Supporting Information for

Reactivity of Biliatresone, a Natural Biliary Toxin, with Glutathione, Histamine, and Amino Acids

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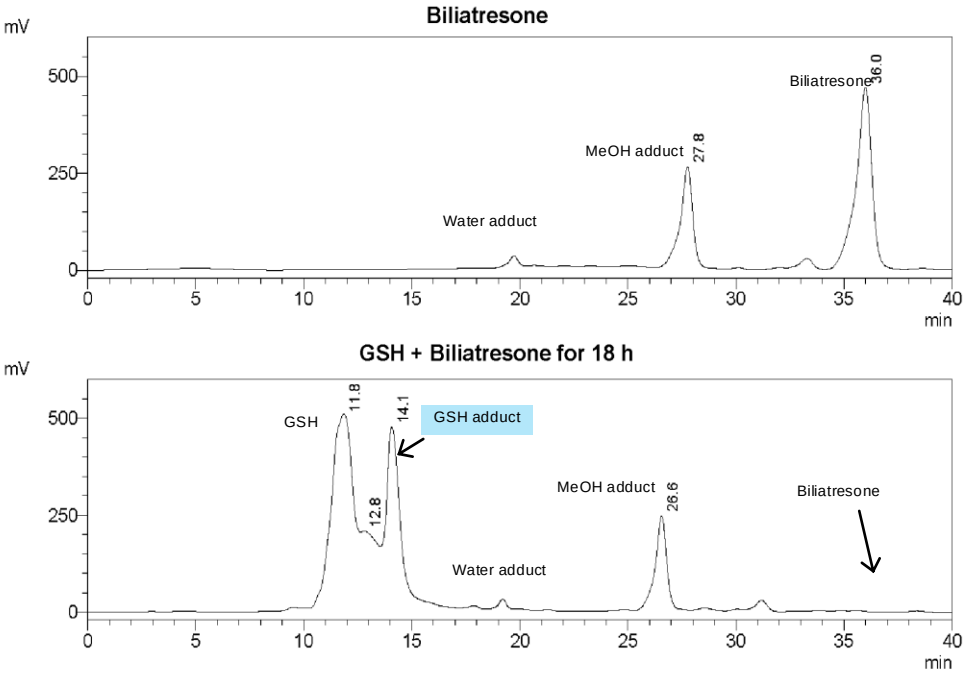

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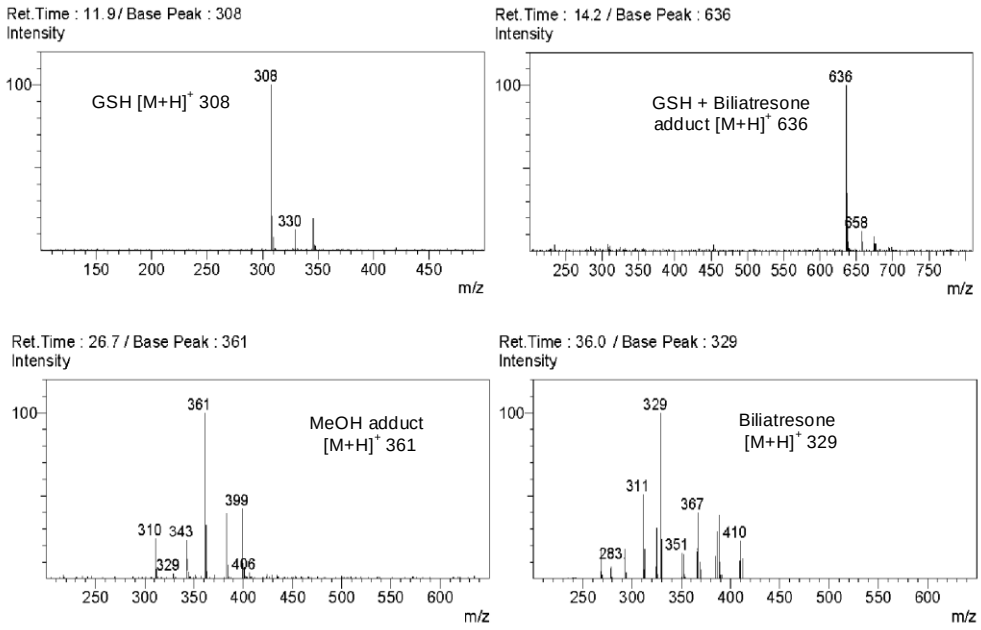
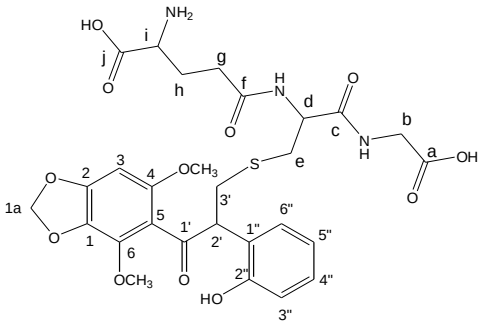


Figure S1. LC-MS (ESI, positive, *m/z*) analysis of the formation of the GSH adduct from a mixture of biliatresone and its MeOH adduct. (A) LC chromatogram monitored at 206 nm; flow rate 0.2 mL/min. (B) MS analysis for each major peak in the LC chromatograms: GSH (t_R 11.8 min), GSH adduct (t_R 14.1 min), MeOH adduct (t_R 26.6 min), and biliatresone (t_R 36.0 min).



Biliatresone Part						Glutathione Part				
#	Group		^{1}H shift (J in Hz)	^{13}C shift (δ in ppm)	HMBC correlations	#	Group	^{1}H shift (J in Hz)	^{13}C shift (δ in ppm)	HMBC correlations
1	C			151.01		a	C=O		172.73	
1a	CH_2		5.86 (d, J=3.2)	101.42	1, 2	b	CH_2	3.94 s	41.13	a
2	C			130.03		c	C=O		171.74	
3	CH		6.32 s	89.10	1. 2, 4, 5	d	CH	4.55 t	55.62	c, e, f
4	C			153.13		e	CH_2	2.88 m	25.42	c, d, 1′
5	C			115.09		f	C=O		174.19	
6	C			140.47		g	CH_2	2.56 m	31.29	h, i, f
1′	C			202.68		h	CH_2	2.15 q	26.16	g, i, j
2′	CH		5.15 (dd, J=7.4, 5, 9)	53.79	1', 3', 1", 2", 6"	i	CH	3.76 t	53.79	g, h, j
3′	CH_2	β	4.14 (dd, J=7.4, 10)	71.95	1', 1"	j	C=O		172.88	
		α	3.61 (dd, J=5.8, 10)							
1″	C			121.62						
2″	C			154.81						
3″	CH		6.79 m	115.09	2", 5"					
4″	CH		7.06 m	128.99	2", 6"					
5″	CH		6.76 m	119.51	1", 3"					
6″	CH		7.03 m	128.46	2", 4"					
	OCH_3		3.64 s	57.74	4					
	OCH_3		3.71 s	59.34	6					

Figure S2. Summary NMR data for the structural elucidation of the GSH adduct of biliatresone in the CD_3OD lock solvent.

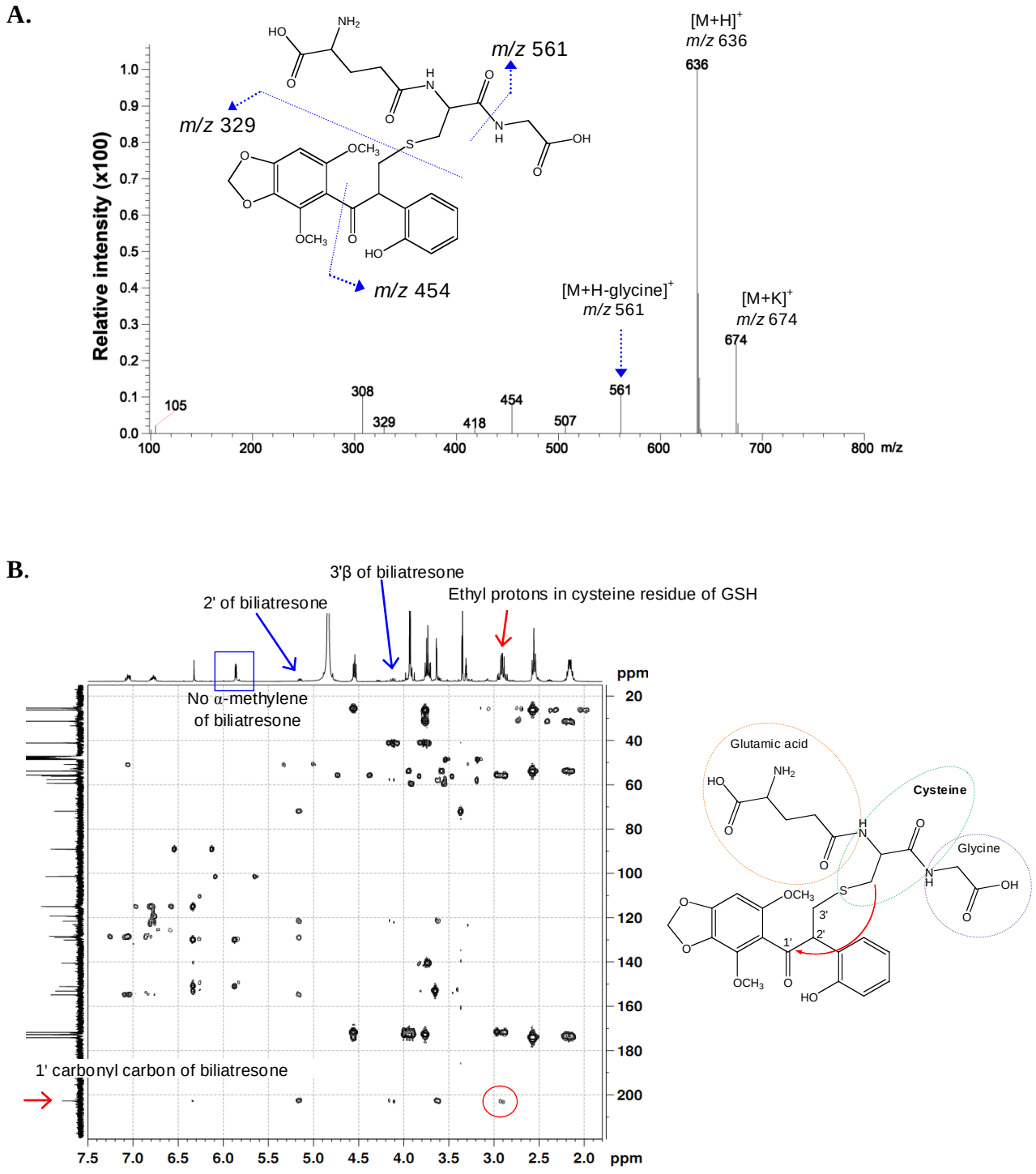


Figure S3. Characterization of the GSH adduct of biliatresone. (A) MS spectrum from the LC-MS (ESI, positive, *m/z*) analysis. (B) 2D NMR HMBC spectrum (100 MHz, CD_3OD) showing the long-range correlation (red circle) between the carbonyl carbon (red arrow) of biliatresone and the ethyl protons (red arrow) in the cysteine residue of the GSH tripeptide.

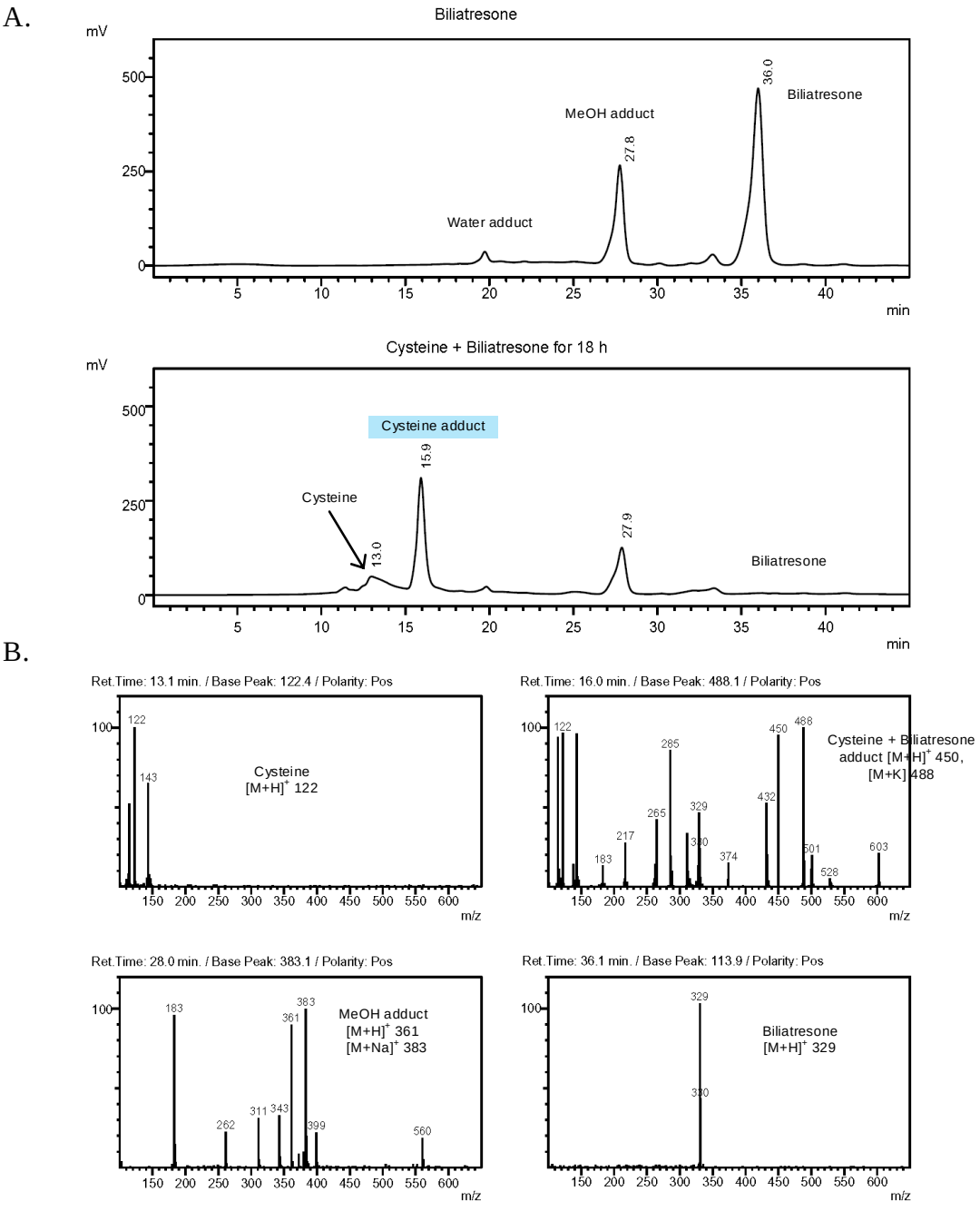


Figure S4. LC-MS (ESI, positive, *m/z*) analysis of the cysteine adduct of biliatresone. (A) LC chromatogram monitored at 206 nm, flow rate 0.2 mL/min. (B) MS analysis for each major peak of the chromatogram: cysteine (t_R 13.1 min), cysteine adduct (t_R 15.9 min), MeOH adduct (t_R 27.8 min), and biliatresone (t_R 36.0 min).

A. B.

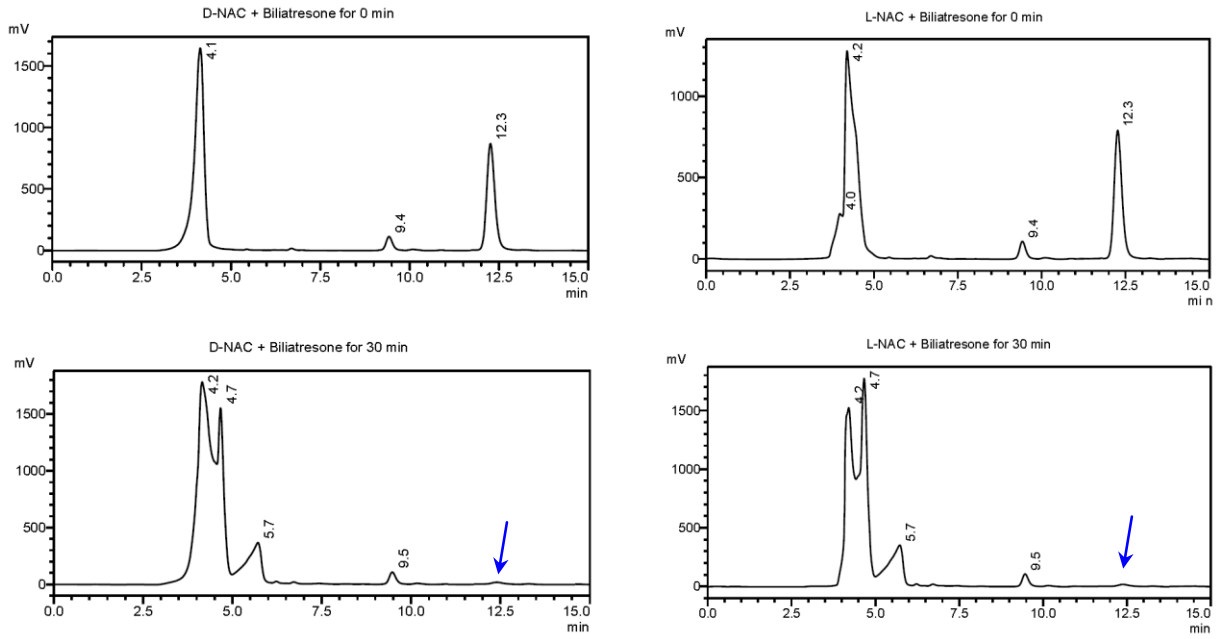


Figure S5. HPLC analyses of the formation of D-NAC and L-NAC adducts of biliatresone. LC chromatogram before and after 30 min of reaction; 206 nm, flow rate 0.5 mL/min. The formation of the D- and L-NAC adducts of biliatresone were complete within 30 min; (A) D-NAC adduct (t_R 4.7 and 5.7 min), MeOH adduct (t_R 9.4-5 min), and biliatresone (t_R 12.3 min) and (B) L-NAC (t_R 4.2 min), L-NAC adduct (t_R 4.7 and 5.7 min), MeOH adduct (t_R 9.4-5 min), and biliatresone (t_R 12.3 min). The blue arrows indicate the biliatresone peaks.

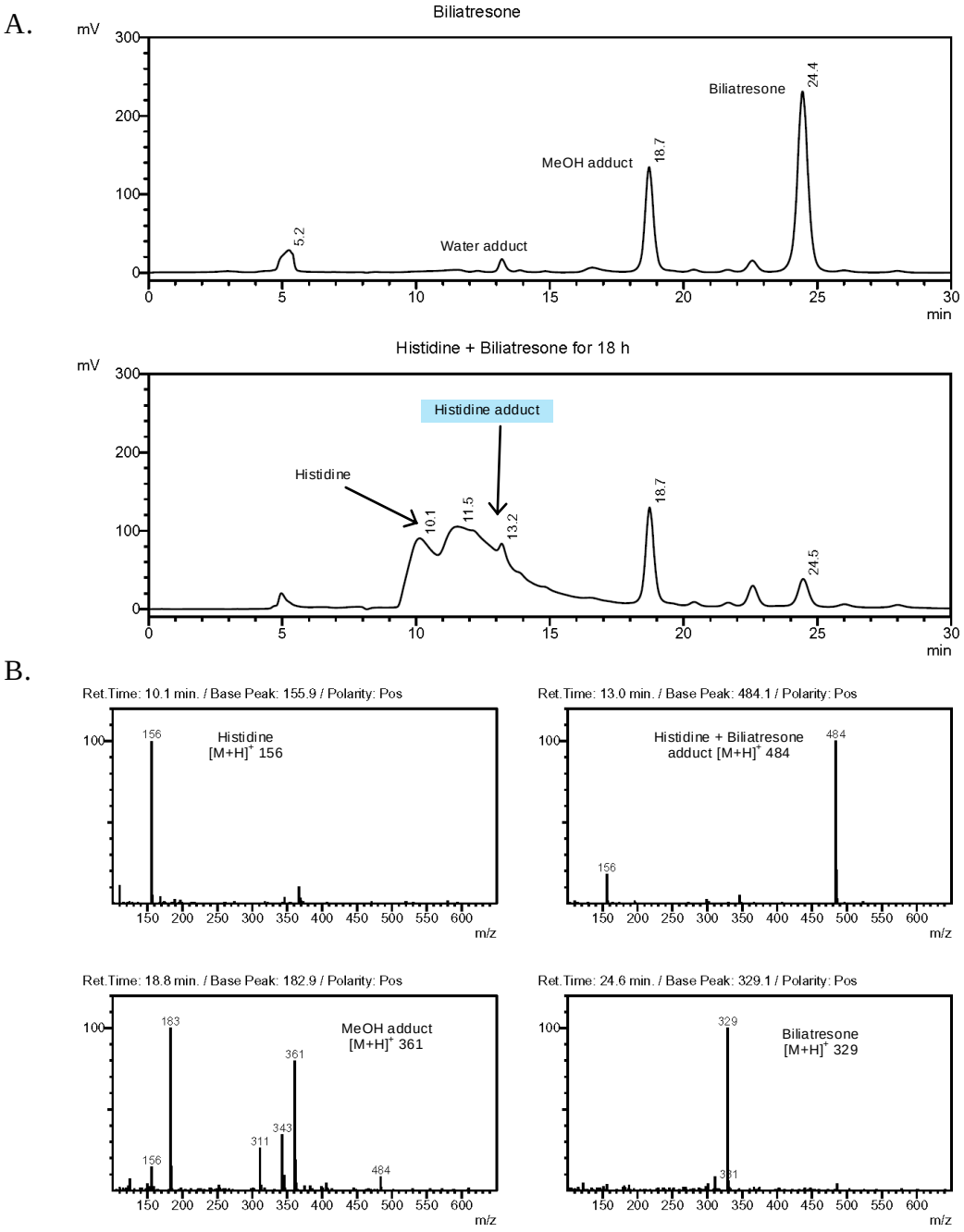
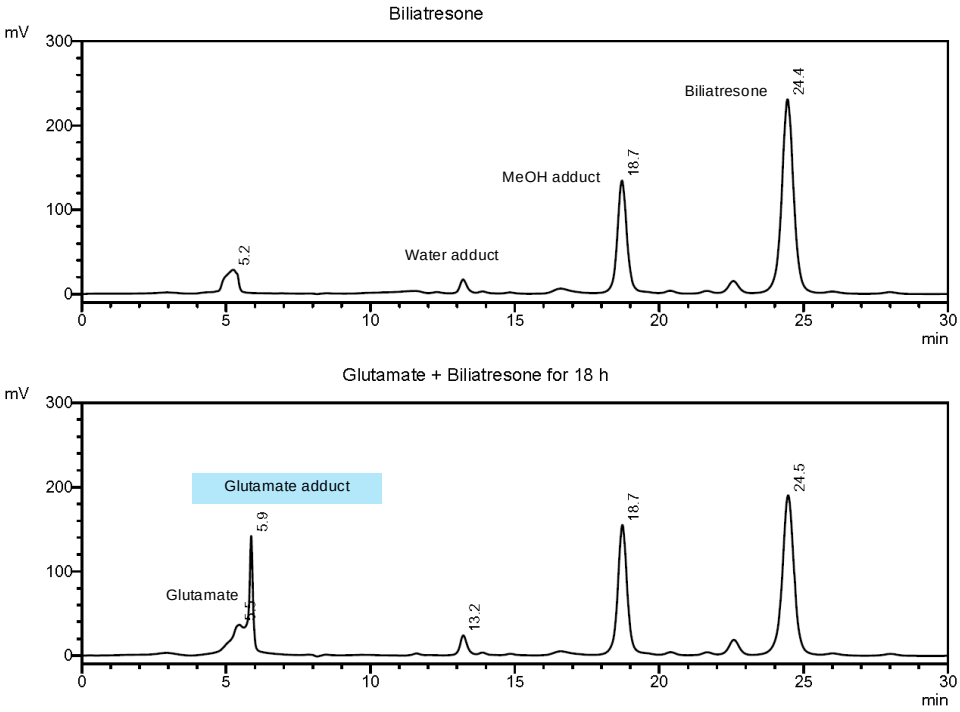


Figure S6. LC-MS (ESI, positive, *m/z)* analysis of the histidine adduct of biliatresone. (A) LC chromatogram of the histidine-biliatresone mixture before and after 18 h of incubation; 206 nm, flow rate 0.3 mL/min. (B) MS analysis for each major peak in the chromatogram: histidine (t_R 10.1 min), histidine adduct (t_R 13.2 min), MeOH adduct (t_R 18.7 min), and biliatresone (t_R 24.5 min).

A.



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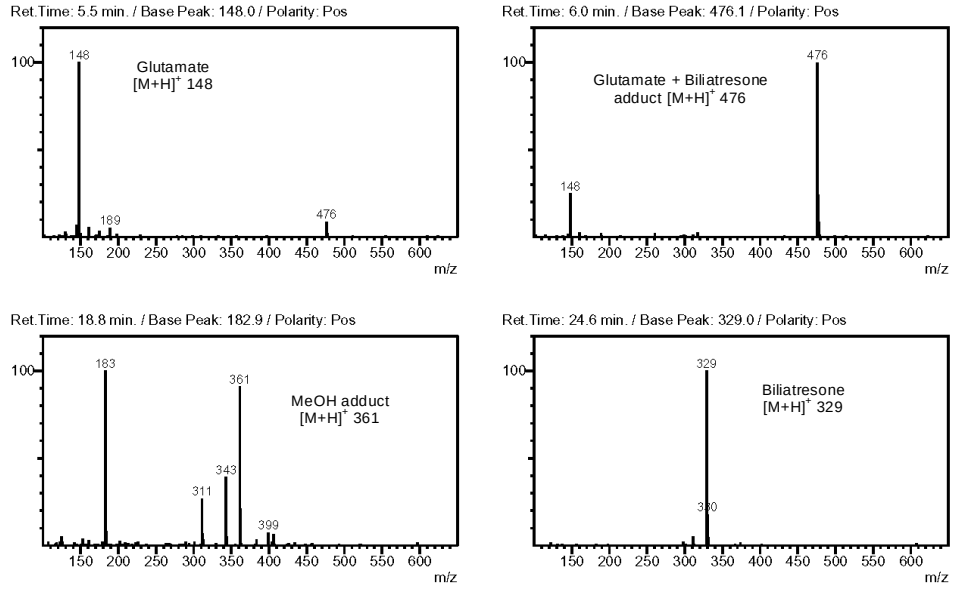


Figure S7. LC-MS (ESI, positive, *m/z*) analysis of the glutamate adduct of biliatresone. (A) LC chromatogram before and after 18 h of reaction; 206 nm, flow rate 0.3 mL/min. (B) MS analysis of each major peak of the chromatogram: glutamate (t_R 5.5 min), glutamate adduct (t_R 5.9 min), MeOH adduct (t_R 18.7 min), and biliatresone (t_R 24.5 min).

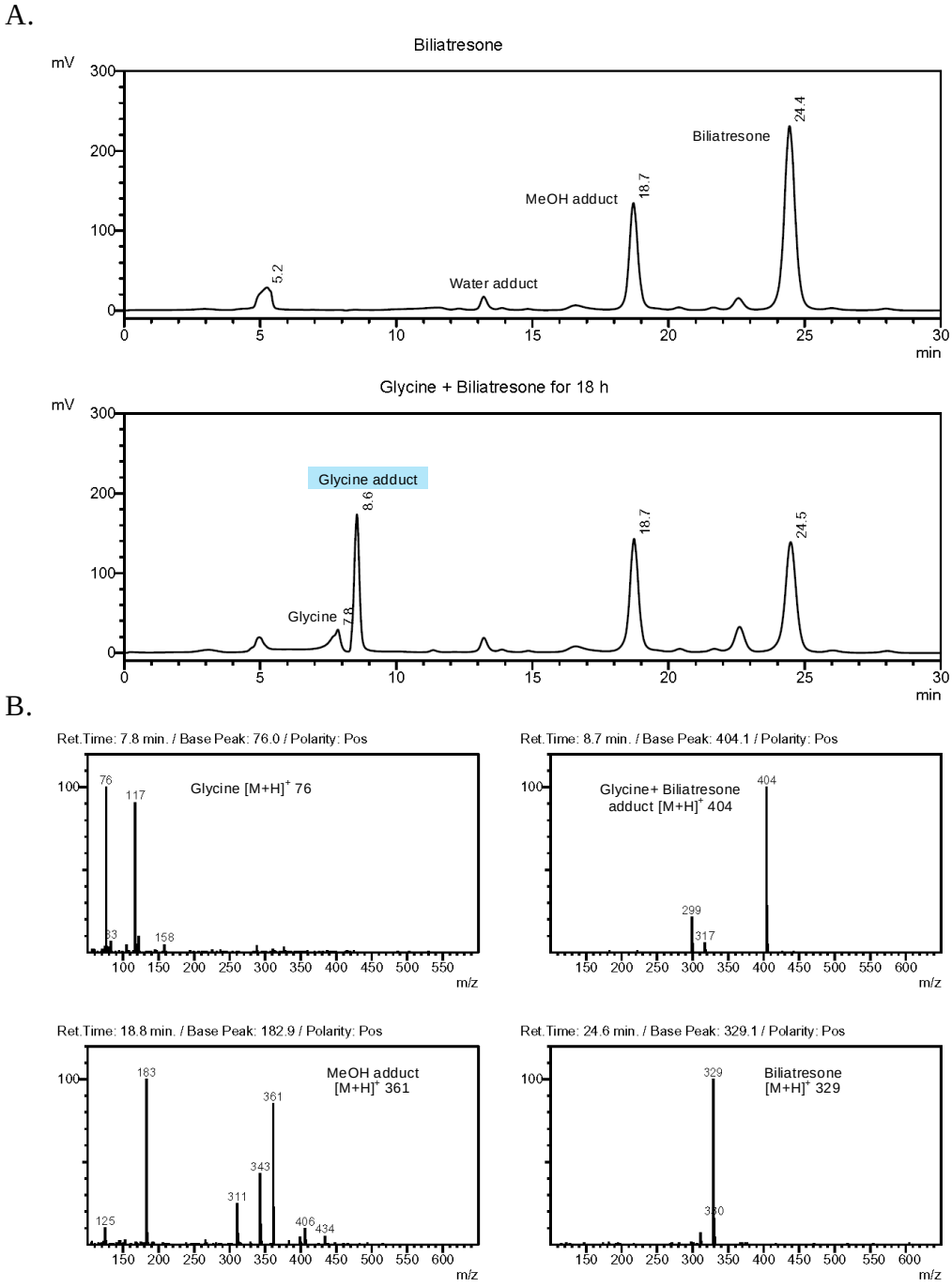


Figure S8. LC-MS (ESI, positive, *m/z*) analysis of the glycine adduct of biliatresone. (A) LC chromatogram before and after 18 h of reaction; 206 nm, flow rate 0.3 mL/min. (B) MS analysis for each major peak of the chromatogram: glycine (t_R 7.8 min), glycine adduct (t_R 8.6 min), MeOH adduct (t_R 18.7 min), and biliatresone (t_R 24.5 min).

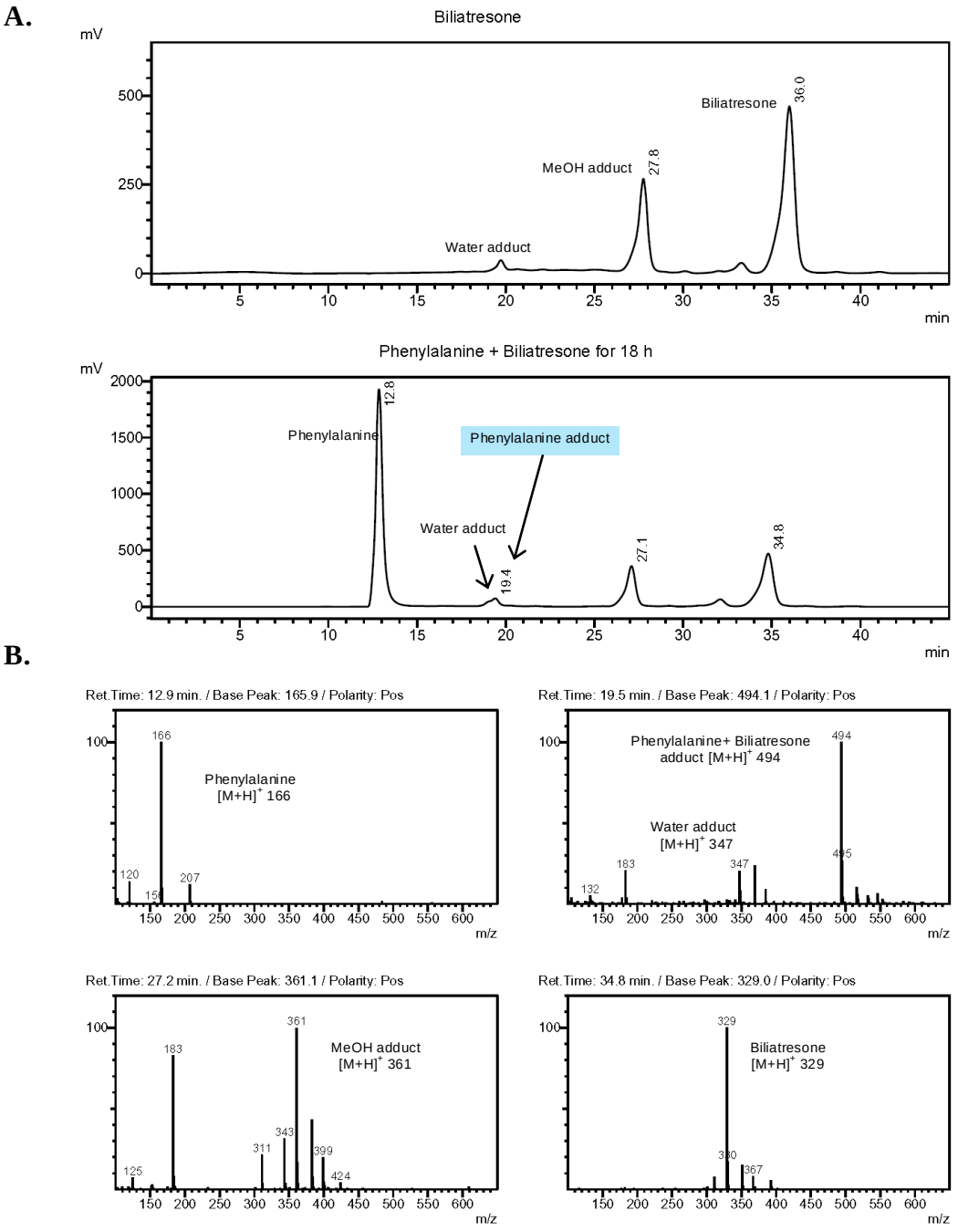
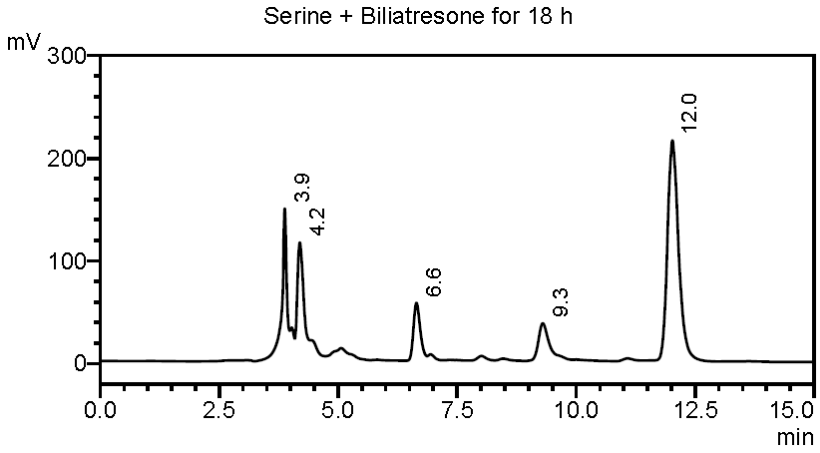


Figure S9. LC-MS (ESI, positive, *m/z*) analysis of the phenylalanine adduct of biliatresone. (A) LC chromatogram before and after 18 h of reaction; 206 nm, flow rate 0.2 mL/min. (B) MS analysis for each major peak of the chromatogram: phenylalanine (t_R 12.8 min), phenylalanine adduct (t_R 19.5 min), MeOH adduct (t_R 27.1 min), and biliatresone (t_R 34.8 min).

A.



B.

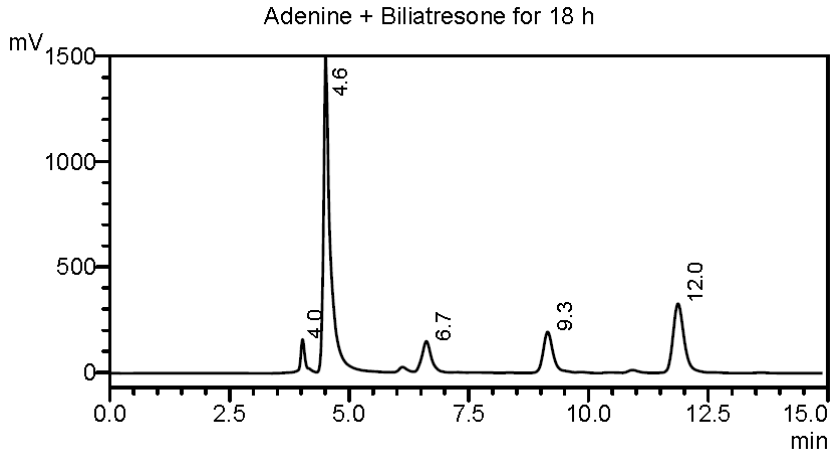


Figure S10. HPLC analyses of the formation of serine and adenine adducts of biliatresone; 206 nm, flow rate 0.5 mL/min. (A) No serine adduct of biliatresone formed in the reaction during 18 h; DL-serine (t_R 3.9 and 4.2 min), water adduct (t_R 6.6 min), MeOH adduct (t_R 9.3 min), and biliatresone (t_R 12.0 min). (B) No adenine adduct of biliatresone formed in the reaction over a period of 18 h; adenine (t_R 4.6 min), water adduct (t_R 6.7 min), MeOH adduct (t_R 9.3 min), and biliatresone (t_R 12.0 min).

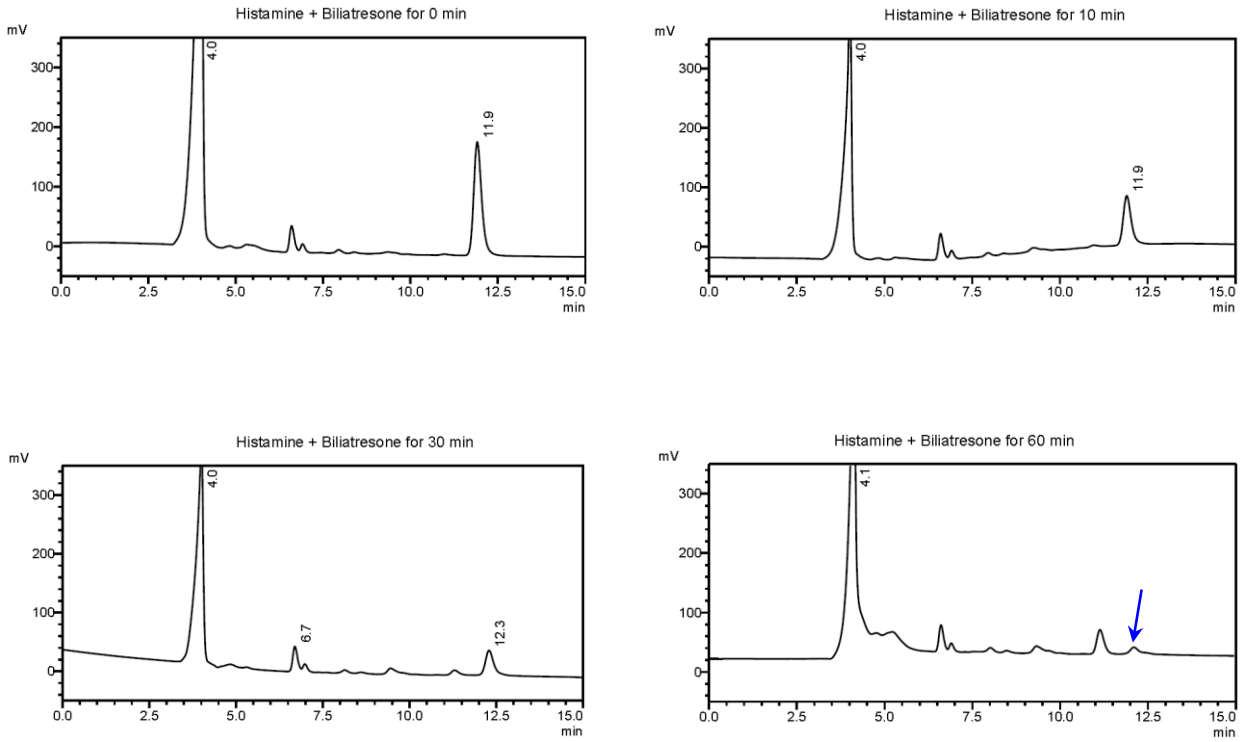


Figure S11. HPLC analyses of the time-dependent conjugation of histamine and biliatresone for 60 min; 206 nm, flow rate 0.5 mL/min. The LC chromatograms were obtained after 0, 10, 20 (not shown), 30 and 60 min of reaction. Histamine adduct (combined with the histamine peak as evidenced by the increasing area of this peak; t_R 4.0 min), water adduct (t_R 6.7 min) and biliatresone (t_R 11.9 min). The blue arrow is the peak of biliatresone.

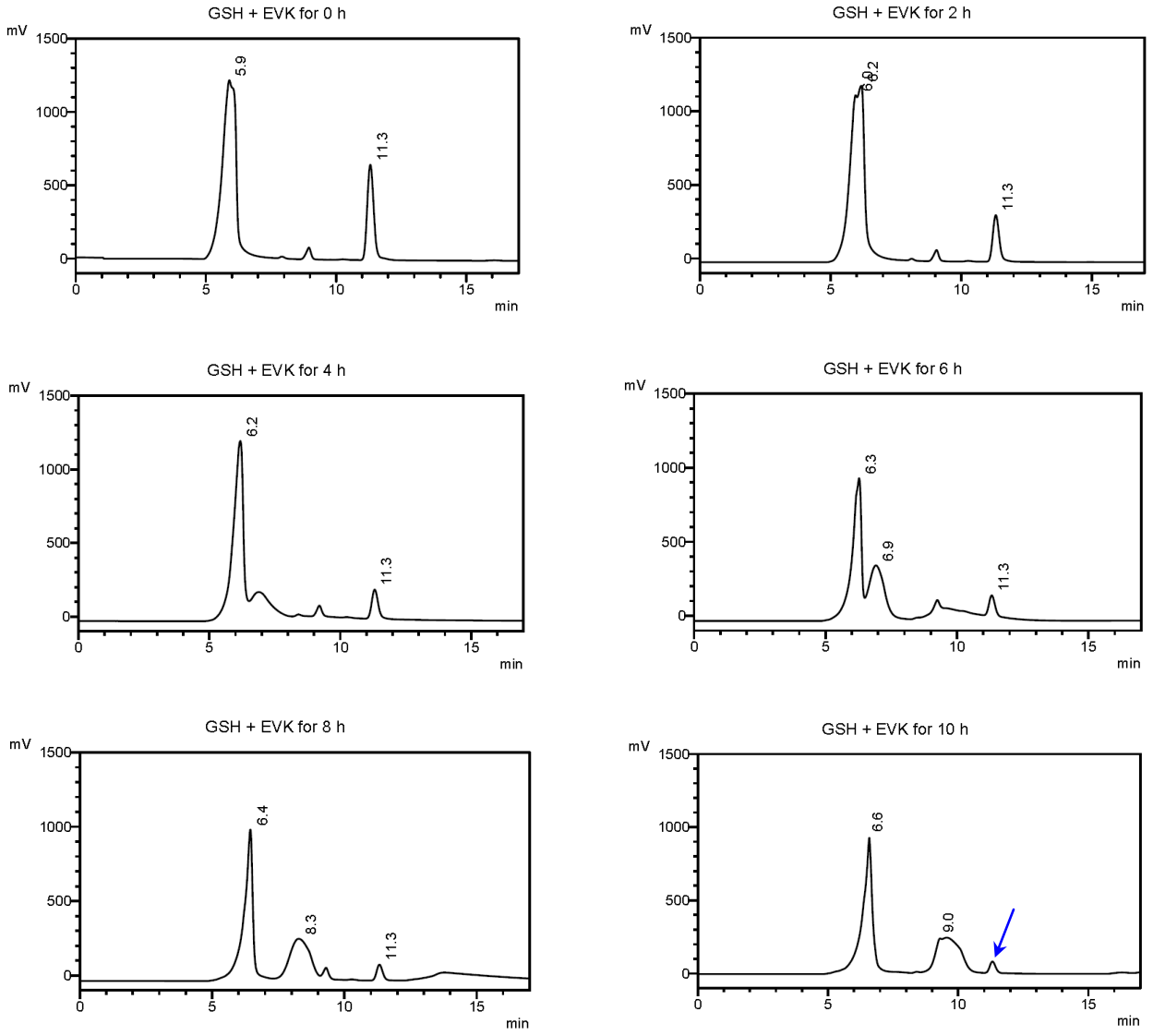


Figure S12. HPLC analysis of the time-dependent conjugation of GSH and ethyl vinyl ketone (EVK; 1-penten-3-one) for 12 h; 206 nm, flow rate 0.3 mL/min. The LC chromatograms were obtained at 1 h reaction intervals. The formation of the EVK adduct of GSH were complete within 11 h; GSH (t_R 5.9-6.6 min), EVK adduct (t_R 6.9-9.0 min), and EVK (t_R 11.3 min). The blue arrows indicate the EVK peak.

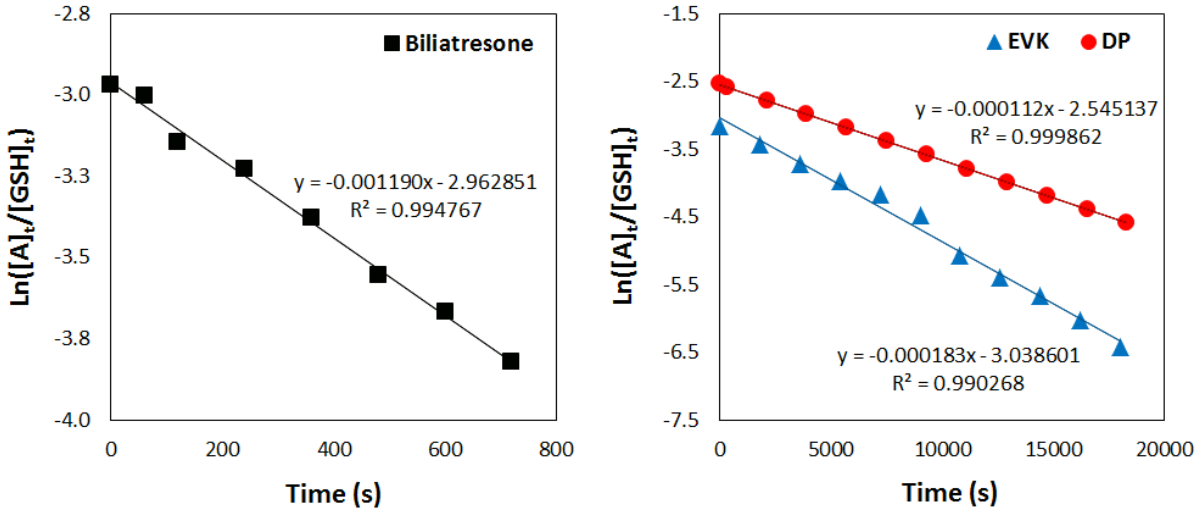


Figure S13. Second-order reaction kinetic plots for the reactivities of biliatresone (black square), DP (red circle), and EVK (blue triangle) toward GSH.