Supplementary information

Stereoselective Pharmacokinetics and Anti-inflammatory Activity of Amygdalin Epimers: Implications for Thermal and pH Stability in Amygdalin -Based Functional Foods

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Table S1 The intra-day and inter-day accuracy and precision, matrix effect and extraction recovery of R-Amy, S-Amy, R-Pru and S-Pru in rat plasma (mean ± SD, n = 6).

Analytes	Concentration (ng/mL)	Matrix effect/%	Extraction recovery/%	Accuracy/%	Precision (RSD%)	
					Intra-day	Inter-day
R-Amy	4.68 (LLOQ)	105.15 ± 16.12	105.09 ± 15.72	102.07 ± 10.39	4.33	4.84
	12.50 (LQC)	108.39 ± 6.28	85.68 ± 2.44	97.39 ± 3.51	1.25	7.54
	3000.00 (MQC)	100.34 ± 3.35	105.45 ± 3.39	97.95 ± 1.67	2.15	2.40
	4500.00 (HQC)	102.38 ± 3.30	105.23 ± 2.93	96.35 ± 1.51	1.23	1.41
S-Amy	4.68 (LLOQ)	102.00 ± 7.51	98.82 ± 9.44	100.00 ± 2.76	9.44	4.33
	12.50 (LQC)	101.39 ± 2.15	98.69 ± 4.94	97.08 ± 2.48	5.24	1.25
	3000.00 (MQC)	101.49 ± 2.26	102.12 ± 2.94	98.69 ± 1.57	1.30	2.15
	4500.00 (HQC)	102.15 ± 3.72	100.32 ± 5.21	97.11 ± 1.15	1.93	1.23
R-Pru	4.68 (LLOQ)	91.89 ± 12.06	101.24 ± 11.38	104.29 ± 6.52	6.25	5.98
	12.50 (LQC)	104.16 ± 8.29	101.36 ± 6.57	102.72 ± 0.96	0.93	4.34
	3000.00 (MQC)	107.27 ± 3.16	103.24 ± 3.37	101.30 ± 3.36	3.32	1.23
	4500.00 (HQC)	102.91 ± 2.73	101.25 ± 4.11	94.53 ± 1.89	2.00	3.01
S-Pru	4.68 (LLOQ)	102.31 ± 6.81	102.23 ± 7.84	97.00 ± 8.85	9.12	8.11
	12.50 (LQC)	101.58 ± 5.78	103.15 ± 1.99	97.61 ± 5.40	5.54	6.19
	3000.00 (MQC)	105.90 ± 1.83	99.71 ± 2.52	101.08 ± 2.40	2.37	0.61
	4500.00 (HQC)	102.48 ± 2.30	100.51 ± 2.73	95.06 ± 2.97	3.12	1.25

Table S2 The stability and dilution effect of R-Amy, S-Amy, R-Pru and S-Pru in rat plasma (mean ± SD, n = 6).

Analytes	Concentration (ng/mL)	Short-term stability (room temperatue for 24h)	Freeze-thaw stability (3 freeze-thaw cycles)	Concentration (ng/mL)	Accuracy/%	RSD%
		Stability (RSD%)		Diluted factors		
R-Amy	12.50 (LQC)	1.55	5.89	1200 (×50)	93.94 ± 2.32	2.47
	4500.00 (HQC)	6.02	2.82	600 (×100)	86.85 ± 1.15	1.32
S-Amy	12.50 (LQC)	4.15	3.01	1200 (×50)	93.12 ± 2.43	2.60
	4500.00 (HQC)	2.11	1.78	600 (×100)	86.39 ± 0.96	1.11
R-Pru	12.50 (LQC)	1.34	6.74	1200 (×50)	95.03 ± 2.27	2.39
	4500.00 (HQC)	5.35	2.80	600 (×100)	88.64 ± 0.81	0.92
S-Pru	12.50 (LQC)	4.78	3.43	1200 (×50)	92.09 ± 1.93	2.10
	4500.00 (HQC)	2.55	2.20	600 (×100)	88.20 ± 3.79	4.29

The profiling and identification of S-Amy were performed using a Triple TOF™ 5600+ system (AB Sciex, CA, USA) coupled to a Shimadzu 30A UHPLC system (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B) delivered at a flow rate of 0.3 mL/min under the following gradient program: 5% B (0–2 min), 5–10% B (2–11 min), 10–95% B (11–13 min), 95% B (13–15 min), 95–5% B (15–15.1 min), and 5% B (15.1–17 min). The column temperature was maintained at 45 °C, and the injection volume was 5 μL. Mass spectrometry was performed on the Triple TOF™ 5600+ system operating in both positive and negative ESI modes. The capillary voltage was set to 5.5 kV in positive mode, with a mass scan range of m/z 100–1200. The turbo spray temperature (TEM) was 500 °C. The nebulizer gas (GS1) and heater gas (GS2) flow rates were both 55 psi, and the curtain gas was 35 psi. The collision energy (CE) was set to 25 eV, with a collision energy spread (CES) of 15 eV.

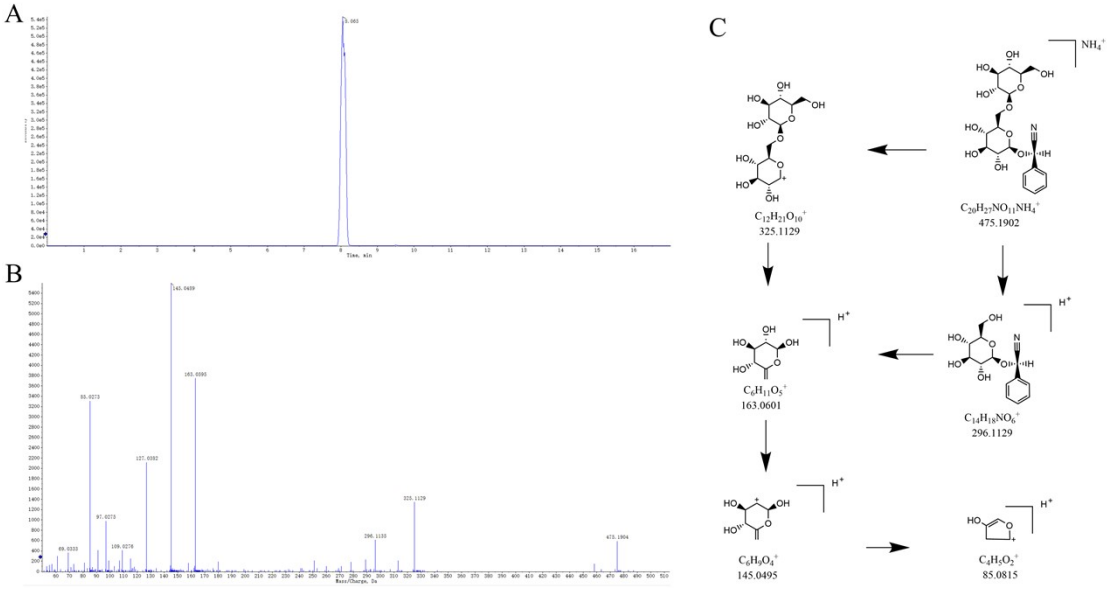

Fig. S1 UPLC-Q-TOF-MS chromatograms of S-Amy.

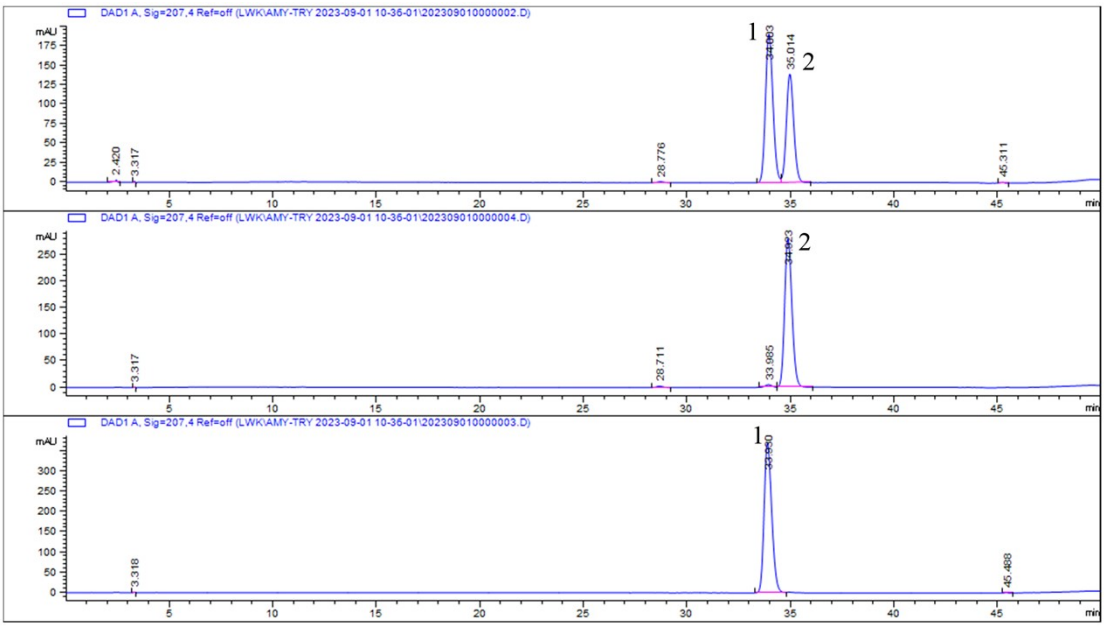

Fig. S2 HPLC-DAD chromatograms of R-Amy and S-Amy (1. S-Amy, 2. R-Amy).

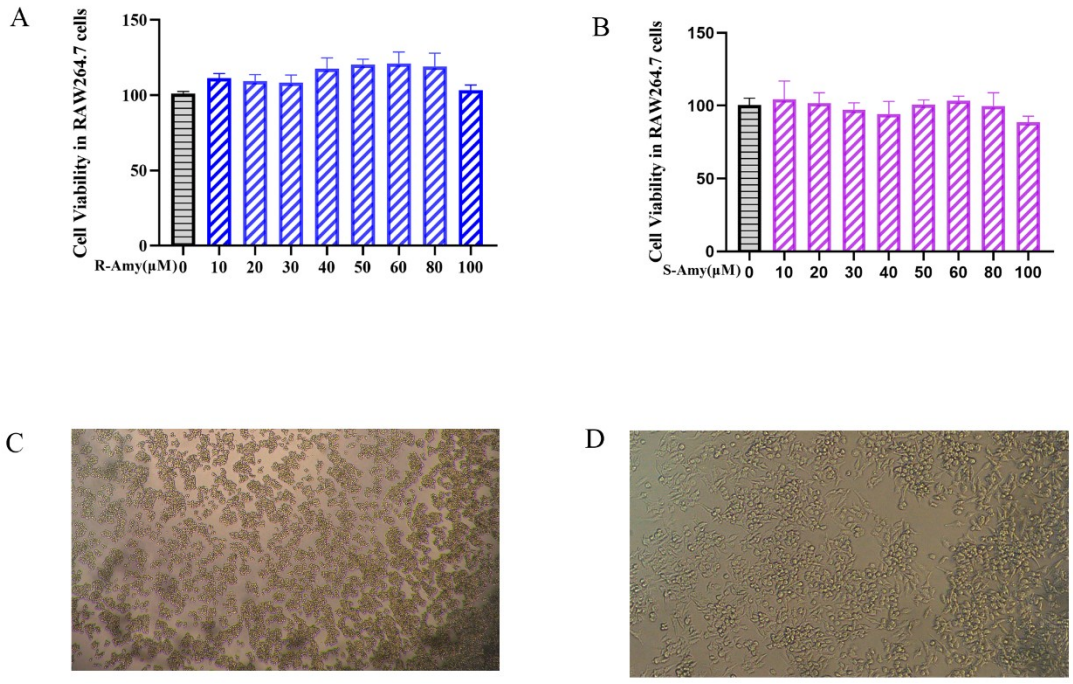

Fig. S3 The effects of R/S-Amy on RAW264.7 cells. (A-B) The CCK-8 of R/S-Amy on RAW264.7 cells. (C-D) LPS-induced morphological changes in RAW264.7 cells（C. Nomral; D.LPS）.

Single-column of the UHPLC-MS/MS condition

MS/MS on a Sciex ExionLC AD UHPLC system (SCIEX, USA) linked to an AB Sciex QTRAP 4500 triple quadrupole mass spectrometer (SCIEX, USA) in negative ion mode, Amy epimers were detected using a an Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm) at 40°C, with a flow rate of

0.5 mL/min and an injection volume of 2 μL. The mobile phase was a gradient system consisting of 0.1% formic acid in water (A) and methanol (B), with the following gradient: 5% B (0 – 2.0 min), 5 - 8% B (2.0 - 7.0 min), 8 - 10% B (7.0 - 14.0 min), 10% B (14.0 - 19.0 min), 10 - 95% B (19.0 – 21.0 min), 95% B (21.0 – 23.0 min), 95 - 5% B (23.0 – 23.1 min), and 5% B (23.1 – 25.0 min).

ESI-MS/MS negative mode conditions were set as follows: capillary voltage, 5.5 kV; turbo spray temperature, 550℃; GS1, 50 psi; GS2, 50 psi; CUR, 35 psi. The optimized MRM parameters were established as follows: D/S-Amy: collision energy (CE) = -30 eV, declustering potential (DP) = -52 V; D/S-Pru: CE = -20 eV, DP = -40 V; internal standard (IS, pNPG): CE = -24 eV, DP = -66 V. The selected precursor-to-product ion transitions were m/z 502.00→323.00 (D/S-Amy), m/z 340.00→160.90 (D/S-Pru).

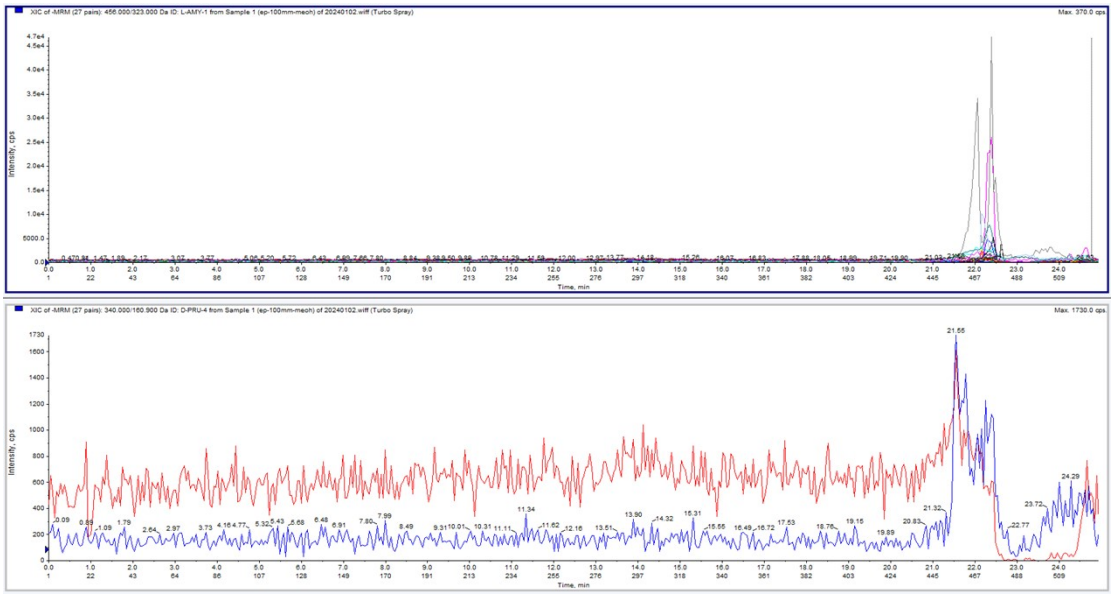

Fig. S4 Blank plasma sample. (A. total ion chromatogram, B. extracted ion chromatogram)

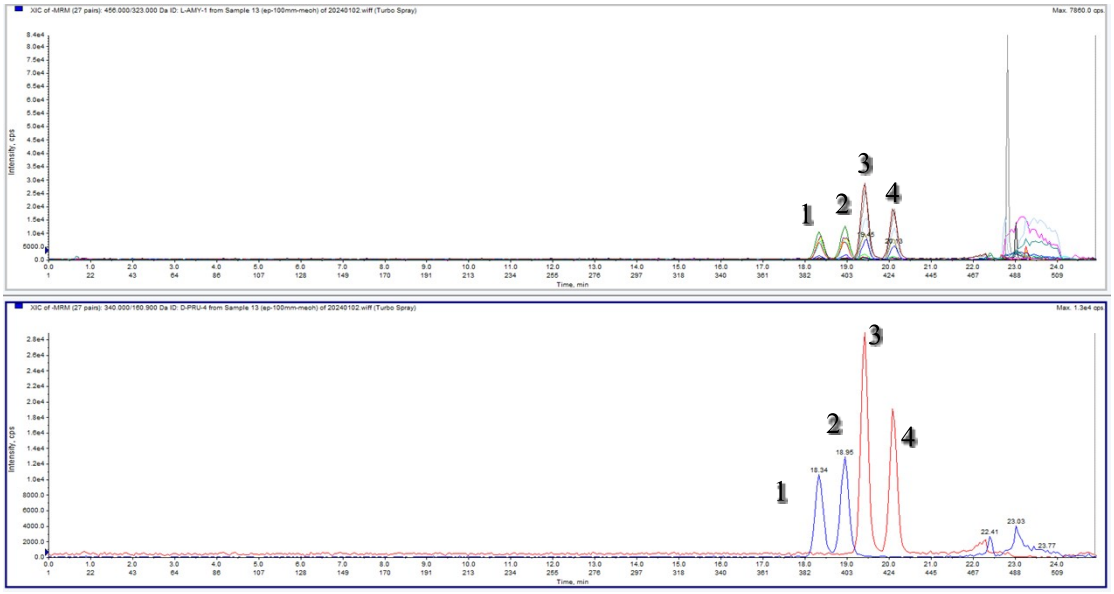

Fig. S5 Representative multiple reaction monitoring chromatograms in blank plasma sample spiked with R-Amy, S-Amy, R-Pru and S-Pru. (A. total ion chromatogram, B. extracted ion chromatogram, 1. S-Amy, 2. R-Amy, 3. R-Pru, 4. S-Pru)