

Electronic Supplementary Information

Supplementary Experimental Procedures

The preparation process of OTP was as follows: Tea polyphenols (the purity is more than 98%) aqueous solution was oxidized with atmosphere oxygen at 37°C under alkaline conditions until the A230nm/A520nm (determined by spectrophotometer, HITACHI, U-2910, the optical path length is 1.0 cm, sample concentration is 500 mg/L) equaled 1.40 (the value of the initial tea polyphenols sample is 10.90). Then, the solution was dried by lyophilization.

The preparation process of TPS was as follows: Fresh green tea was extracted by deionized water and the aqueous extract was concentrated. Then, alcohol was added to the concentrated solution until the percentage of alcohol reached 80%. This process lasted for 3 hours. The alcohol sedimentation process was repeated twice. Finally, the sediment was dissolved by deionized water and dried by lyophilization.

The preparation process of PTPS began with alcohol being added to fermented Pu-er tea aqueous extract until it reached an alcohol concentration of 80%, a process which lasted for 3 hours. The alcohol sedimentation process was repeated twice. Finally, the sediment was dissolved by deionized water and dried by lyophilization.

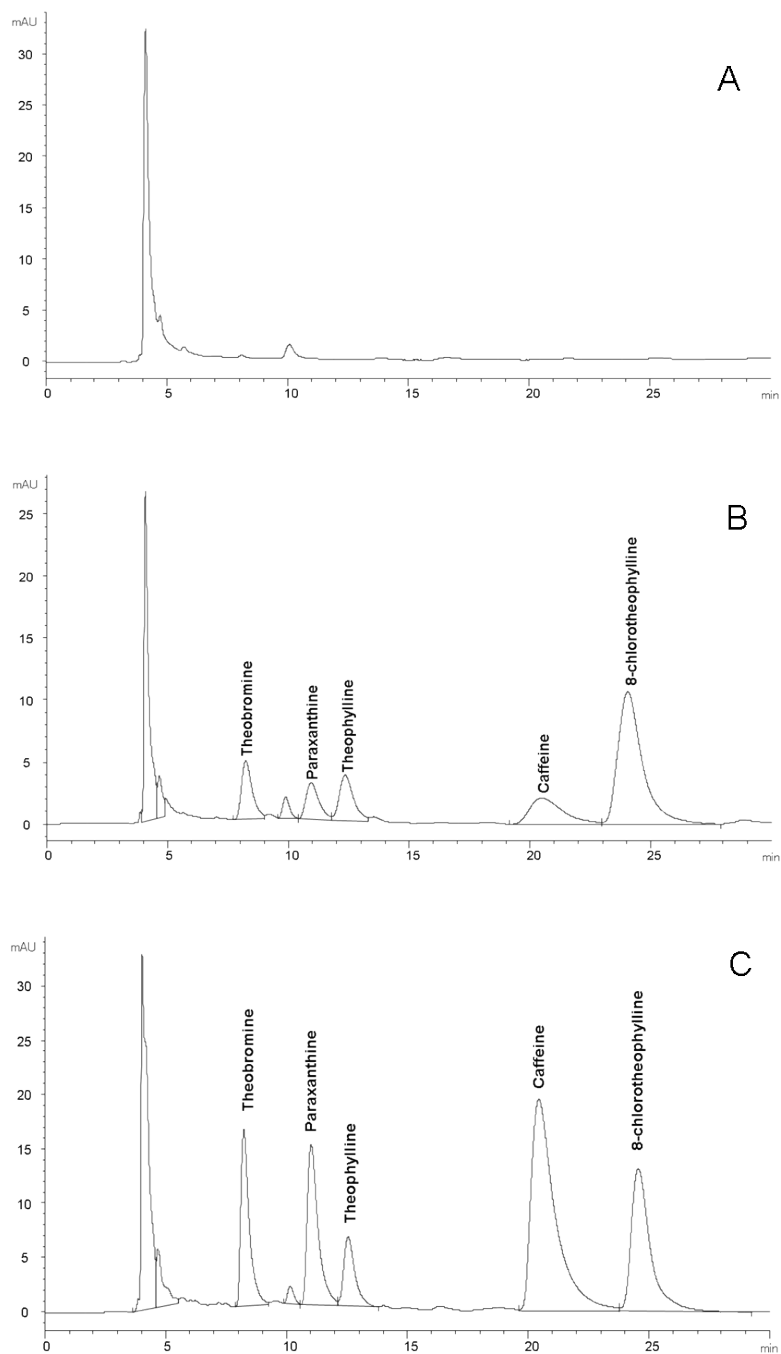
The preparation process of PTTB was initiated by having fermented Pu-er tea aqueous being extracted by isopyknic chloroform, ethyl acetate and n-butyl alcohol three times, separately. The final aqueous phase was accomplished by lyophilization.

Supplemental data

“Assay Validation” for Levels of caffeine and its metabolites in plasma

Chromatography

Typical chromatograms of extracted drug-free plasma, appropriate standard and sample from a subject 240 min following a single dose of caffeine (50 mg/kg) are shown in Supplemental Figure 1. In plasma, caffeine, paraxanthine, theophylline, theobromine and 8-chlorotheophylline (internal standard) eluted at 20.0 min, 10.8 min, 12.3 min, 8.1 min and 23.9 min, respectively. Run time for the plasma samples was 30.0 min. The chromatograms from eight different caffeine-free plasma sources revealed no interfering peaks at retention times of interest.



Supplemental Figure 1. HPLC chromatograms for: (A) Caffeine-free plasma. (B) Plasma samples with caffeine (42.41 mg/L), paraxanthine, theophylline (19.44 mg/L), theobromine (5.08 mg/L) and 8-chlorotheophylline (19.83 mg/L). (C) Plasma sample taken at 240 min following administration of caffeine (50 mg/kg) with added internal standard (3.33 mg/L).

Extraction Recovery

The mean(n=6) overall extraction recovery from human plasma of caffeine, paraxanthine, theophylline and theobromine was 85.28 ± 2.31 %, 57.13 ± 0.87 %, 60.10 ± 0.39 % and 72.84 ± 0.87 %, respectively. The

recovery for the internal standard (8-chlorotheophylline) was 90.89 ± 1.07 %.

Linearity and Sensitivity

The relationship between caffeine, paraxanthine, theophylline and theobromine concentrations and peak area ratio was linear over the range of 0.05–80 mg/L, 0.10–80 mg/L, 0.105–80 mg/L and 0.035–80 mg/L, respectively. The calibration curves demonstrated that linearity of response ($r^2 > 0.9998$) was obtained for caffeine, paraxanthine, theophylline and theobromine.

Resulted in peak area responses greater than 10 times those observed in the equivalent caffeine-free samples at retention times relevant to the analytes, the limit of quantitation for caffeine, paraxanthine, theophylline and theobromine in plasma was 0.05 mg/L, 0.10 mg/L, 0.105 mg/L and 0.035 mg/L, respectively. The limit of detection, determined by a signal-to-noise ratio of 3:1, for caffeine, paraxanthine, theophylline and theobromine in plasma was approximately 0.025 mg/L, 0.045 mg/L, 0.060 mg/L, 0.015 mg/L, respectively.

Precision and Accuracy

The intra-day precision (percentage relative standard deviation, % RSD) and accuracy (% CV) for caffeine, paraxanthine, theophylline and theobromine in plasma was assessed. The precision and accuracy for the analytes in plasma were within the acceptable range (< 7 %). Moreover, caffeine, paraxanthine, theophylline and theobromine in plasma was stable for at least 24 h at 4°C.

Response factors

The solution containing 10 mg/L of the analytes (caffeine, paraxanthine, theophylline and theobromine) and the internal standard (8-chlorotheophylline) are detected by HPLC and the response factor, F , is calculated by the following formula.

$$F = \frac{Area_{analyte} / M_{analyte}}{Area_{IS} / M_{IS}}$$

F : Response factor.

$Area_{analyte}$: Peak area of analyte.

$M_{analyte}$: The concentration (molarity, M) of analyte.

$Area_{IS}$: Peak area of the internal standard.

M_{IS} : The concentration (molarity, M) of the internal standard.

The results showed that the response factor of caffeine, paraxanthine, theophylline and theobromine was 1.005, 0.456, 0.514 and 0.906, respectively.

Supplemental Table 1. The content of caffeine, theophylline, theobromine and paraxanthine of the samples¹

Sample type	Sample name	Caffeine, mg/L	Theophylline, mg/L	Theobromine, mg/L	Paraxanthine, mg/L
fermented pu-erh tea	Gold bud tribute pu-erh tea	2360.09±12.50	62.91±1.87	170.40±0.08	6.17±0.01
	TAETEA pu-erh tea (7592)	1813.7±0.40	12.47±0.49	105.08±0.02	7.77±0.08
	Brick pu-erh tea	1685.94±0.21	81.48±0.18	59.66±0.05	9.10±0.04
non-fermented pu-erh tea	Moonlight White pu-erh tea	2667.94±0.32	63.96±0.11	176.69±0.02	0.00±0.00
	Bangwei pu-erh tea	1431.63±5.25	33.67±0.97	87.68±0.13	8.80±0.68
black tea	Changning black tea(first grade)	1381.45±1.15	104.54±2.55	100.58±0.22	2.08±0.11
	MAKEMY black tea	2316.04±1.48	127.45±0.16	116.30±0.10	0.00±0.00
	Dianhong black tea	1835.20±1.03	106.07±0.13	204.83±0.08	2.25±0.01
oolong tea	Tieguanyin oolong tea	1070.55±5.53	40.17±0.57	26.90±0.09	10.45±1.34
	Da Hung Pao oolong tea	1633.92±1.56	47.47±0.44	40.10±0.04	0.00±0.00
green tea	Longjing green tea	1278.28±4.26	68.65±0.73	99.33±0.07	19.21±0.10
	Biluochun green tea	1671.55±0.85	31.94±0.84	132.48±0.10	13.36±0.17
	Huilong green tea	1506.84±0.65	47.88±0.12	259.17±0.13	10.61±0.09
	Yunnan green tea	1585.98±0.42	56.67±0.40	123.43±0.94	20.64±0.47
coffee	Nescafe coffee	564.70±0.46	34.24±0.21	33.88±0.02	6.22±0.12
	Maxwell House coffee	687.14±0.20	36.34±0.14	29.48±0.10	2.79±0.04
	Yunnan Pasteral coffee	678.24±0.55	32.56±0.10	52.77±0.03	7.66±0.15
instant fermented pu-erh tea	Deepure instant essence of pu-erh tea	1568.02±3.83	25.21±0.88	109.31±0.17	0.00±0.00
TPS	TPS	28.68±0.56	1.21±0.06	6.22±0.41	1.84±0.07
OTP	OTP	191.66±1.63	27.74±0.56	19.03±0.03	0.00±0.00
PTPS	PTPS	483.92±0.52	4.81±0.25	50.76±0.21	0.36±0.01
PTTB	PTTB	9.92±1.63	0.45±0.42	15.02±0.02	0.12±0.02
mixture of TPS and caffeine	caffeine added TPS	1176.98±0.73	0.00±0.00	8.00±0.20	1.73±0.07

mixture of OTP and caffeine	Caffeine added OTP	1284.56±3.04	29.56±0.44	22.18±0.08	1.02±0.52
mixture of PTTB and caffeine	caffeine added PTTB	1240.76±2.34	0.78±0.24	14.99±0.03	0.14±0.01

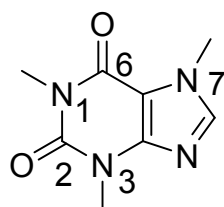
¹Data were presented as mean ± SEM of three independent experiments. TPS, tea polysaccharides; OTP, oxidative tea polyphenols; PTPS, Pu-er tea polysaccharide; PTTB, Pu-er tea theabrownin.

Supplemental Table 2. The percent of precipitate volume of the samples¹

sample type	sample name	precipitate volume, %
fermented pu-erh tea	Gold bud tribute pu-erh tea	9.42±0.08
	TAETEA pu-erh tea (7592)	8.67±0.33
	Brick pu-erh tea	6.88±0.96
non-fermented pu-erh tea	Moonlight White pu-erh tea	0.58±0.08
	Bangwei pu-erh tea	0.33±0.33
black tea	Changning black tea(first grade)	0.46±0.46
	MAKEMY black tea	0.54±0.54
	Dianhong black tea	0.58±0.17
oolong tea	Tieguanyin oolong tea	0.33±0.33
	Da Hung Pao oolong tea	0.92±0.25
green tea	Longjing green tea	0.33±0.17
	Biluochun green tea	0.12±0.12
	Huilong green tea	0.00±0.00
	Yunnan green tea	0.00±0.00
coffee	Nescafe coffee	0.29±0.12
	Maxwell House coffee	0.83±0.17
	Yunnan Pasteral coffee	0.17±0.17
instant fermented pu-erh tea	Deepure instant essence of pu-erh tea	9.33±0.58
OTP	OTP	20.54±1.54
PTPS	PTPS	13.62±0.17
mixture of TPS and caffeine	caffeine added TPS	2.03±0.18
mixture of OTP and caffeine	caffeine added OTP	20.75±0.50
mixture of PTTB and caffeine	caffeine added PTTB	12.67±0.29
caffeine	caffeine	0.02±0.01

¹Data were presented as mean ± SEM of three independent experiments. C-CAF, complex caffeine; F-CAF, free caffeine; OTP, oxidative tea polyphenols; PTPS, Pu-er tea polysaccharide; PTTB, Pu-er tea theabrownin; TPS, tea polysaccharides.

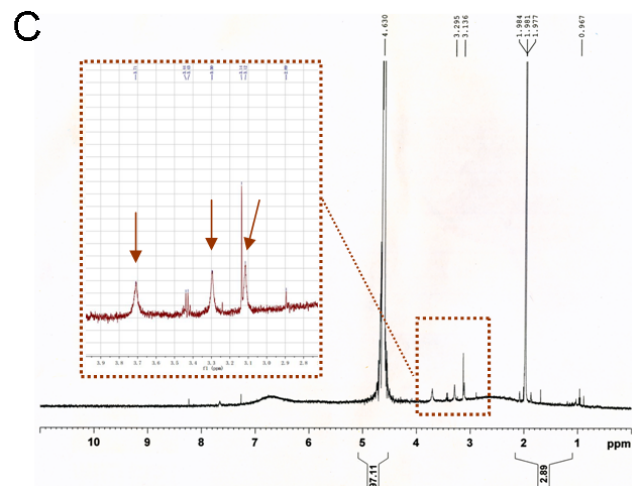
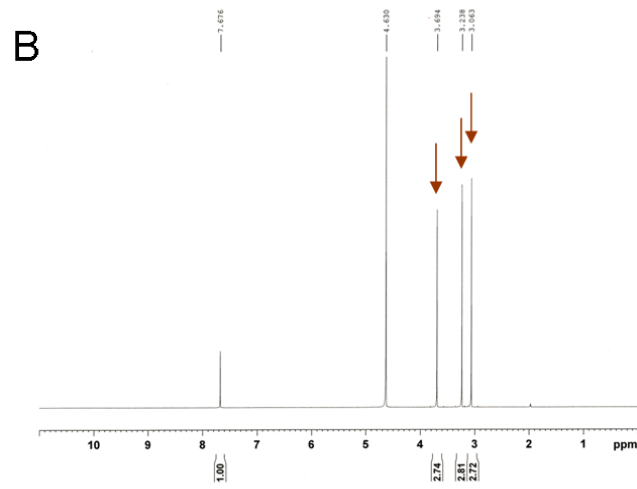
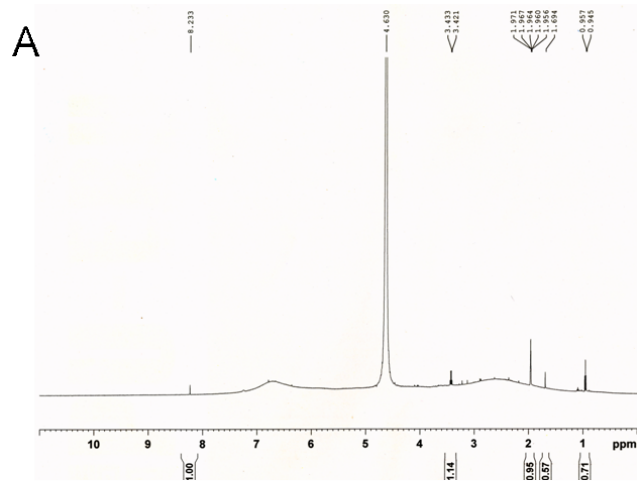
¹H NMR spectra data for caffeine



Supplemental Figure 2. Structure of caffeine

Supplemental Table 3. δ H value of free caffeine and complex of OTP & caffeine

No. / Position	Free caffeine	Complex of OTP & caffeine
1	3.06	3.12
3	3.24	3.30
7	3.69	3.71



Supplemental Figure 3. ^1H NMR spectra for: (A) OTP, (B) Caffeine and (C) Complex of OTP & caffeine. Red arrows indicate the peak of methyl group of caffeine. OTP, oxidative tea polyphenols.