components in rat plasma under different storage conditions ( $n = 6$ )

| Components     | Nominal conc.<br>(ng mL <sup>-1</sup> ) | Room temperature<br>for 6 h |        | In autosampler vials<br>for 24 h |        | Three freeze-thaw<br>cycles |        | −80 °C for 31 days |        |
|----------------|---|-----------------------------|--------|----------------------------------|--------|-----------------------------|--------|--------------------|--------|
|                |   | RSD (%)                     | RE (%) | RSD (%)                          | RE (%) | RSD (%)                     | RE (%) | RSD (%)            | RE (%) |
| Camphene       | 3.00                                    | 10.6                        | −0.9   | 4.3                              | −2.9   | 4.0                         | −0.1   | 6.6                | −4.4   |
|                | 400                                     | 4.1                         | −4.1   | 7.1                              | −1.8   | 2.5                         | −2.2   | 7.2                | 1.5    |
| β-Phellandrene | 3.00                                    | 7.3                         | −6.3   | 7.6                              | −3.9   | 6.9                         | −5.4   | 2.1                | −7.6   |
|                | 400                                     | 2.9                         | −9.2   | 9.3                              | −2.4   | 2.0                         | −6.8   | 7.3                | −3.6   |
| Eucalyptol     | 3.00                                    | 7.0                         | −2.4   | 10.1                             | 0.0    | 2.7                         | 0.3    | 11.4               | −2.2   |
|                | 400                                     | 2.9                         | −6.6   | 7.6                              | −3.9   | 9.1                         | −4.2   | 2.0                | −0.3   |
| Copaene        | 3.00                                    | 5.1                         | −6.9   | 5.4                              | −5.5   | 8.8                         | −5.0   | 3.3                | −7.9   |
|                | 400                                     | 2.4                         | −9.8   | 9.4                              | −2.7   | 6.6                         | 0.1    | 7.3                | −3.7   |
| Borneol        | 1.50                                    | 6.6                         | −6.0   | 3.4                              | −6.2   | 5.9                         | −3.8   | 3.3                | 2.9    |
|                | 200                                     | 5.3                         | −8.0   | 7.9                              | −6.0   | 4.6                         | −6.4   | 6.7                | 2.7    |
| Zingiberene    | 3.00                                    | 3.9                         | −0.7   | 11.0                             | −0.3   | 7.5                         | −1.3   | 7.4                | 2.1    |
|                | 400                                     | 5.7                         | −2.2   | 3.1                              | −5.3   | 3.3                         | −3.0   | 8.6                | −0.5   |
| Curcumene      | 3.00                                    | 2.6                         | −8.8   | 5.6                              | −10.7  | 9.7                         | −4.8   | 3.7                | −5.5   |
|                | 400                                     | 2.8                         | −9.2   | 3.0                              | −9.4   | 2.9                         | −4.5   | 7.0                | −3.4   |
| Attractylone   | 24.0                                    | 6.0                         | −2.8   | 5.7                              | −5.6   | 5.8                         | 4.1    | 4.0                | −2.2   |
|                | 3200                                    | 3.5                         | −6.0   | 4.3                              | −3.9   | 3.1                         | −2.2   | 6.5                | −3.3   |

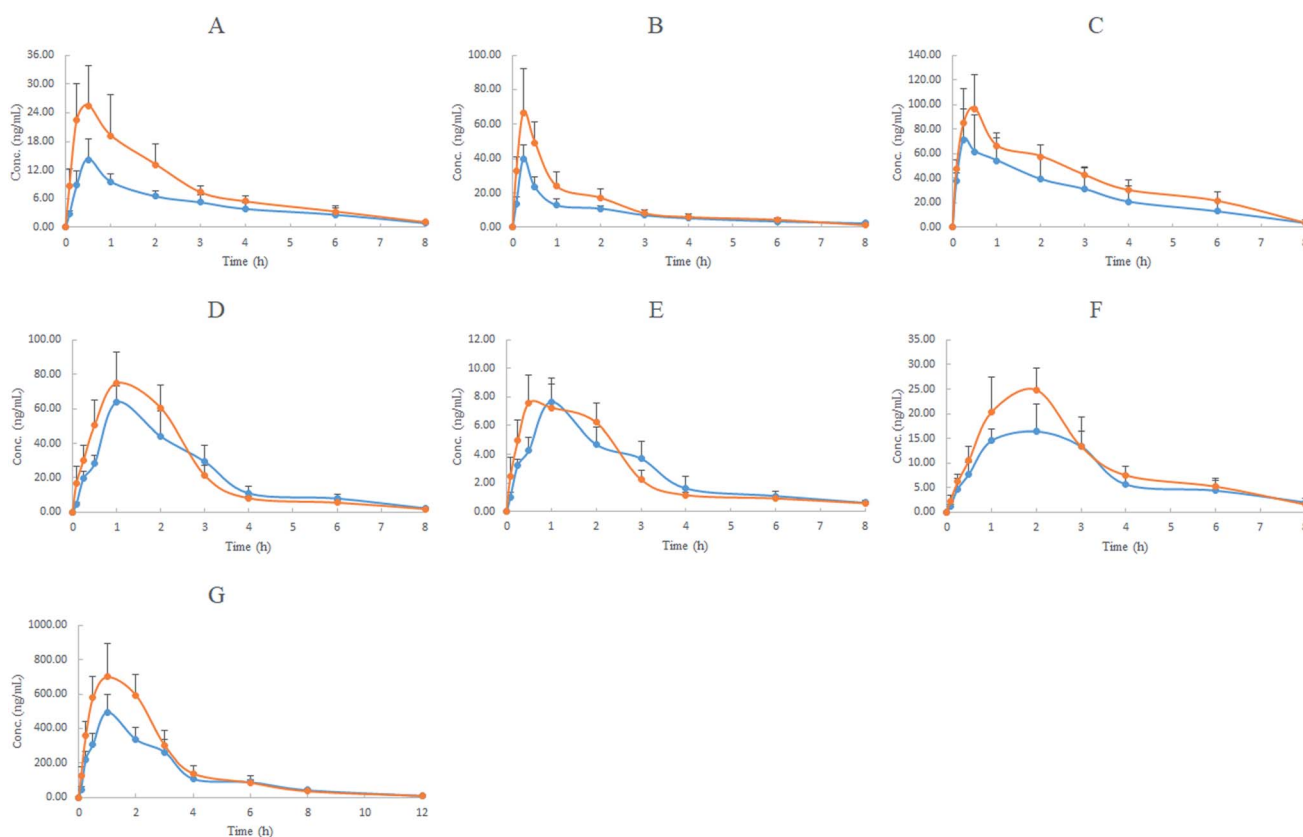


Fig. 5 Profiles of mean plasma concentration–time of (A) camphene, (B) β-phellandrene, (C) eucalyptol, (D) zingiberene, (E) curcumene, (F) copaene and (G) attractylone after a single oral dose of 4.8 g kg<sup>-1</sup> Yinchenzhu decoction (YCZFD, orange line) and 29.2 mg kg<sup>-1</sup> YCZFD volatile oil (VO, blue line) in rats (mean ± SD,  $n = 6$ ).

characteristics of the influence of water extract on the pharmacokinetics of volatile components were firstly clarified, which can provide valuable information for understanding

the potential effect of YCZFD components. However, to date, to the best of our knowledge, there have been no studies on the CYP enzymes and transporters involved in these volatile



**Table 8** Pharmacokinetic parameters of volatile components in rat plasma after oral administration of 4.8 g kg<sup>-1</sup> Yinchenzhufu decoction (YCZFD) and 29.2 mg kg<sup>-1</sup> YCZFD volatile oil (VO) (*n* = 6, mean  $\pm$  SD)<sup>a</sup>

| Components     | Group | <i>T</i> <sub>max</sub> (h)  | <i>C</i> <sub>max</sub> <sup>d</sup> (ng mL <sup>-1</sup> ) | <i>C</i> <sub>max</sub> /dose <sup>e</sup> (μg mL <sup>-1</sup> ) | <i>T</i> <sub>1/2</sub> (h)  | AUC <sub>0-t</sub> <sup>d</sup> (ng h mL <sup>-1</sup> ) | AUC <sub>0-t</sub> /dose <sup>e</sup> (μg h mL <sup>-1</sup> ) |
|----------------|-------|------------------------------|---|---|------------------------------|--|--|
| Camphene       | VO    | 0.50 $\pm$ 0.00              | 14.4 $\pm$ 4.1  | 303 $\pm$ 86  | 2.20 $\pm$ 0.35              | 38.5 $\pm$ 9.0   | 811 $\pm$ 189  |
|                | YCZFD | 0.42 $\pm$ 0.13              | 26.0 $\pm$ 8.0 <sup>c</sup>                                 | 535 $\pm$ 165 <sup>c</sup>  | 1.78 $\pm$ 0.21              | 66.5 $\pm$ 17.9 <sup>c</sup>                             | 1370 $\pm$ 377 <sup>c</sup>                                    |
| β-Phellandrene | VO    | 0.25 $\pm$ 0.00              | 40.2 $\pm$ 4.1  | 332 $\pm$ 34  | 3.59 $\pm$ 1.48              | 63.7 $\pm$ 10.4  | 526 $\pm$ 86   |
|                | YCZFD | 0.29 $\pm$ 0.10              | 68.0 $\pm$ 23.9 <sup>b</sup>                                | 581 $\pm$ 204 <sup>b</sup>  | 1.77 $\pm$ 0.19 <sup>b</sup> | 99.2 $\pm$ 22.5 <sup>b</sup>                             | 848 $\pm$ 192 <sup>b</sup>                                     |
| Eucalyptol     | VO    | 0.42 $\pm$ 0.30              | 81.7 $\pm$ 19.9   | 3620 $\pm$ 881  | 1.84 $\pm$ 0.30              | 216 $\pm$ 98   | 9560 $\pm$ 4340  |
|                | YCZFD | 0.46 $\pm$ 0.10              | 101 $\pm$ 27  | 4390 $\pm$ 1170   | 1.69 $\pm$ 0.23              | 305 $\pm$ 43   | 13 300 $\pm$ 1870  |
| Copaene        | VO    | 2.00 $\pm$ 0.63              | 17.7 $\pm$ 4.2  | 1510 $\pm$ 359  | 2.39 $\pm$ 0.65              | 64.0 $\pm$ 19  | 5470 $\pm$ 1620  |
|                | YCZFD | 1.83 $\pm$ 0.41              | 25.1 $\pm$ 4.5 <sup>b</sup>                                 | 2110 $\pm$ 378 <sup>b</sup>                                       | 1.72 $\pm$ 0.17 <sup>b</sup> | 82.2 $\pm$ 16.2  | 6910 $\pm$ 1360  |
| Zingiberene    | VO    | 1.00 $\pm$ 0.00              | 63.8 $\pm$ 9.6  | 716 $\pm$ 108   | 1.76 $\pm$ 0.81              | 171 $\pm$ 38   | 1920 $\pm$ 426   |
|                | YCZFD | 1.00 $\pm$ 0.00              | 75.0 $\pm$ 18.3   | 823 $\pm$ 203   | 1.59 $\pm$ 0.28              | 191 $\pm$ 41   | 2120 $\pm$ 455   |
| Curcumene      | VO    | 1.00 $\pm$ 0.00              | 7.66 $\pm$ 1.26   | 323 $\pm$ 53  | 2.57 $\pm$ 0.57              | 21.8 $\pm$ 4.2   | 920 $\pm$ 177  |
|                | YCZFD | 0.58 $\pm$ 0.20 <sup>c</sup> | 7.94 $\pm$ 1.66   | 321 $\pm$ 67  | 4.14 $\pm$ 0.89              | 22.3 $\pm$ 4.7   | 903 $\pm$ 190  |
| Atractylon     | VO    | 0.92 $\pm$ 0.20              | 495 $\pm$ 105   | 1070 $\pm$ 227  | 1.86 $\pm$ 0.34              | 1610 $\pm$ 330   | 3480 $\pm$ 713   |
|                | YCZFD | 1.08 $\pm$ 0.49              | 721 $\pm$ 153 <sup>c</sup>                                  | 1570 $\pm$ 334 <sup>c</sup>                                       | 1.84 $\pm$ 0.20              | 2230 $\pm$ 460 <sup>b</sup>                              | 4870 $\pm$ 1000 <sup>b</sup>                                   |

<sup>a</sup> Data are expressed as the mean  $\pm$  SD, *n* = 6. <sup>b</sup> *p* < 0.05 vs. VO group. <sup>c</sup> *p* < 0.01 vs. VO group. <sup>d</sup> The observed value. <sup>e</sup> Dose-normalized value.

compounds. The mechanism of the pharmacokinetic interactions between the components of the WE and volatile components of YCZFD is still unclear and requires further research.

## 4. Conclusions

In the present study, 85 volatile compounds were identified in the VO of YCZFD, and 11 highly abundant volatile components in YCZFD and YCZFD VO were quantified using an established GC-MS/MS method. An HS-SPDE-GC-MS/MS method for the simultaneous quantification of camphene, β-phellandrene, eucalyptol, copaene, borneol, zingiberene, curcumene, and atractylon in rat plasma was developed, which provides a methodological basis for the pharmacokinetic study of volatile components in YCZFD and other related Chinese *materia medica*. The pharmacokinetic laws of camphene, β-phellandrene, eucalyptol, copaene, zingiberene, curcumene, and atractylon from YCZFD, as well as the characteristics of exposure of volatile components increased by WE of YCZFD, were established. These results elucidate the potentially effective components of YCZFD that can be used to promote progression in the development of YCZFD.

## Author contributions

Bin Zan performed the experiments and completed this manuscript. Yuanyuan Li guided the experiment. Xiaoshu Sun and Tianming Wang assisted the experiments. Rong Shi guided the experiment and revised the article. Yueming Ma designed the experiment, guided and revised the article.

## Conflicts of interest

The authors declare no conflict of interests, financial or otherwise.

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## References

- Q. Wang, R. Shi, Y. M. Ma, P. Jiang, J. Zhong, H. Y. Cui, P. Liu and C. H. Liu, Content determination of the major constituents of Yinchenzhufu decoction *via* ultra high-performance liquid chromatography coupled with electrospray ionisation tandem mass spectrometry, *J. Pharm. Biomed. Anal.*, 2013, **77**, 88–93.
- D. W. Mao, N. Tang, Y. Q. Chen, L. H. Liu, N. Wang and S. Wang, A clinical study on the efficacy of modified Yinchenzhufu decoction in chronic liver failure, *Chin. J. Integr. Tradit. West. Med. Liver Dis.*, 2015, **25**, 74–76.
- C. J. Qu, A. Z. Wu, W. L. Wang, W. Qin and J. Qu, Experimental study of Yinchenzhufu decoction on jaundice animal model with Yin yellow syndrome, *J. Liaoning Univ. Tradit. Chin. Med.*, 2006, **8**, 89–90.
- G. F. Wang, Y. Y. Li, R. Shi, T. M. Wang, Y. F. Li, W. K. Li, M. Zheng, F. B. Fan, J. Zou, B. Zan, J. S. Wu and Y. M. Ma, Yinchenzhufu decoction protects against alpha-naphthylisothiocyanate-induced acute cholestatic liver injury in mice by ameliorating disordered bile acid homeostasis and inhibiting inflammatory responses, *J. Ethnopharmacol.*, 2020, **254**, 112672.
- Q. Wang, P. Jiang, F. Y. Ye, R. Shi, Y. M. Ma, J. Zhong, J. S. Wu, P. Liu, C. H. Liu and Y. Q. Jia, Identification and pharmacokinetics of multiple constituents in rat plasma after oral administration of Yinchenzhufu decoction, *J. Ethnopharmacol.*, 2014, **153**, 714–724.
- J. D. Cha, S. E. Moon, H. Y. Kim, J. C. Lee and K. Y. Lee, The essential oil isolated from *Artemisia capillaris* prevents LPS-induced production of NO and PGE2 by inhibiting MAPK-



- mediated pathways in RAW 264.7 macrophages, *Immunol. Invest.*, 2009, **38**, 483–497.
- 7 J. D. Cha, M. R. Jeong, S. I. Jeong, S. E. Moon, J. Y. Kim, B. S. Kil and Y. H. Song, Chemical composition and antimicrobial activity of the essential oils of *Artemisia scoparia* and *A. capillaris*, *Planta Med.*, 2005, **71**, 186–190.
  - 8 C. Yang, D. H. Hu and Y. Feng, Essential oil of *Artemisia vestita* exhibits potent *in vitro* and *in vivo* antibacterial activity: Investigation of the effect of oil on biofilm formation, leakage of potassium ions and survival curve measurement, *Mol. Med. Rep.*, 2015, **11**, 2852–2860.
  - 9 B. Sabulal, M. Dan, A. J. J. R. Kurup, N. S. Pradeep, R. K. Valsamma and V. George, Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity, *Phytochemistry*, 2006, **67**, 2469–2473.
  - 10 G. Singh, I. P. Kapoor, P. Singh, C. S. de Heluani, M. P. de Lampasona and C. A. Catalan, Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*, *Food Chem. Toxicol.*, 2008, **46**, 3295–3302.
  - 11 A. J. Akinyemi and P. A. Adeniyi, Effect of essential oils from ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) rhizomes on some inflammatory biomarkers in cadmium induced neurotoxicity in rats, *J. Toxicol.*, 2018, **2018**, 4109491.
  - 12 S. Gu, L. Li, H. Huang, B. Wang and T. Zhang, Antitumor, Antiviral, and Anti-Inflammatory Efficacy of Essential Oils from *Atractylodes macrocephala* Koidz. Produced with Different Processing Methods, *Molecules*, 2019, **24**(16), 2956.
  - 13 K. Vaillancourt, G. LeBel, L. Yi and D. Grenier, Arch. *In vitro* antibacterial activity of plant essential oils against *Staphylococcus hyicus* and *Staphylococcus aureus*, the causative agents of exudative epidermitis in pigs, *Microbiol.*, 2018, **200**, 1001–1007.
  - 14 Y. El Atki, I. Aouam, F. El Kamari, A. Taroq, K. Nayme, M. Timinouni, B. Lyoussi and A. Abdellaoui, Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics, *J. Adv. Pharm. Technol. Res.*, 2019, **10**, 63–67.
  - 15 C. Pannee, I. Chandhane and L. Wacharee, Antiinflammatory effects of essential oil from the leaves of *Cinnamomum cassia* and cinnamaldehyde on lipopolysaccharide-stimulated J774A.1 cells, *J. Adv. Pharm. Technol. Res.*, 2014, **5**, 164–170.
  - 16 L. Sun, S. B. Zong, J. C. Li, Y. Z. Lv, L. N. Liu, Z. Z. Wang, J. Zhou, L. Cao, J. P. Kou and W. Xiao, The essential oil from the twigs of *Cinnamomum cassia* Presl alleviates pain and inflammation in mice, *J. Ethnopharmacol.*, 2016, **194**, 904–912.
  - 17 C. J. Qu, A. Z. Wu, W. L. Wang, W. Qin and J. Qu, Study the effect of Yinchenzhufu Decoction against Yin jaundice syndrome in an animal model, *Journal of Liaoning College of Traditional Chinese Medicine*, 2006, **8**, 89–90.
  - 18 K. Heberger, M. Gorgenyi and T. Kowalska, Temperature dependence of Kováts indices in gas chromatography revisited, *J. Chromatogr. A*, 2002, **973**, 135–142.
  - 19 D. Lenz, L. Kroner and M. A. Rothschild, Determination of gamma-hydroxybutyric acid in serum and urine by headspace solid-phase dynamic extraction combined with gas chromatography-positive chemical ionization mass spectrometry, *J. Chromatogr. A*, 2009, **1216**, 4090–4096.
  - 20 M. Z. Hou, L. L. Chen, C. Chang, J. F. Zan and S. M. Du, Pharmacokinetic and tissue distribution study of eight volatile constituents in rats orally administrated with the essential oil of *Artemisia argyi* Folium by GC-MS/MS, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2021, **1181**, 122904.
  - 21 H. Yan, Y. Sun, Y. Ma, B. Ji, X. Hou, Z. Yu and Y. Zhao, Determination of atractylon in rat plasma by a GC-MS method and its application to a pharmacokinetic study, *J. Pharm. Anal.*, 2015, **5**, 327–331.
  - 22 T. T. Zhao, L. L. Zhu, M. Chen and Q. Zhou, Is it appropriate regarding patient preference to take Myrtol standardized enteric-coated soft capsules after a meal rather than at fasted state? A food-drug pharmacokinetic interaction study in healthy Chinese volunteers, *Patient Prefer. Adherence*, 2016, **10**, 2031–2037.
  - 23 C. Sa, J. Liu, Y. Dong, L. Jiang, G. Gentana and A. Wurita, Quantification of eucalyptol(1,8-cineole) in rat serum by gas chromatography-mass/mass spectrometry and its application to a rat pharmacokinetic study, *Biomed. Chromatogr.*, 2021, **35**, e5080.

