Supplementary materials

Quantitative determination of maslinic acid and oleanolic acid in olive pomace using high-speed shear extraction and liquid chromatography

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1. Method validation

1.1 Standard curve, linear range, detection limit, and quantification limit

A series of standard solutions of MA and OA at various concentrations were prepared and analyzed under the aforementioned liquid chromatography conditions. Linear regression was performed by plotting the peak areas of the analytes on the Y-axis against the corresponding standard solution concentrations on the X-axis. The results are presented in Fig S1. As shown in the figure, MA exhibits a good linear relationship within the concentration range of 1.5–500 μg/mL, and OA shows a good linear relationship within the concentration range of 0.75–500 μg/mL, with linear correlation coefficients (r^2) greater than 0.999.

Appropriate volumes of MA and OA standard solutions were taken and serially diluted with the diluent. The diluted solutions were injected following the established detection method, and the signal-to-noise ratio (S/N) for each chromatographic peak was calculated. The limit of detection (LOD) was defined as the mass concentration at which S/N≥3, and the limit of quantification (LOQ) was defined as the mass concentration at which S/N≥10. The linear equations, r^2, LODs, and LOQs for MA and OA are summarized in Table S1.

Table S1 Linear equation, linear correlation coefficient, limit of detection and limit of quantitation of maslinic acid and oleanolic acid

Compound	Linear range（μg/mL）	Linear equation	Linear correlation coefficient（r^2）	Limit of detection（μg/mL）	Limit of quantitation（μg/mL）
MA	1.5-500	Y = 9.0793X + 60.858	0.9998	0.0035	0.012

OA	0.75-500	Y = 8.5263X + 31.147	0.9994	0.0038	0.014

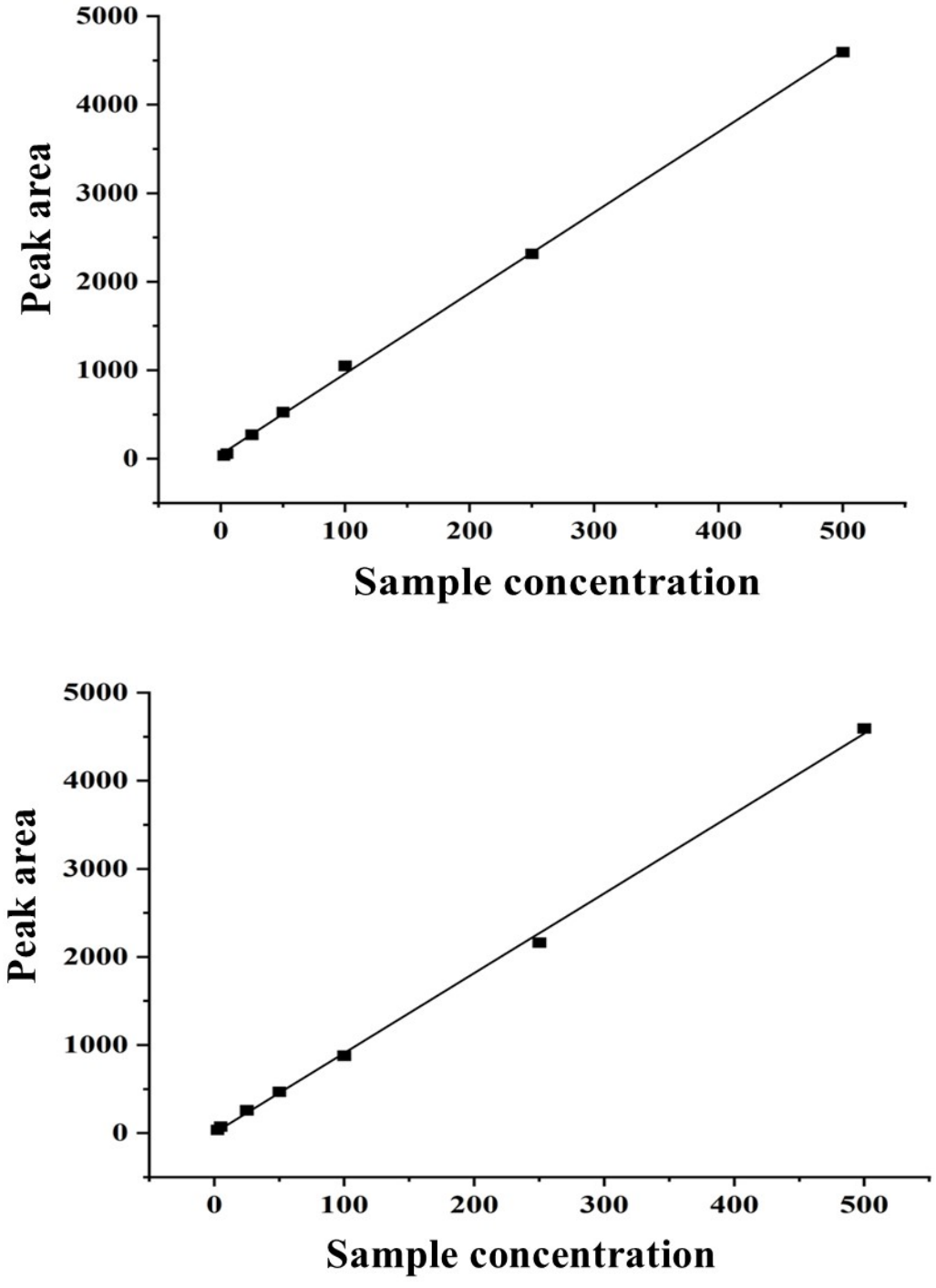

Fig S1 Standard curves of maslinic acid and oleanolic acid

1.2 Method precision

The precision of this detection method was evaluated by examining the repeatability of peak areas after repeated injections of the same processed sample. Five consecutive injections of the corresponding standard solution were performed at equal time intervals under the liquid chromatography conditions to assess the intra-day precision. Continuous injections over three consecutive days were carried out to evaluate the inter-day precision. The relative standard deviation (RSD) was used as the evaluation metric. The results are presented in Table S2. The intra-day precision RSD values for the two analytes were less than 2.95%, and the inter-day precision RSD values were less than 2.9%, indicating that the method exhibits good precision and meets the detection requirements for actual samples in the experiment.

Table S2 Inspection of precision of maslinic acid and oleanolic acid

Compound	RSD（%）	
	Intraday precision	Daytime precision
MA	2.07	2.90
OA	2.95	2.85

1.3 Recovery

In this study, the recovery was determined by the standard addition method. Nine samples of olive pomace were taken and divided into three groups. Standard solutions of MA and OA were spiked at levels of 0.8×, 1.0×, and 1.2× of their respective original contents in the samples. After extraction under the optimized HSE conditions, HPLC analysis was performed. The contents of MA and OA in each sample solution were calculated based on the peak area results, and the recovery as well as the relative standard deviation (RSD) were calculated. The results are shown in Table S3. As

presented in the table, the recoveries ranged from 98.34% to 109.25%, with RSD values below 3.06%. These results indicate that this method meets the detection requirements.

Table S3 Recovery rate investigation of olive pomace

Compound	spike concentration（%）	Recovery（%）	RSD（%）
MA	80	98.34	3.01
	100	99.45	2.88
	120	106.21	2.95
OA	80	99.12	2.96
	100	102.13	3.06
	120	109.25	2.85