Supplementary Material

1. Quantitation of 11 volatile components in YCZFD

1.1 Methods

1.1.1 Preparation of the standard and quality control (QC) samples

Standard stock solutions of α-pinene (2.07 mg/mL), camphene (2.44 mg/mL), β-phellandrene (6.20 mg/mL), eucalyptol (1.33 mg/mL), copaene (2 mg/mL), caryophyllene (4.71 mg/mL), borneol (2.08 mg/mL), zingiberene (1 mg/mL), curcumene (1.4 mg/mL), trans-cinnamaldehyde (5.94 mg/mL), atractylon (7.9 mg/mL) and naphthalene (I.S., 1 mg/mL) were prepared in methyl tert-butyl ether : dichloromethane (50:50, *v/v*) and stored at -20 ℃. Then, the 11 stock solutions were mixed and diluted with methyl tert-butyl ether: dichloromethane (50:50, *v/v*) to prepare a final mixed standard solution containing α-pinene (20.7 μg/mL), camphene (24.4 μg/mL), β-phellandrene (62 μg/mL), eucalyptol (13.3 μg/mL), copaene (20 μg/mL), caryophyllene (47.1 μg/mL), borneol (20.8 μg/mL), zingiberene (10 μg/mL), curcumene (14 μg/mL), trans-cinnamaldehyde (59.4 μg/mL), atractylon (158 μg/mL). The mixture was subsequently serially diluted with methyl tert-butyl ether: dichloromethane (50:50, *v/v*) to prepare solutions exhibiting nominal concentration ranges of 0.323–20.7 μg/mL for α-pinene, 0.381–24.4 μg/mL for camphene, 0.969–62 μg/mL for β-phellandrene, 0.208–13.3 μg/mL for eucalyptol, 0.313–20 μg/mL for copaene, 0.156–10 μg/mL for zingiberene, 0.219–14 μg/mL for curcumene, 0.736–47.1 μg/mL for caryophyllene, 0.325–20.8 μg/mL for borneol, 0.927–59.4 μg/mL for trans-cinnamaldehyde and 2.47–158 μg/mL for atractylon. The QC samples of α-pinene, camphene, β-phellandrene, eucalyptol, copaene, caryophyllene, borneol, zingiberene, curcumene, trans-cinnamaldehyde, atractylon at low (0.970, 1.144, 2.906, 0.623, 0.938, 2.208, 0.975, 0.469, 0.656, 2.782 and 7.404 μg/mL, respectively), medium (3.234, 3.813, 9.688, 2.078, 3.125, 7.359, 3.250, 1.563, 2.188, 9.273 and 24.68 μg/mL, respectively) and high levels (16.56, 19.52, 49.6, 10.64, 16, 37.68, 16.64, 8, 11.2, 47.48 and 126.36 μg/mL, respectively).

1.1.2 Method validation

1.1.2.1 Calibration curves and linearity

Calibration curves were constructed by diluting the initial stock solution with methyl tert-butyl ether: dichloromethane (50:50, *v/v*) to afford seven standard samples with different concentrations; then, 90 μl each sample was mixed with 10 μl IS solution for GC–MS/MS analysis. Each standard curve was created by plotting the analyte/IS peak area ratios vs. the analyte concentrations.

1.1.2.2 Precision and accuracy

The intra-day precision and accuracy were evaluated by measuring five replicates of the QC samples at three concentrations levels on the same day, whereas the inter-day precision and accuracy were estimated using three validation batches on three consecutive days. The precision was calculated as the relative standard deviation (RSD%), and the accuracy was defined as the relative error (RE%).

1.1.2.3 Recovery

The recovery at all levels was used to further evaluate the accuracy of the method. Accurate amounts of 11 standards were added to the YCZFD volatile oil sample, and then, it was processed and analysed. The amount of each component was calculated using the corresponding calibration curve. The recovery of each component was calculated according to the following equation: accuracy (%) = (amount$_{detected}$ − amount$_{original}$)/amount$_{spiked}$ × 100%).

1.1.2.4 Repeatability and stability

To investigate the repeatability of the method, five different solutions of YCZFD volatile oil were analysed, and the RSD was considered as a measure of reproducibility. The same sample solution was stored at 4 ℃ and analysed at 24 h to investigate the stability of the solution.

1.2 Results

Typical MRM chromatograms are presented in **Fig. S1**. In the chromatograms, all components and IS were clearly detected. This method displayed a good specificity. The regression equations, correlation coefficients and linear ranges as well as LOD and LOQ values of the 11 components are shown in **Table S1**. All calibration curves

exhibit good linearity ($r^2 > 0.9962$) between the peak area ratio and the concentration. The precision of the methods are shown in **Table S2**. The precision of the intra- and inter-day variation for the detection levels of the investigated components is less than 6.92%. **Table S3** lists the mean recoveries (92.05% to 104.97%) of the 11 components, with RSD values < 11.05%. The RSD values of the repeatability test were less than 3.87% for all components. When the solution was stored at 4 ℃, the 11 components were found to be stable for 48 h (RSD < 9.65%). The results indicate that the established method was specific, sensitive, satisfactory, accurate and reliable for the quantitation of the 11 volatile components of YCZFD and YCZFD VO.

2. Quantification of the 11 volatile components in YCZFD in rat plasma

2.1 Method validation

2.1.1 Specificity

Specificity was investigated by comparing blank rat plasma from six different sources, plasma samples spiked with working solution and IS, plasma samples after the oral administration of YCZFD VO, and plasma samples after the oral administration of YCZFD.

2.1.2 Linearity

Linearity was investigated by analyzing two independent calibration curves in three batches. The accepted correlation coefficient (r), which was obtained using the regression model of plotting analyte/IS peak area ratios versus the nominal concentrations with $1/x^2$ weighting factor, should be >0.995, and the accuracy of the back-calculated calibration standard concentrations have to be within ±15% (±20% for LLOQ) deviation of the nominal concentration, the precision for each concentration point (n=6) should be within 15% (20% for LLOQ).

2.1.3 Precision and accuracy

The intra-batch precision and accuracy were estimated by analyzing QC samples at three concentration with six determinations for each level in one batch, whereas the inter-batch precision and accuracy were investigated by analyzing three

validation batches in three different days. Precision was calculated as the relative standard deviation (RSD%) should be within 15%, and the accuracy was defined as the relative error (RE%) should be within ±15%.

2.1.4 Recovery

The recovery was estimated by comparing the peak area of the extracted standard in the blank plasma with the standards in the absence of the matrix based on three determinations at three QC levels for each group.

2.1.5 Stability

The stability of the 11 analytes in the rat plasma was assessed by performing three freeze-thaw cycles, storing at room temperature for 48 h, and storing in a -80 °C freezer for 30 days on low and high QC concentrations with six replicates of samples. The analytes can be considered stable when the relative error within ± 15% of the nominal concentration, and the standard deviation of six replicates of each concentration within 15%.

Figure captions
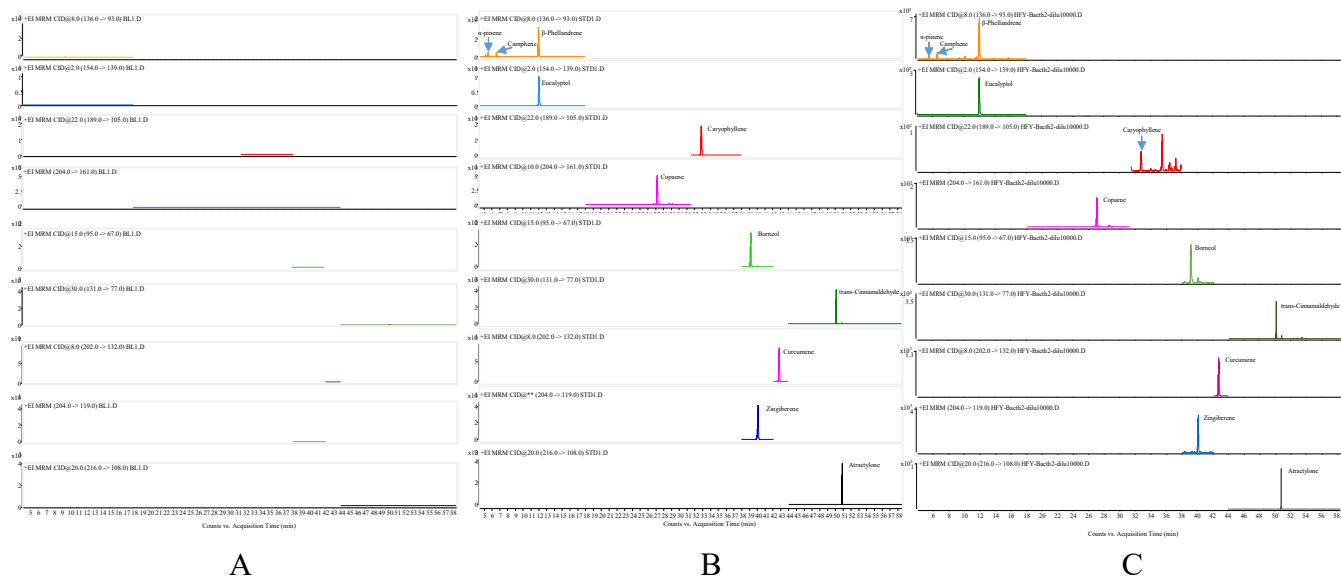
A   B   C

Fig. S1 Typical MRM chromatograms of 11 components (α-Pinene, Camphene, β-Phellandrene, Eucalyptol, Borneol, Copaene, Caryophyllene, Zingiberene, Curcumene, trans-Cinnamaldehyde, Atractylone in volatile oil of YCZFD. Panels show (A) blank sample, (B) standard sample, (C) YCZFD volatile oil sample.

Table S1 Calibration curves, LODs and LOQs of 11 volatile components

Components	Calibration curve	r^2	Linear range (µg/mL)	LOQ (ng/mL)	LOD (ng/mL)
α-pinene	0.00104*X+0.0000644	0.9990	0.323-20.700	20.0	5.00
Camphene	0.000571*X+0.000047	0.9971	0.381-24.400	20.0	5.00
β-Phellandrene	0.00358*X+0.000422	0.9994	0.969-62.000	10.0	3.00
Eucalyptol	0.000547*X+0.000018	0.9984	0.208-13.300	20.0	5.00
Copaene	0.00181*X+0.00003	0.9994	0.313-20.000	30.0	10.0
Caryophyllene	0.000307*X-	0.9996	0.736-47.100	40.0	10.0
Borneol	0.0102*X+0.000221	0.9995	0.325-20.800	20.0	5.00
Zingiberene	0.00254*X-0.0000647	0.9962	0.156-10.000	6.00	2.00
Curcumene	0.00382*X+0.0000782	0.9967	0.219-14.000	3.00	1.00
trans-	0.016*X-0.00119	0.9997	0.927-59.400	15.0	5.00
Atractylone	0.00528*X+0.00104	0.9991	2.470-158.000	9.00	3.00

Table S2 Intra- and inter-day variability for the assay of 11 volatile components

Components	Conc. (μg/mL)	Intra-day (n=5) Mean (μg/mL)			RSD (%)	Inter-day (n=15) Mean (μg/mL)			RSD (%)
α-pinene	0.970	0.945	±	0.05	5.32	0.931	±	0.034	3.70
	3.234	3.045	±	0.11	3.82	3.110	±	0.147	4.72
	16.560	16.21	±	0.83	5.17	16.21	±	0.798	4.92
Camphene	1.144	1.107	±	0.02	2.52	1.118	±	0.053	4.78
	3.813	3.604	±	0.23	6.38	3.654	±	0.232	6.36
	19.520	18.10	±	0.93	5.17	18.54	±	0.978	5.27
β-Phellandrene	2.906	2.785	±	0.09	3.59	2.806	±	0.139	4.95
	9.688	9.554	±	0.34	3.57	9.689	±	0.294	3.04
	49.600	46.91	±	2.75	5.88	47.45	±	2.440	5.14
Eucalyptol	0.623	0.605	±	0.03	5.53	0.605	±	0.032	5.28
	2.078	2.305	±	0.07	3.68	2.039	±	0.074	3.64
	10.640	10.04	±	0.57	5.73	10.27	±	0.553	5.38
Copaene	0.938	0.879	±	0.02	2.99	0.901	±	0.04	4.45
	3.125	2.956	±	0.11	3.76	3.047	±	0.154	5.06
	16.000	15.26	±	0.92	6.05	15.41	±	0.821	5.33
Caryophyllene	2.208	2.217	±	0.05	2.32	2.129	±	0.090	4.22
	7.359	6.957	±	0.42	6.14	7.156	±	0.393	5.49
	37.680	37.44	±	1.30	3.47	37.08	±	1.480	3.99
Borneol	0.975	0.963	±	0.03	3.49	0.945	±	0.040	4.21
	3.250	3.246	±	0.10	3.32	3.218	±	0.113	3.51
	16.640	16.19	±	0.97	5.99	16.06	±	0.750	4.67
Zingiberene	0.469	0.445	±	0.01	3.67	0.446	±	0.018	4.06
	1.563	1.504	±	0.08	5.61	1.530	±	0.065	4.26
	8.000	8.537	±	0.53	6.21	7.970	±	0.518	6.50
Curcumene	0.656	0.621	±	0.04	6.92	0.628	±	0.037	5.87
	2.188	2.124	±	0.12	5.91	2.140	±	0.105	4.93
	11.200	11.19	±	0.69	6.19	10.92	±	0.469	4.30
trans-	2.782	2.641	±	0.10	3.77	2.613	±	0.092	3.51
	9.273	8.841	±	0.34	3.88	8.878	±	0.311	3.51
	47.480	45.45	±	2.75	6.06	45.75	±	2.941	6.43
Atractylone	7.404	7.180	±	0.43	6.01	7.174	±	0.250	3.48
	24.68	23.01	±	0.94	4.10	23.33	±	0.848	3.63
	126.36	127.9	±	2.19	1.72	124.0	±	5.756	4.64

Table S3 Recovery and reproducibility levels of 11 volatile components

Components	Recovery (n=3)		Reproducibility (n=5)	
	Accuracy (%)	RSD (%)	Mean	RSD (%)
α-pinene	103.54	1.51	0.94	1.88
Camphene	98.52	3.64	3.46	2.20
β-Phellandrene	101.62	4.87	8.27	2.28
Eucalyptol	104.97	0.86	1.59	2.30
Copaene	94.49	3.80	0.87	3.87
Caryophyllene	97.45	0.82	0.99	3.29
Borneol	98.80	5.01	1.64	3.64
Zingiberene	95.66	10.18	7.02	3.55
Curcumene	92.05	1.43	1.71	1.76
trans-	102.87	11.05	4.97	3.87
Atractylone	98.37	2.39	32.97	1.46