

Supplementary information

Simultaneous Qualitative and Quantitative Analysis of Tyloxapol in Tobramycin Eye Drops by LC-Q-TOF and HPLC-ELSD

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Table S1 The m/z ranges of tyloxapol at three retention times

	9.5min (m/z)	15.48-15.58min (m/z)	33.6-34.0 min (m/z)		
Tyloxapol Reference Standard	488.3595- 840.5698 Z=1	560.3988- 802.543 Z=2	970.6837- 1146.7889 Z=1	630.4244- 821.2045 Z=3	912.1282- 1088.2328 Z=2
Tobramycin Eye Drops	488.359- 840.569 Z=1	560.3983- 802.5424 Z=2	970.6832- 1146.7876 Z=1	630.4242- 821.2042 Z=3	912.1276- 1088.2323 Z=2
Tyloxapol 03	488.359- 840.5692 Z=1	560.3984- 802.5426 Z=2	970.683- 1146.7879 Z=1	630.424- 821.2045 Z=3	912.1277- 1088.2322 Z=2
Tyloxapol 01	444.3327- 708.4898 Z=1	538.3851- 714.4897 Z=2	926.6564- 1146.7874 Z=1	615.7476- 762.5021 Z=3	890.1144- 1044.2032 Z=2
Tyloxapol 02	444.3329- 708.4906 Z=1	538.3854- 714.4901 Z=2	926.6571- 1146.7881 Z=1	615.7487- 762.5022 Z=3	890.1144- 1044.2054 Z=2

Table S2 Isotope peak m/z

	9.5min	Peak 1 at about 15 min	Peak 2 at about 15 min	Peak 1 at about 33 min	Peak 2 at about 33 min
Tyloxapol sample 03	664.465	670.464	1014.7095	718.1417	977.6653
	665.4676	670.9656	1015.713	718.4764	978.1668
Eye drops	664.4647	670.4638	1014.7095	718.1419	977.6654
	665.4675	670.9655	1015.7128	718.4763	978.167

Table S3 ELSD Parameter Optimization Results

Parameter settings		Peak area	S/N
Gas flow rate(mL/min)	2	18545.375	259.8526
	2.4	18340.265	247.5538
	2.8	15207.451	178.1449
	3.2	15099.615	606.8205
	3.6	11501.717	377.0122
	4	10506.28	125.3302
Drift tube temperature(°C)	90	8963.199	1217.2496
	95	10029.05	3039.6778
	100	10061.896	881.37243
	105	11745.428	1421.3634
	110	12586.887	536.04533
	115	15099.615	1606.82055
	120	14341.154	501.8270

Table S4 LinearityTable

Linearity level	Concentration in µg/mL	Peak Area
L1	243.9000	4108.097
L2	390.2400	9283.716
L3	487.8000	14015.772
L4	585.3600	18556.875
L5	731.7000	25677.050
regression equation	y=44.67316x-7463.2640	
regression coefficient	0.9954	

Table S5 Stability

	Time (h)	Peak area	Measured concentration(µg/mL)	Rate of change (%)
System	0	14145.117	501.9046	/
Suitability Solution	76	13244.336	498.8454	-0.6

Eye drop	0	13798.100	494.5594	/
sample	69	14096.702	517.5872	4.7
solution				

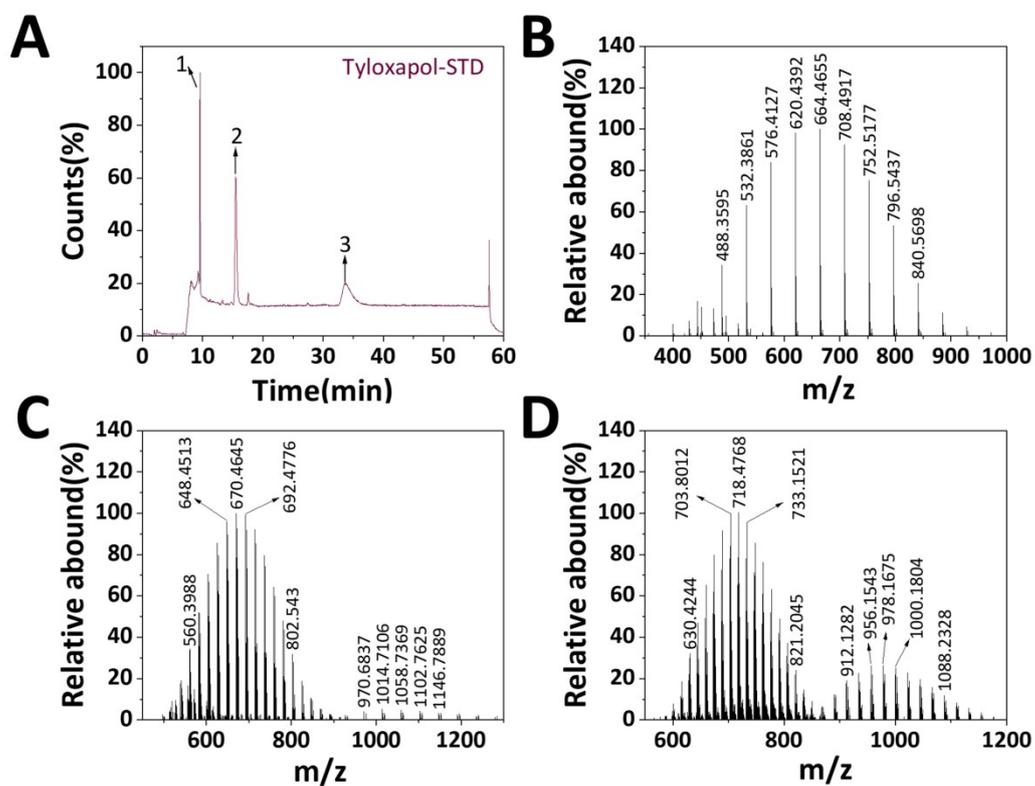


Fig. S1 (A) Extracted ion chromatogram (EIC) of tyloxapol reference standard; (B) Mass spectrum of peak 1; (C) Mass spectrum of peak 2; (D) Mass spectrum of peak 3.

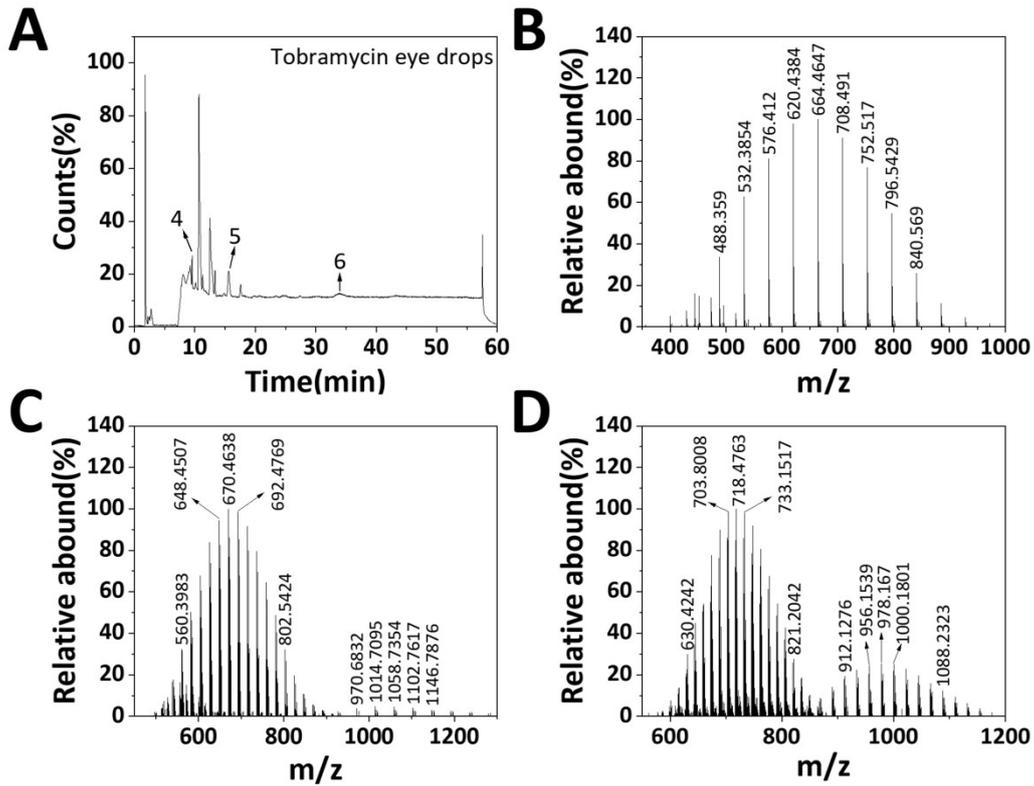


Fig. S2 (A) Extracted ion chromatogram (EIC) of Eye Drops ; (B) Mass spectrum of peak 4; (C) Mass spectrum of peak 5; (D) Mass spectrum of peak 6.

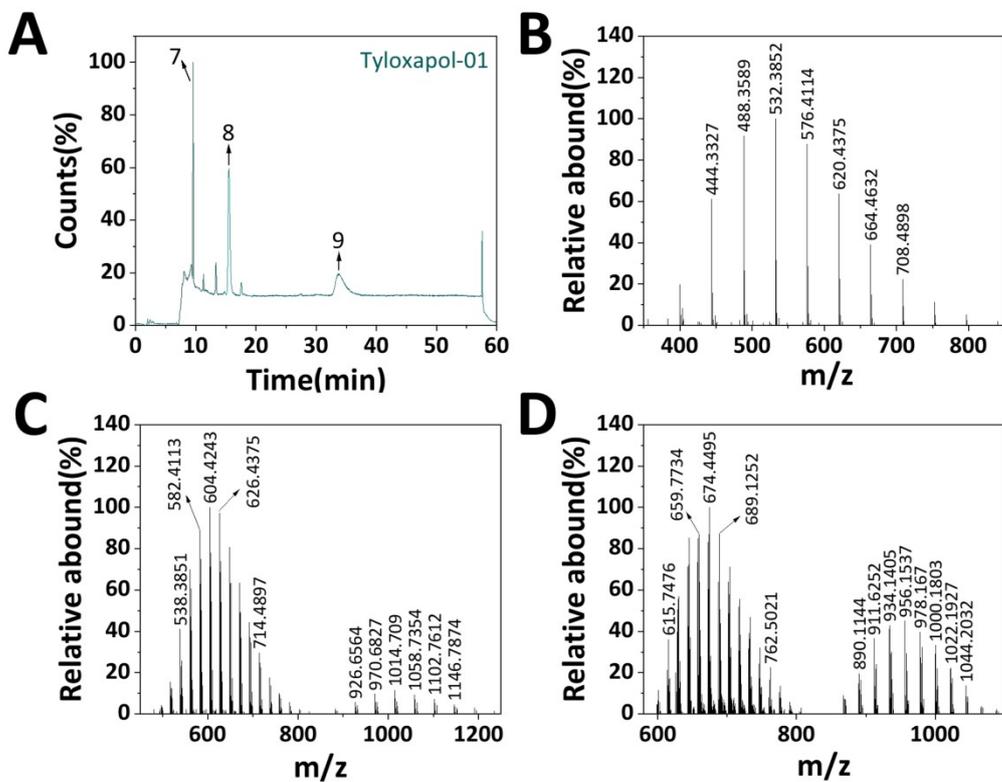


Fig. S3 (A) Extracted ion chromatogram (EIC) of Tyloxapol Sample 01; (B) Mass

spectrum of peak 7; (C) Mass spectrum of peak 8; (D) Mass spectrum of peak 9.

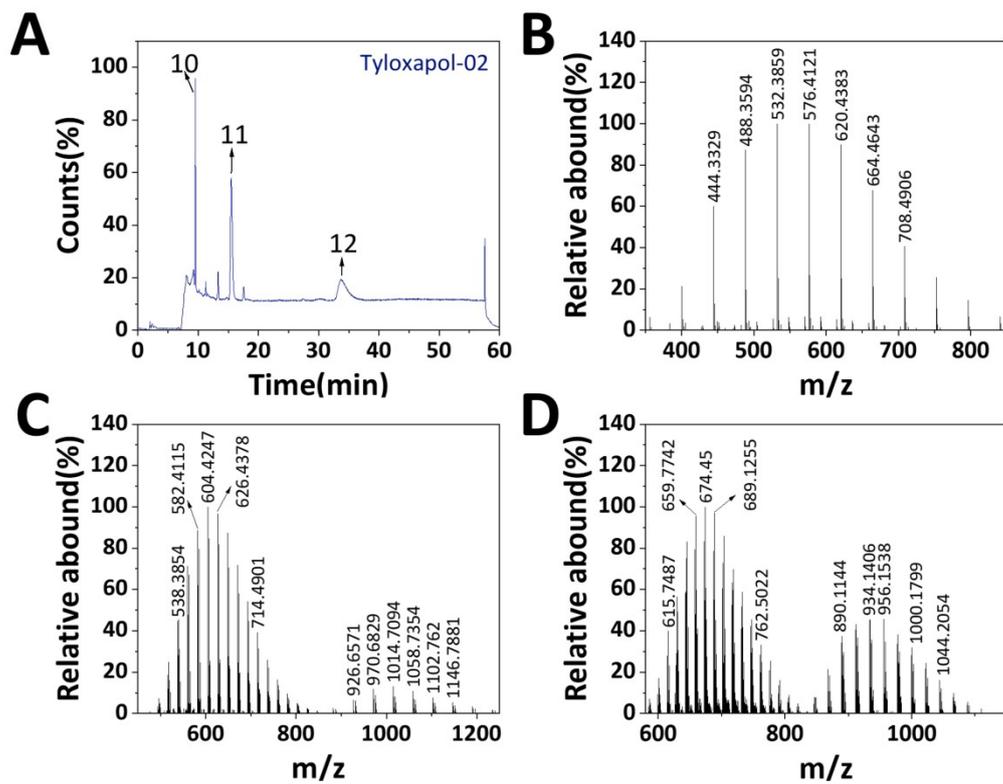


Fig. S4 (A) Extracted ion chromatogram (EIC) of Tyloxapol Sample 02; (B) Mass spectrum of peak 10; (C) Mass spectrum of peak 11; (D) Mass spectrum of peak 12.

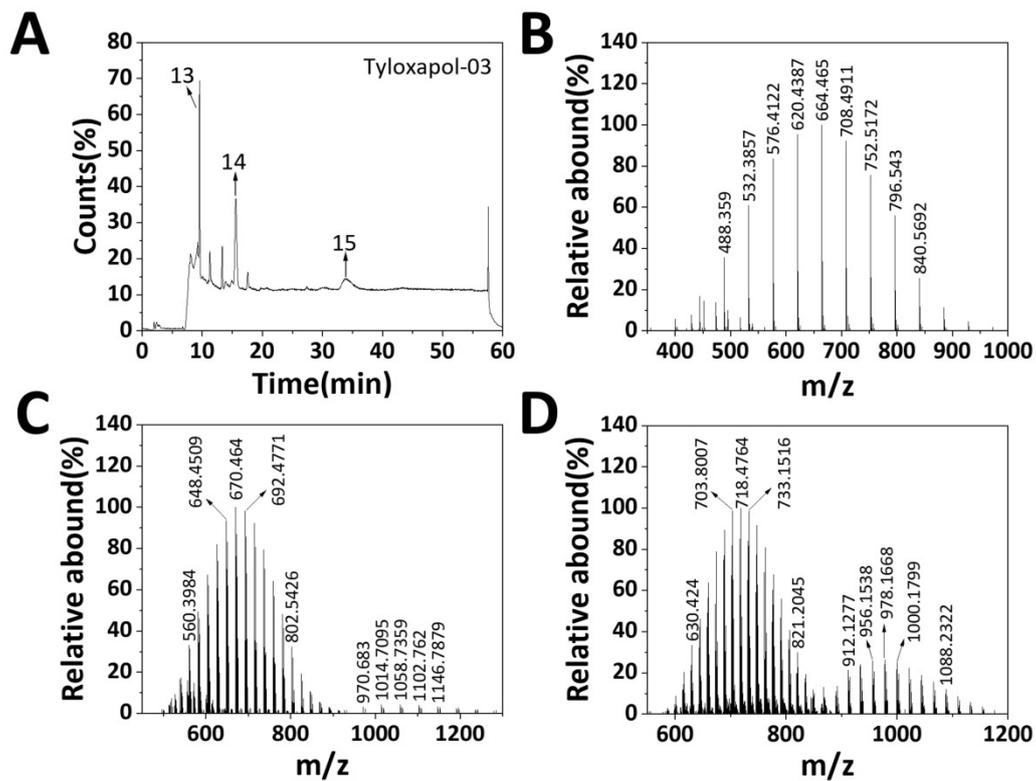


Fig. S5 (A) Extracted ion chromatogram (EIC) of Tyloxapol Sample 03; (B) Mass spectrum of peak 13; (C) Mass spectrum of peak 14; (D) Mass spectrum of peak 15.

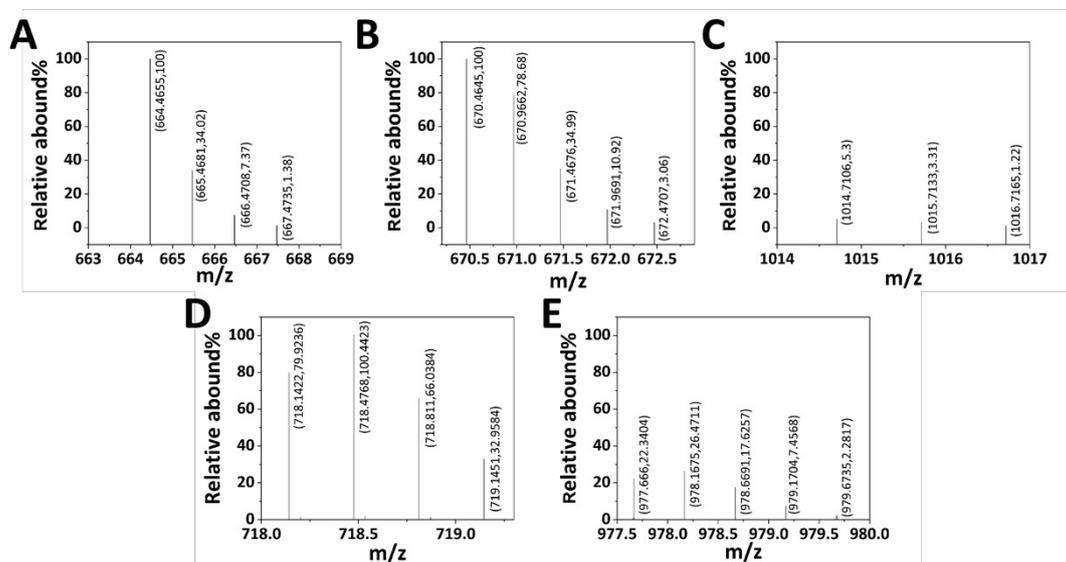


Fig. S6. Isotopic distributions of the reference substance. (A) Isotopic pattern of the highest peak at approximately 9 min; (B) Isotopic pattern of the highest peak of the first cluster at 15 min; (C) Isotopic pattern of the highest peak of the second cluster at 15 min; (D) Isotopic pattern of the highest peak of the second cluster at 33 min; (E) Isotopic pattern of the highest peak of the second cluster at 33 min.

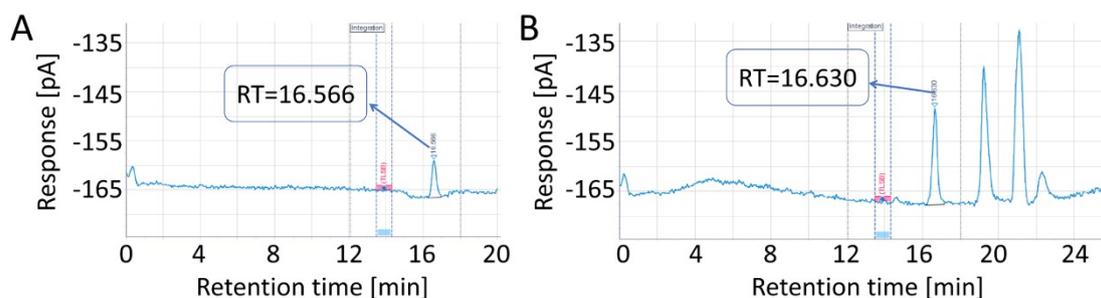


Fig. S7 Experimental study was conducted using a BioPulite Protein Perfect SEC column (7.8 mm × 30 cm): (A) Tyloxapol stock solution I had a retention time of 16.566 min and a tailing factor of 1.3. (B) Tobramycin eye drop solution had a retention time of 16.630 min and a tailing factor (T) of 1.1.

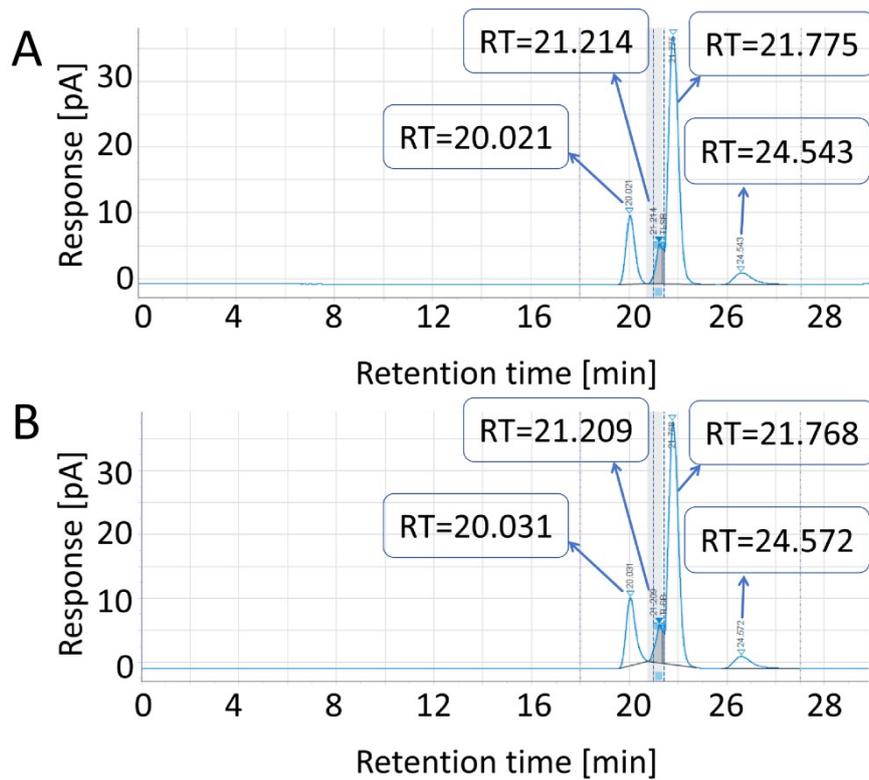


Fig. S8 Experimental study conducted using a TSKgel G4000PWXL column (7.8 mm × 30 cm): (A) Tyloxapol blank excipient had a retention time of 21.214 min and a tailing factor (T) of 0.7. (B) Tyloxapol spiked blank excipient had a retention time of 21.209 min and a tailing factor (T) of 0.7.

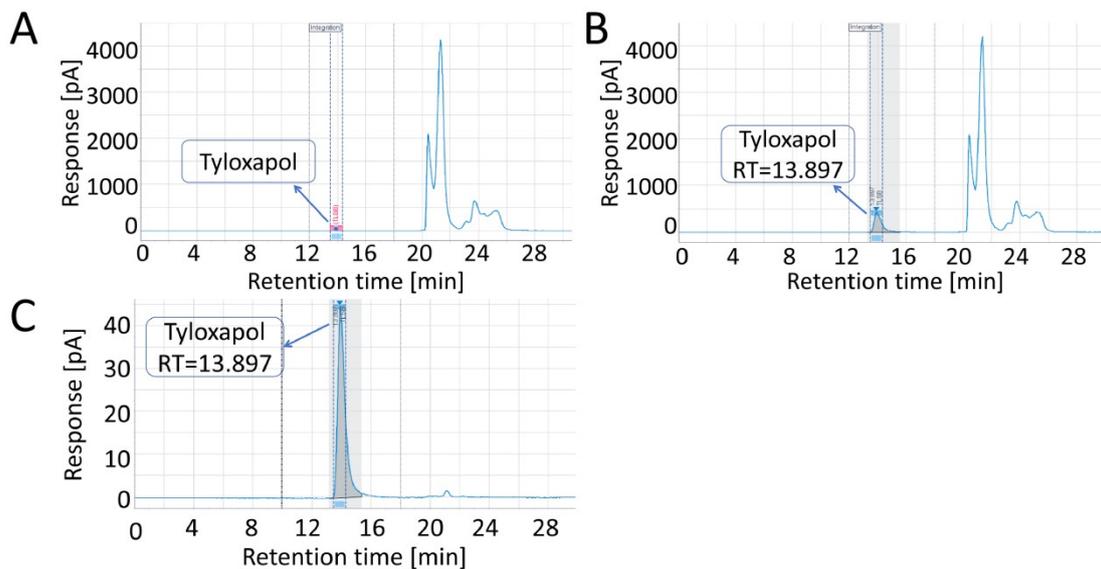


Fig. S9. Experimental test conducted using a TSKgel G2000PWXL column (7.8 mm × 30 cm): (A) Tyloxapol blank excipient solution; (B) The peak area at 13.962 min in the spiked blank excipient solution was 14285.376 pA, with a tailing factor of 1.50578. (C)

The peak area at 13.968 min in the reference solution was 14123.224 pA, with a tailing factor of 1.49.

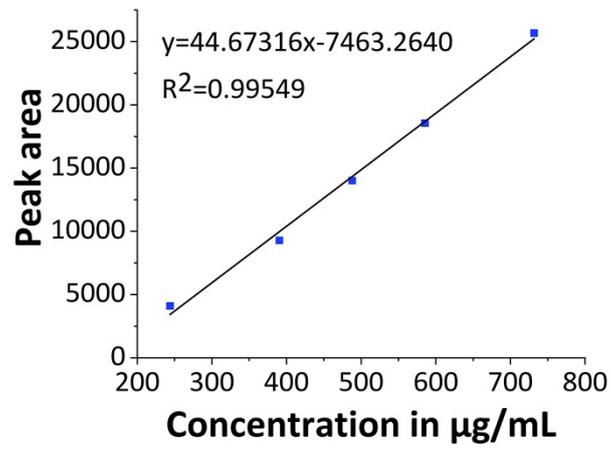


Fig. S10 Linear Plot of Tyloxapol