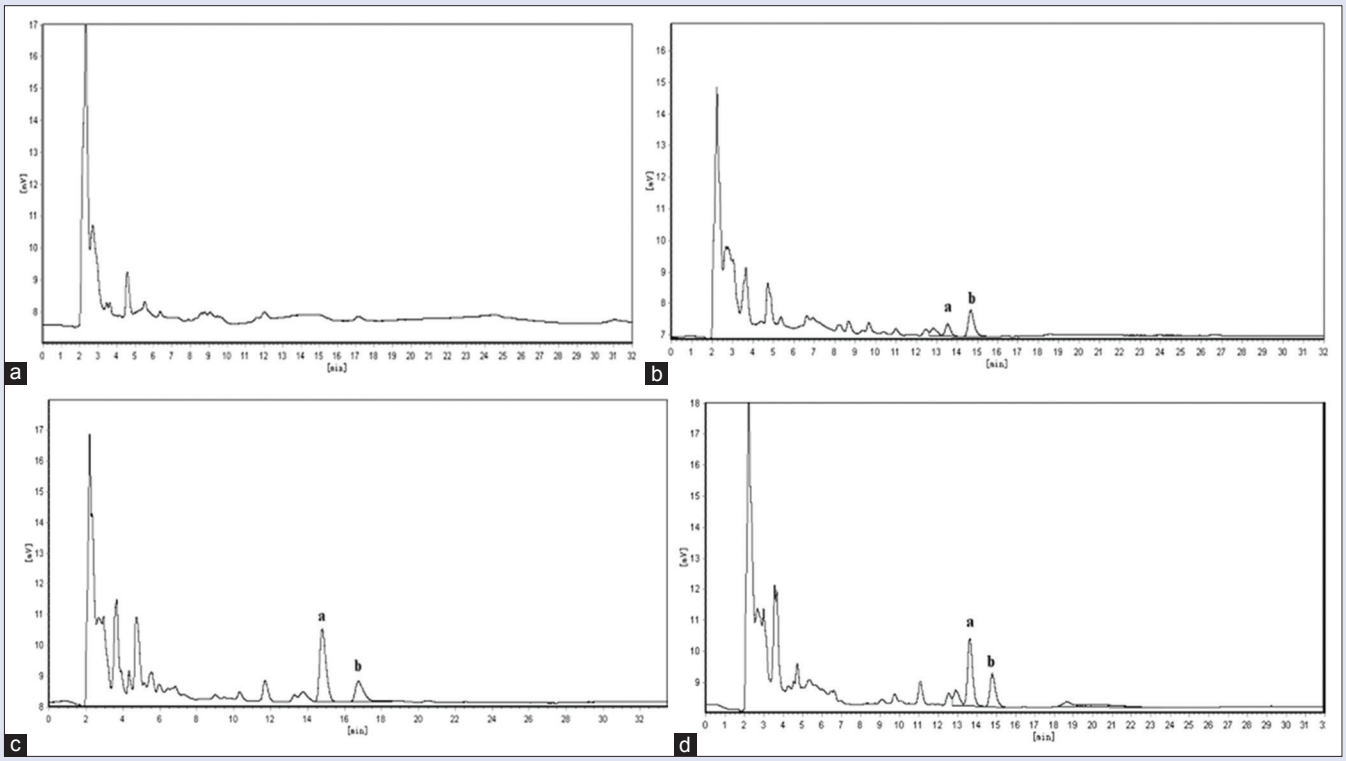

Figure 1: (a) Chromatograms of blank tissue homogenate. (b) Blank tissue homogenate with atractylodin 20 μL and IS 20 μL. (c) Spleen sample (1.5 h) after oral administration of raw *Atractylodis rhizoma* 20 g/kg. (d) Spleen sample (1.5 h) after oral administration of processed *Atractylodis rhizoma* 20 g/kg

Table 1: The linear regression analysis of atracylodin in rat tissue

Tissues	Standard curves	*r*	Linear range(μg/mL)
Heart	Y=2.846X+0.102	0.9955	0.029～5.80
Liver	Y=3.732X+0.207	0.9908	0.029～5.80
Spleen	Y=3.175X-0.140	0.9950	0.029～5.80
Lung	Y=2.965X-0.055	0.9993	0.029～5.80
Kidney	Y=2.286X+0.055	0.9943	0.029～5.80
Large intestine	Y=2.153X+0.428	0.9902	0.029～5.80
Small intestine	Y=3.373X-0.013	0.9966	0.029～5.80
Stomach	Y=2.700X+0.048	0.9932	0.029～5.80

Data analyses

HPLC analysis procedure was applied to analyze tissue distribution of atractylodin.

RESULTS

Method validation

Specificity

Figure 1 shows that no interference peaks from endogenous constituents were detected.

Linearity of calibration curve

The calibration curves were linear over the concentration range of 0.029~5.80 μg/mL in tissue homogenates of atractylodin. The correlation coefficient values of the calibration curve were over 0.9900. Typical linear regression equations and correlation coefficients in each tissue are listed in Table 1.

Recovery and stability

The extraction recoveries of atractylodin ranged from 82.402% to 89.744% in tissue samples. The data are listed in Table 2. The stock solution stabilities for the analyte and IS did not significant differences. The data are listed in Table 3.

Table 2: The recovery of atractylodin in rat tissue (*n*=3)

	Concentration (μg/mL)	Mean(%)	RSD (%)
Heart	0.029	85.635	6.996
	0.580	82.402	3.945
	5.800	89.744	2.111
Liver	0.029	83.266	3.742
	0.580	85.913	2.136
	5.800	85.461	5.174
Spleen	0.029	85.462	3.393
	0.580	85.924	2.242
	5.800	88.027	3.265
Lung	0.029	87.884	1.923
	0.580	85.419	4.327
	5.800	85.202	3.005
Kidney	0.029	85.613	3.485
	0.580	85.064	2.453
	5.800	84.435	1.019
Large intestine	0.029	86.054	3.459
	0.580	85.949	3.944
	5.800	87.814	1.832
Small intestine	0.029	83.529	1.476
	0.580	87.015	3.810
	5.800	88.195	3.648
Stomach	0.029	85.788	2.841
	0.580	85.394	2.390
	5.800	87.915	2.992

Stability of analysis showed no significant sample loss over 24 h at room temperature, three freeze-thaw cycles, and 30 days storage condition. The RSD of three conditions was within ± 15%. The data are listed in Table 4.

Table 3: The stock solution stabilities for atractylodin and the IS (*n*=6)

Time (h)/Peak area (A)	0	4	8	12	24	48	Mean	RSD%
Atractylodin	8803.1	8811.5	8790.2	8781.1	8799.4	8791.3	8796.1	0.12
IS	21745	21721	21733	21716	21672	21659	21708	0.15

Table 4: The stability of atractylodin in rat tissue (*n*=5)

	RSD		
	0.029 μg/mL	0.580 μg/mL	5.800 μg/mL
Heart	7.002	1.730	2.504
Liver	6.170	2.007	2.357
Spleen	3.752	1.361	2.316
Lung	3.232	1.913	1.626
Kidney	4.156	2.445	2.106
Large intestine	3.980	1.386	1.307
Small intestine	2.738	2.383	1.743
Stomach	4.337	1.375	2.067

Table 5: The tissue concentrations of atractylodin after oral administration raw Atractylodis rhizoma at a dose of 20 g/kg to rats (*n*=5)

Tissue (μg/g)	10 min	1.5h	4h	8h
Heart	283.7813	253.1313	202.0188	183.1500
Liver	1137.5563	692.9063	209.1688	728.8438
Spleen	326.3188	245.5688	601.7063	164.2000
Lung	585.9875	502.8125	410.7438	1428.8250
Kidney	153.7438	291.9563	359.7063	138.6938
Large intestine	232.5125	749.4000	290.0313	560.6625
Small intestine	2249.6750	527.9063	462.5938	318.2438
Stomach	4717.8625	4446.9063	3436.4688	2516.8688

Table 6: The tissue concentrations of atractylodin after oral administration processed Atractylodis rhizoma at a dose of 20 g/kg to rats (*n*=5)

Tissue (μg/g)	10 min	1.5h	4h	8h
Heart	216.0563	112.8250	102.3500	180.9875
Liver	1143.7063	271.1500	201.3313	305.9938
Spleen	307.3500	210.3938	505.6938	242.4438
Lung	193.0875	179.6563	220.8188	14.4938
Kidney	189.7125	169.5750	172.5688	211.4938
Large intestine	59.7438	287.4125	227.1813	556.2188
Small intestine	1285.3813	406.8688	557.8250	482.9813
Stomach	4666.1625	2666.6875	3168.8813	1530.4750

Accuracy and precision

Accuracy was assessed by analyzing six aliquots of low, medium, and high concentration samples. Accuracy of atractylodin in tissues ranged from 85.00% to 96.80%. The precision data for atractylodin were not exceed 5%.

Tissue distribution study

The tissue concentrations of atractylodin determined at 10 min, 1.5, 4, and 8 h after oral administration raw and processed *A. rhizoma* a dose of 20 g/kg is shown in Tables 5, 6 and Figures 2, 3.

DISCUSSION

The assay was applied to a tissue distribution experiment in the rat after oral administration of 20 g/kg raw and processed *A. rhizoma*, respectively. The tissue distribution was shown in Tables 5 and 6. The atractylodin in raw and processed *A. rhizoma* was distributed in all tissues, such as heart, liver, spleen, lung, kidney, large intestine, small intestine, and stomach. The concentration of atractylodin in raw and processed *A. rhizoma* is the highest in the stomach and small intestine which proved that the theory of *A. rhizoma* can strengthen spleen-stomach and improve its function of digestion and elimination. The concentration of atractylodin in different tissues after oral processed *A. rhizoma* decreased, the reason needs further research. Atractylodin is one of the main components in volatile oils of *A. rhizoma*. Volatile oils are both "dry" (side effect) components and active components.[12] After being processed, the content of volatile oils was decreased, so the "dry" effects can be weaken while the therapeutic effects can be improved relatively.[6,13] In this study, the IS of rhubarb, emodin, and physcion was studied. Finally, the moderate retention time and no interference peaks from endogenous constituents are physcion.

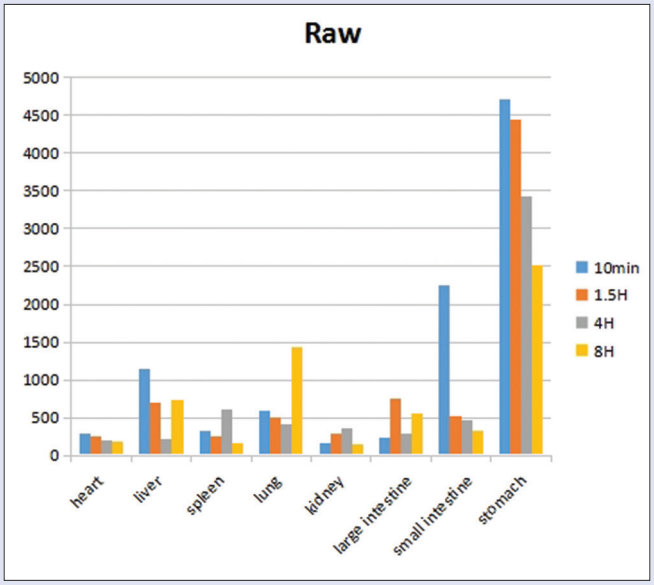

Figure 2: The concentration-time profile of atractylodin after oral administration raw *Atractylodis rhizoma* at a dose of 20 g/kg to rats

CONCLUSIONS

A simple, specific, and rapid reversed phase-HPLC method with ultraviolet detection for quantification of atractylodin in rat tissue has been developed for the first time. It has been successfully applied to a tissue distribution study of atractylodin after oral administration of 20 g/kg raw and processed *A. rhizoma,* respectively. The atractylodin in raw and processed *A. rhizoma* was distributed in all tissues and the concentration of atractylodin is the highest in the stomach and small intestine. The concentration of atractylodin in processed *A. rhzoma* decreased, but its relative concentration is higher in the stomach and small intestine than other tissue.

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Financial support and sponsorship

Nil.

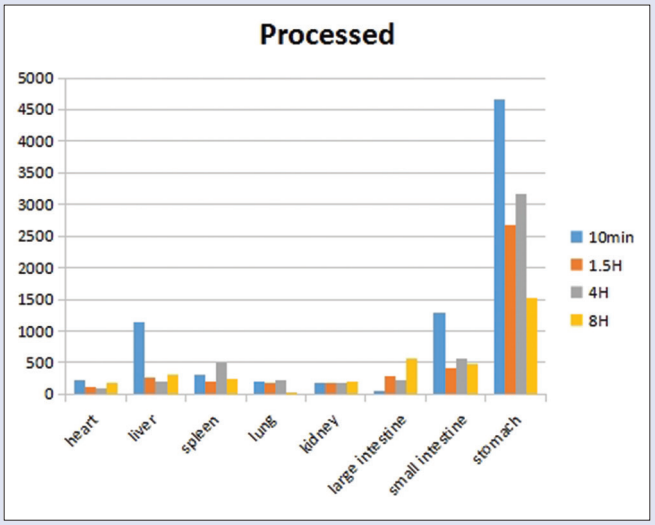

Figure 3: The concentration-time profile of atractylodin after oral administration processed *Atractylodis rhizoma* at a dose of 20 g/kg to rats

Conflicts of interest

There are no conflicts of interest.

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