SUPPLEMENTARY MATERIALS

Investigation and Optimization of the Analytical Method

Investigation of detection wavelength

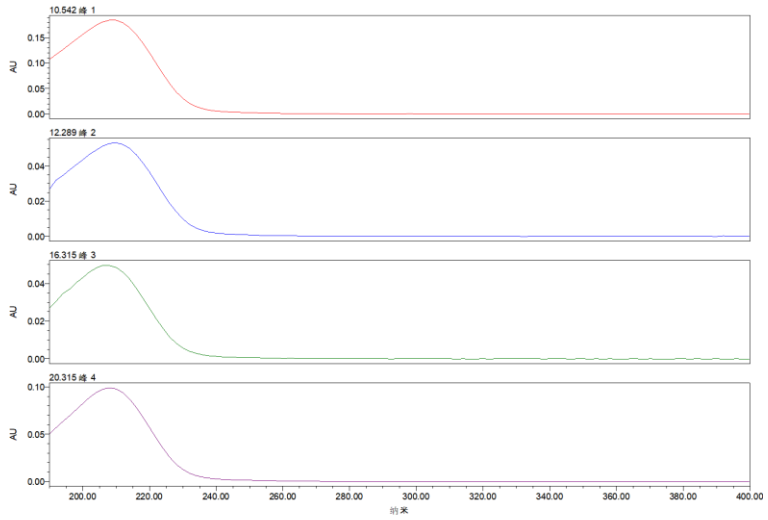

Figure S1. UV Spectrum of the reference standards

Under these experimental conditions, the main focus was on alkaloids in Areca Semen. Alkaloids such as arecoline showed maximum absorption at around 210 nm.

Selection of Extraction Method

Various extraction methods were compared.

Method 1:

A 0.5 g portion of Areca Semen powder was taken and moistened with 2 mL of ammonia solution for 30 min. Then, 25 mL of methanol was accurately added and weighed. Ultrasonic extraction was performed for 30 minutes (power 500 W, frequency 40 kHz, at room temperature). After cooling to room temperature, methanol was added to compensate for weight loss, followed by filtration. 10.0 mL of the filtrate was concentrated under reduced pressure to near dryness. The residue was dissolved in 50% acetonitrile (containing 0.1% phosphoric acid), diluted to 10 mL, and filtered through a 0.22 μm microporous membrane to obtain the sample solution.

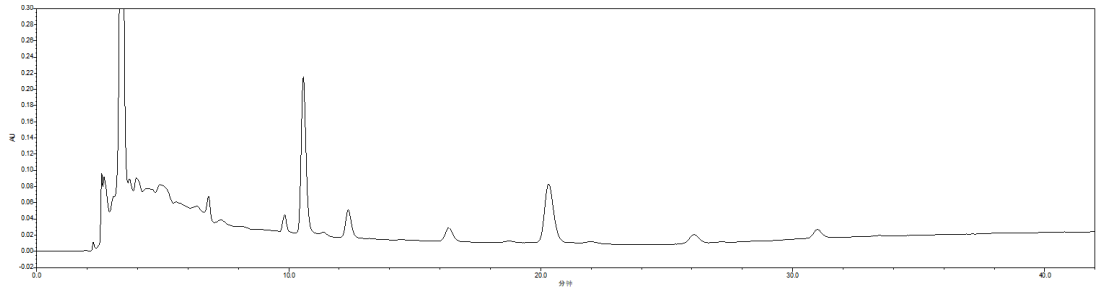

Figure S2.1*. Chromatogram obtained using Extraction Method 1*

Method 2:

Approximately 0.5 g of the powdered sample was accurately weighed and placed in a stoppered conical flask. 50 mL of ethyl ether was added, followed by 3 mL of carbonate buffer solution (prepared by dissolving 1.91 g of sodium carbonate and 0.56 g of sodium bicarbonate in water and diluting to 100 mL). The mixture was allowed to stand for 30 min with occasional shaking.

The mixture was then heated under reflux for 30 minutes. The ethyl ether layer was separated and transferred into a flat-bottom flask containing 1 mL of phosphoric acid solution (5→1000). The residue was further extracted twice under reflux with ethyl ether (30 mL and 20 mL, respectively), each for 15 minutes.

All ethyl ether extracts were combined in the same evaporating dish, and the ether was evaporated to dryness. The residue was dissolved in 50% acetonitrile (containing 0.1% phosphoric acid), transferred to a 25 mL volumetric flask, diluted to volume with the same solvent, mixed well, filtered, and the subsequent filtrate was used as the sample solution.

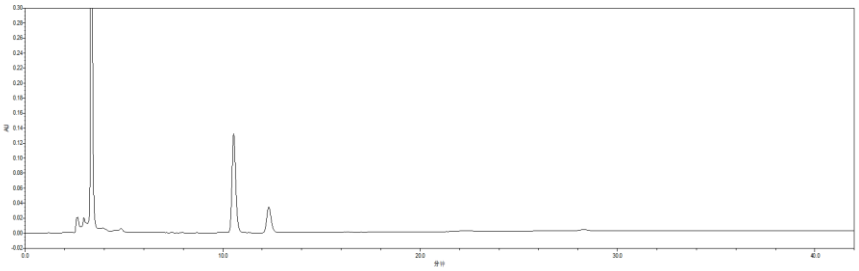

***Figure S2.2**. Chromatogram obtained using Extraction Method 2*

Method 3:

Pulverize the sample into fine powder. Accurately weigh about 0.5 g and place it into a stoppered conical flask. Add 2 mL of ammonia solution to moisten, then add 50 mL of ether, shake gently, and allow to stand for 30 minutes to ensure complete wetting of the powder. Reflux on the water bath for 30 minutes, separate the ether layer, and transfer it into a flat-bottom flask containing 1 mL of phosphoric acid solution (5→1000).

The residue is further extracted twice with ether under reflux (30 mL and 20 mL), 15 minutes each time. Combine the ether extracts in the same evaporating dish and evaporate ether to dryness. Dissolve the residue with 50% acetonitrile (containing 0.1% phosphoric acid), transfer into a 25 mL volumetric flask, and dilute to volume with 50% acetonitrile (containing 0.1% phosphoric acid). Shake well, filter, and use the subsequent filtrate as the test solution.

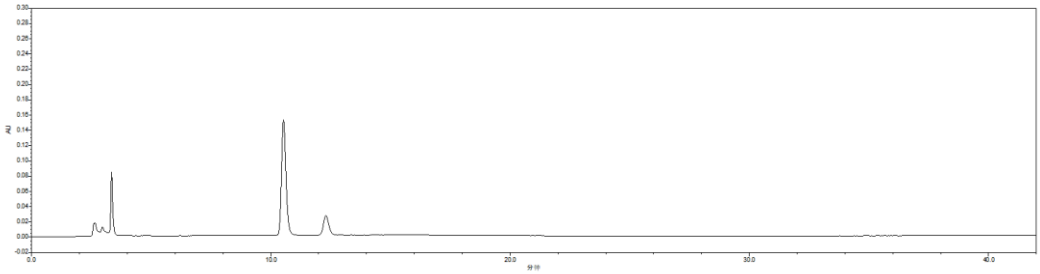

***Figure S2.3**. Chromatogram obtained using Extraction Method 3*

Method 4:

Take 0.5 g of Areca Semen powder, add 25 mL of methanol, reflux for 30 minutes, filter, and collect the subsequent filtrate to obtain the test solution.

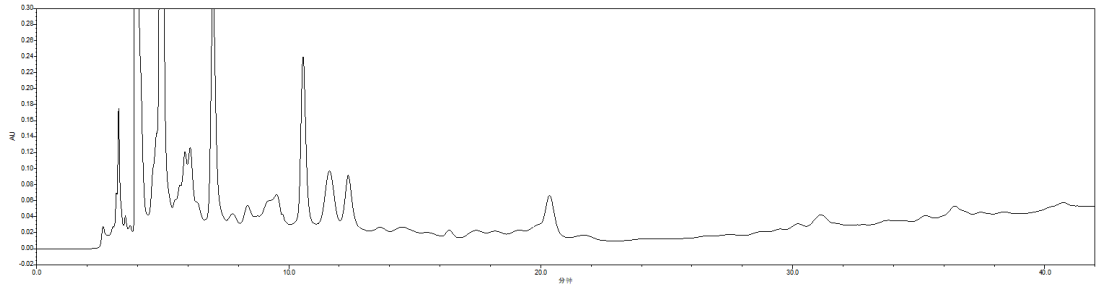

Figure S2.4*. Chromatogram obtained using Extraction Method 4*

Method 5:

Take 0.5 g of Areca nut powder, add 2 mL of ammonia solution to moisten for 30 minutes, then accurately add 25 mL of methanol and weigh. Perform ultrasonic extraction for 30 minutes (power 500 W, frequency 40 kHz, room temperature). Cool to room temperature and compensate the weight loss with methanol, then filter.

Take 10.0 mL of the filtrate, concentrate under vacuum to near dryness, dissolve the residue in 50% acetonitrile, dilute to 10 mL, and filter through a 0.22 μm microporous membrane to obtain the test solution.

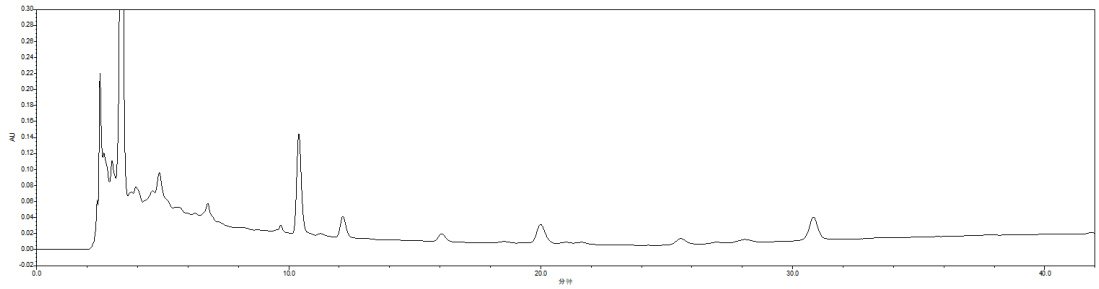

Figure S2.5*. Chromatogram obtained using Extraction Method 5*

Method 6:

Approximately 0.5 g of the powdered sample was accurately weighed and placed in a stoppered conical flask. 50 mL of ethyl ether was added, followed by 3 mL of carbonate buffer solution (prepared by dissolving 1.91 g of sodium carbonate and 0.56 g of sodium bicarbonate in water and diluting to 100 mL). The mixture was allowed to stand for 30 min with occasional shaking.

The mixture was then heated under reflux for 30 minutes. The ethyl ether layer was separated and transferred into a flat-bottom flask. The residue was further extracted twice under reflux with ethyl ether (30 mL and 20 mL), each for 15 minutes.

All ethyl ether extracts were combined in the same flat-bottom flask, the ether was evaporated off, and the residue was dissolved in 50% acetonitrile solution. The solution was transferred to a 25 mL volumetric flask, diluted to volume with 50% acetonitrile, mixed well, filtered, and the subsequent filtrate was used as the sample solution.

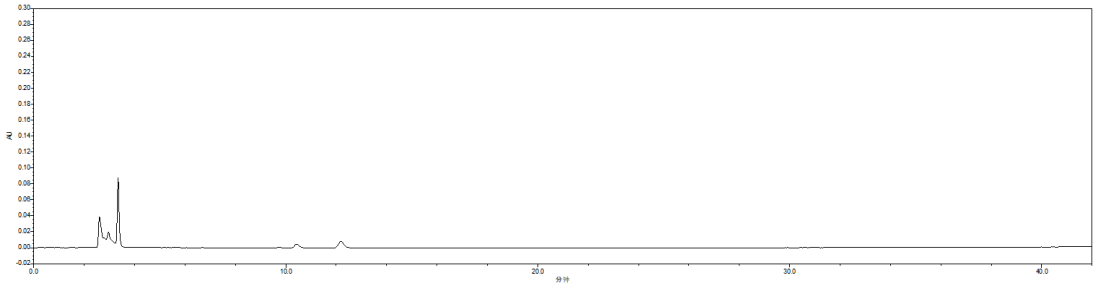

Figure S2.6*. Chromatogram obtained using Extraction Method 6*

Method 7:

Pulverize the sample into fine powder. Accurately weigh about 0.5 g and place it into a stoppered conical flask. Add 2 mL of ammonia solution to moisten, then add 50 mL of ether, shake gently, and allow to stand for 30 minutes to ensure complete wetting of the powder. Reflux for 30 minutes, separate the ether layer, and transfer it into a flat-bottom flask.

The residue is further extracted twice with ether under reflux (30 mL and 20 mL), 15 minutes each time. Combine the ether extracts in the same flat-bottom flask and evaporate ether to dryness. Dissolve the residue with 50% acetonitrile solution, transfer into a 25 mL volumetric flask, dilute to volume with 50% acetonitrile, shake well, filter, and use the subsequent filtrate as the test solution.

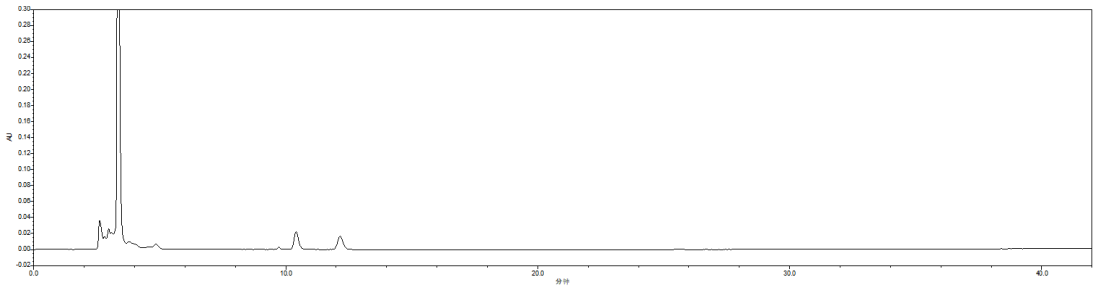

Figure S2.7*. Chromatogram obtained using Extraction Method 7*

Method 8:

Take 0.5 g of Areca Semen powder, add 25 mL of 50% methanol, reflux for 30 minutes, filter, and collect the subsequent filtrate to obtain the test solution.

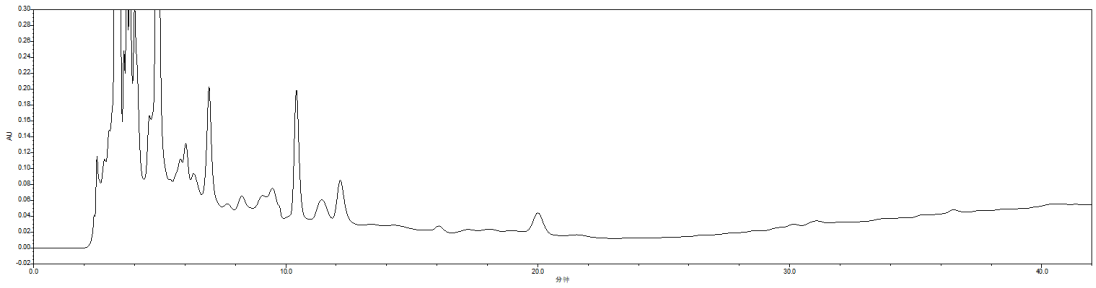

Figure S2.8*. Chromatogram obtained using Extraction Method 8*

Eight different extraction methods were compared. The results showed that **Method 1** produced the largest peak areas for the four alkaloids and also the greatest number of peaks. Detailed results are presented in Table S1. The extraction procedures were as follows:

Table S1. *Comparison of eight extraction methods*

Method	Arecoline (%)	Guvacoline (%)	Arecaidine (%)	Guvacine (%)
1	**0.282**	**0.083**	**0.055**	**0.236**
2	0.273	0.077	/	/
3	0.251	0.069	/	/
4	0.233	0.072	0.021	0.233
5	0.205	0.052	0.033	0.157
6	0.005	0.001	/	/
7	0.012	0.003	/	/
8	0.152	0.045	0.035	0.121

Optimization of Extraction Time

The extraction efficiencies at different extraction times (15, 30, and 60 minutes) were investigated. The results indicated that extraction for 30 minutes was sufficient to achieve relatively complete extraction of the alkaloid constituents in Arecae Semen. The results are presented in Table S2.

Table S2*. Effect of extraction time*

Extraction Time (min)	Arecoline (%)	Guvacoline (%)	Arecaidine (%)	Guvacine (%)
15	0.225	0.072	0.049	0.217
30	**0.282**	**0.083**	**0.055**	**0.236**
60	0.281	0.085	0.057	0.233

Optimization of Sample Amount

The effects of different sample amounts (0.2 g, 0.5 g, and 1.0 g) on extraction efficiency were evaluated. The results demonstrated that a sample amount of 0.5 g provided the optimal extraction performance. The results are shown in Table S3.

Table S3*. Effect of sample amounts*

Sample Amount (g)	Arecoline (%)	Guvacoline (%)	Arecaidine (%)	Guvacine (%)
0.2	0.278	0.080	0.048	0.225
0.5	**0.282**	**0.083**	**0.055**	**0.236**
1.0	0.285	0.082	0.056	0.234