Supplementary Information

A pseudotargeted peptidomics strategy for screening natural signature peptides in animal-derived drugs: taking Pheretima as a case

Figure captions:

Table S1. The information of 24 batches of Pheretima samples.

FigureS1. The result of the optimization of extraction solvents.

FigureS2. The pie plot of the number of detected ion pairs in total ion list by dMRM method in positive mode.

FigureS3. The histogram plot of length distribution of protein sequences of the two theoretical protein databases.

FigureS4. The length distribution of the identified natural peptides of Pheretima by database search.

FigureS5. The 3D waterfall plot of peak area of different proportion samples of PA-MM and PV-MM detected by dMRM method.

Table S1. The information of 24 batches of Pheretima samples, which were identified by the means of macroscopic and microscopic, together with the means of DNA barcode and signature enzymatic peptides.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| No. | Sample number | Species | Signature peptide | Origin | Abbr |
| 1 | MM1 | *Metaphire magan* | P1 P6 | Guangdong | MM |
| 2 | MM4 | *Metaphire magan* | P1 P6 | Hainan | MM |
| 3 | MM24 | *Metaphire magan* | P1 P6 | Guangdong | MM |
| 4 | MM38 | *Metaphire magan* | P1 P6 | Hainan | MM |
| 5 | MM40 | *Metaphire magan* | P1 P6 | Weizhi | MM |
| 6 | MM42 | *Metaphire magan* | P1 P6 | Weizhi | MM |
| 7 | MM52 | *Metaphire magan* | P1 P6 | Hainan | MM |
| 8 | MM53 | *Metaphire magan* | P1 P6 | Weizhi | MM |
| 9 | MM55 | *Metaphire magan* | P1 P6 | Hainan | MM |
| 10 | MM61 | *Metaphire magan* | P1 P6 | Weizhi | MM |
| 11 | PA7 | *Pheretima aspergillum* | P1 P2 | Guangdong | PA |
| 12 | PA32 | *Pheretima aspergillum* | P1 P2 | Guangxi | PA |
| 13 | PA34 | *Pheretima aspergillum* | P1 P2 | Guangxi | PA |
| 14 | PA54 | *Pheretima aspergillum* | P1 P2 | Guangxi | PA |
| 15 | PA57 | *Pheretima aspergillum* | P1 P2 | Guangxi | PA |
| 16 | PV2 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 17 | PV5 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 18 | PV11 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 19 | PV15 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 20 | PV27 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 21 | PV28 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 22 | PV29 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 23 | PV35 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |
| 24 | PV46 | *Pheretima vulgaris* | P3 P4 P5 | Shanghai | PV |

P1: VLSEFSAK; P2: LLEVHIR; P3: FGGVSVPR; P4: LVDIEAVSK; P5: ESIPALVEDYVSGK; P6: LSFEEFR



Figure S1. The result of the optimization of extraction solvents. a, The chromatograms of HRMS data of QC sample processed by four different solvents. b, The histogram plot of the number of peptides identified by PEAS Studio in different solvent extract conditions.



Figure S2. The pie plot of the number of detected ion pairs in total ion list by dMRM method in positive mode.



Figure S3. The histogram plot of length distribution of protein sequences of the two theoretical protein databases.



Figure S4. The length distribution of the identified natural peptides of Pheretima by database search.



Figure S5. The 3D waterfall plot of peak area of different proportion samples of PA-MM and PV-MM detected by dMRM method.