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ORIGINAL RESEARCH

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Rapid Determination of Andrographolide and Dehydroandrographolide in *Andrographis paniculata* by HPLC-UV at Equal Absorption Wavelength

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Supplementary Materials

Table S1 The impacts of different EAWs on AP and DAP separation efficiency (bandwidth of 2 nm).

Wavelength/nm	AP peak area	DAP peak area	*RSD*/%
239	114.075	106.217	3.80
	115.299	107.208	
	114.628	107.756	
240	109.061	109.709	0.27
	109.414	109.728	
	109.154	109.649	
241	103.103	115.001	5.91
	103.614	112.975	
	102.078	115.567	

AP: andrographolide; DAP: dehydroandrographolide; *RSD*: relative standard deviation.

Table S2 The impacts of different bandwidths on AP and DAP separation efficiency (λ=240 nm).

Bandwidth/nm	AP peak area	DAP peak area	*RSD*/%
1	108.090	112.726	1.66
	108.393	111.007	
	108.536	110.069	
2	109.061	109.709	0.27
	109.414	109.728	
	109.154	109.649	
4	108.529	109.231	0.86
	109.351	109.834	
	107.902	110.545	

	108.041	109.441	
8	108.806	109.789	0.88
	107.819	110.212	

AP: andrographolide; DAP: dehydroandrographolide; *RSD*: relative standard deviation.

Table S3 The retention time difference and relative retention time of DAP in different HPLC columns.

HPLC columns	Retention time difference/min	Relative retention time
Agilent Poroshell 120 EC-C_{18}	2.181	2.692
Waters CORTECS C_{18}	2.115	3.409
ACE UltraCore Super C_{18}	2.201	2.724
Average value	2.165	2.942
RSD/%	2.08	13.77

AP: andrographolide; DAP: dehydroandrographolide; *RSD*: relative standard deviation.

Table S4 The repeatability results of sample S1.

AP/%	*RSD*/%	DAP/%	*RSD*/%
0.39		0.43	
0.39		0.43	
0.37	1.72	0.41	1.91
0.37		0.41	
0.38		0.42	
0.38		0.42	

AP: andrographolide; DAP: dehydroandrographolide; *RSD*: relative standard deviation.

Table S5 The stability results of sample S1.

Time/h	AP peak area	*RSD*/%	DAP peak area	*RSD*/%
0	108.049		119.997	
4	108.109		116.033	
8	107.356		116.211	
12	108.763	0.41	116.397	1.36
16	108.338		116.608	
20	108.496		116.738	
24	108.200		119.185	

AP: andrographolide; DAP: dehydroandrographolide; *RSD*: relative standard deviation.

Table S6 The content results of current developed method (A) and the Chinese Pharmacopoeia method (B).

NO.	AP/%		DAP/%	
	Method A	Method B	Method A	Method B
1	0.39	0.38	0.43	0.43
2	0.39	0.38	0.43	0.43
3	0.37	0.39	0.41	0.43
4	0.37	0.37	0.41	0.40

5	0.38	0.37	0.42	0.41
6	0.38	0.37	0.42	0.41
Mean ± SD	0.38 ± 0.01	0.38 ± 0.01	0.42 ± 0.01	0.42 ± 0.01

AP: andrographolide; DAP: dehydroandrographolide.

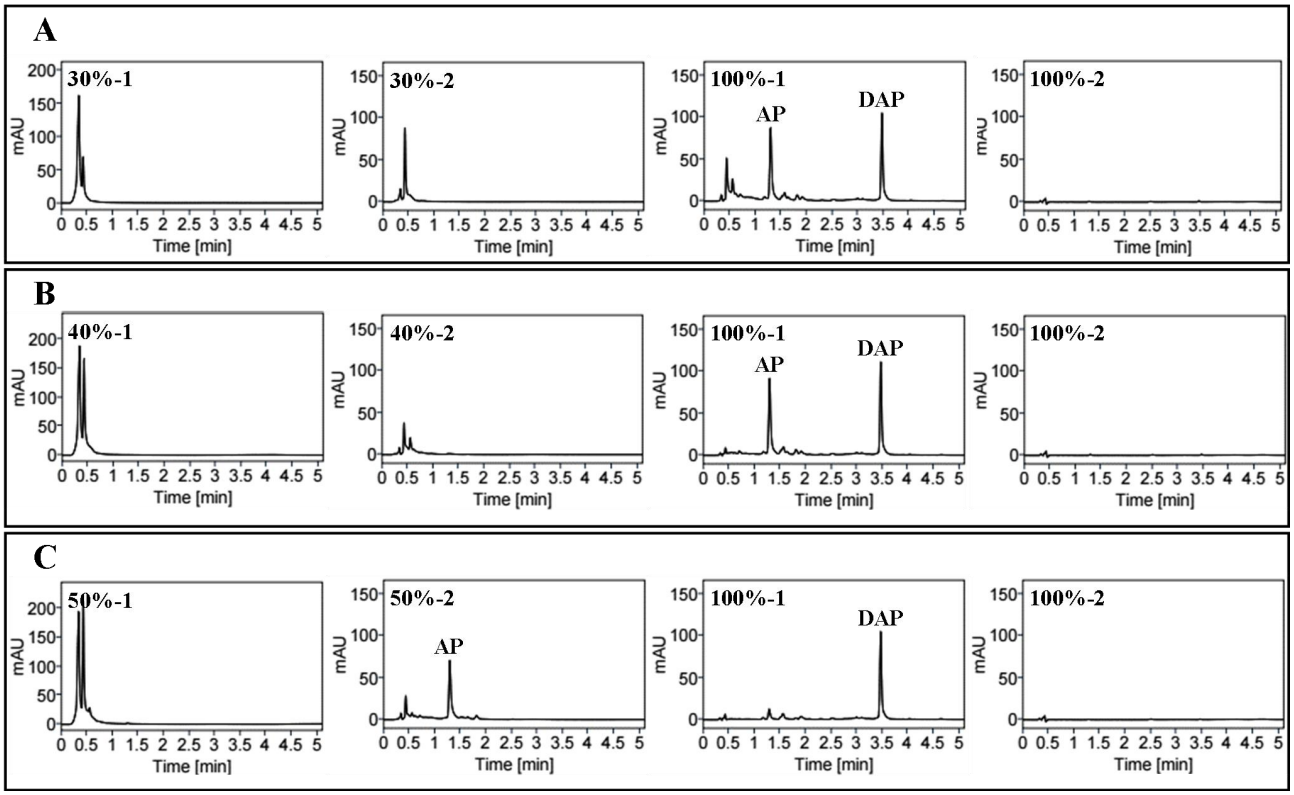
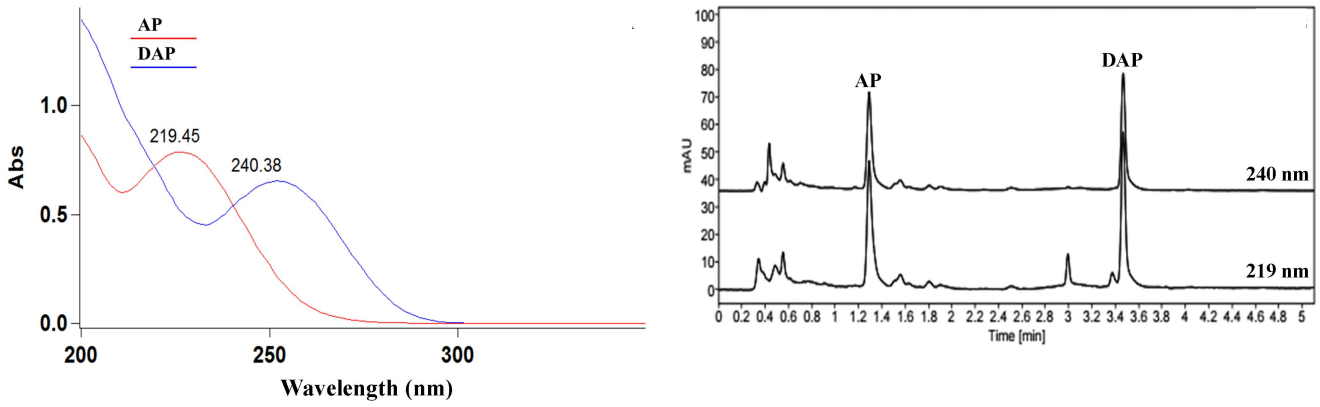


Figure S1 The impacts of methanol at different concentrations on AP and DAP separation efficiency. (AP: andrographolide; DAP: dehydroandrographolide; A: 30% and 100% methanol elution; B: 40% and 100% methanol elution; C: 50% and 100% methanol elution. Elution at each concentration for 2 mL/times, a total of 2 times, i.e. 4 mL.)



(A) (B)

Figure S2 The UV spectrum of AP and DAP (A) and HPLC chromatogram of sample (B). (AP: andrographolide; DAP: dehydroandrographolide.)

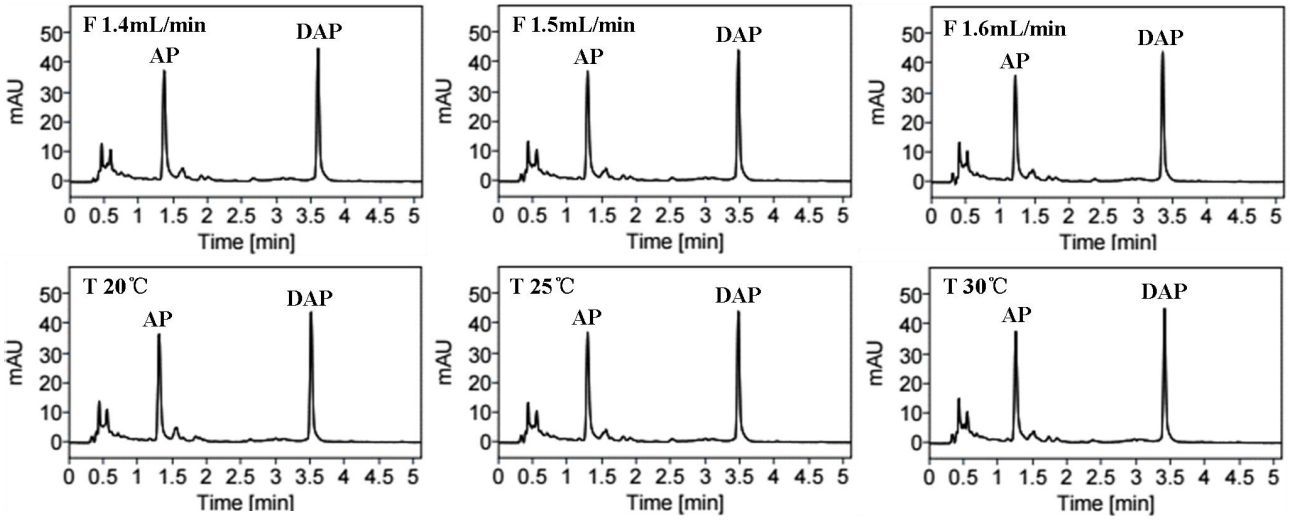


Figure S3 The impacts of different flow rates (F) and temperatures (T) on AP and DAP separation efficiency. (AP: andrographolide; DAP: dehydroandrographolide.)

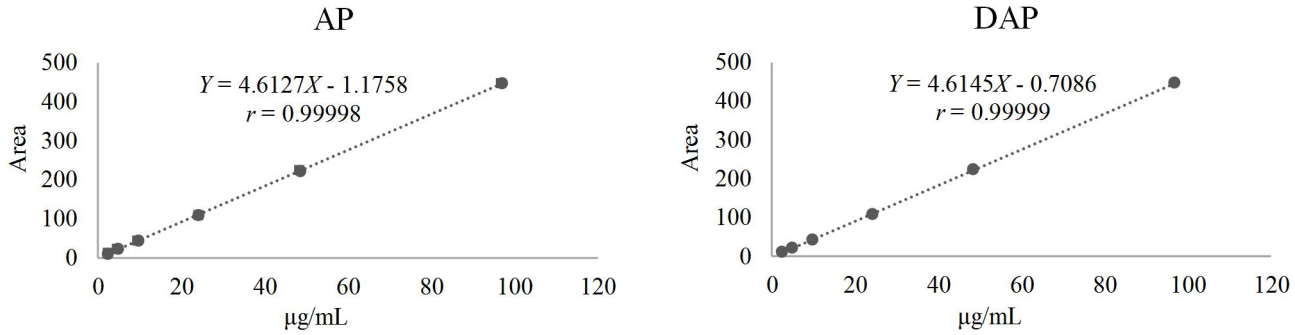


Figure S4 The calibration curves of AP and DAP. (AP: andrographolide; DAP: dehydroandrographolide.)

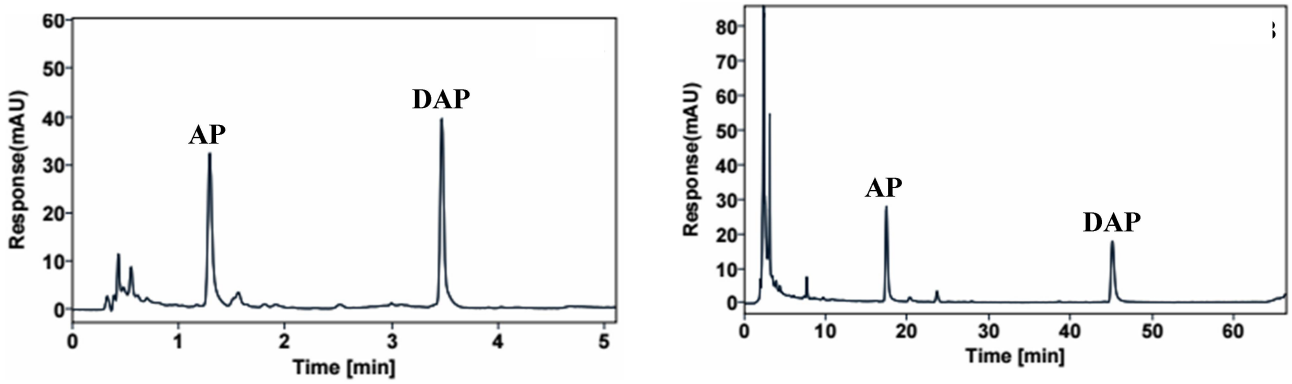


Figure S5 Chromatograms of current developed method (A) and the Chinese Pharmacopoeia method (B). (AP: andrographolide; DAP: dehydroandrographolide.)