MS and HPLC analysis

Peptides:
1) Citropin 1.1
2) Pal-KK-NH\(_2\)
3) Pal-RR-NH\(_2\)
4) Pexiganan
5) Temporin A

Mass spectrometry
Equipment: Waters, ACQUITY QDa mass detector.
Range: 50-1250 m/z.
Positive scan, Cone voltage 15V, Sampling rate - 2 points/sec, capillary voltage 1.0 kV.

HPLC analysis
Equipment: Varian ProStar HPLC system,
Phenomenex, Luna® C18(2) column (100x3 mm, 5 \(\mu\)m, 100Å).
Mobile phase: Acetonitrile and water both containing 0.1 % of TFA (v/v).
Method: gradient of acetonitrile – 10-100% in 15 minutes. The data were collected for 16 min.
Detection: UV-Vis detector; absorbance at 214 nm.

Results
A\ MS analysis

A1: Citropin 1.1
Monoisotopic mass of the Citropin 1.1 is 1613.987 Da.

| \(z\) | Calculated m/z | Measured m/z |
|---|---|---|
| 1 | 1614.99 | - |
| 2 | 808.00 | 808.72 |
| 3 | 539.00 | 539.60 |

A2: Pal-KK-NH\(_2\)
Monoisotopic mass of the Pal-KK-NH\(_2\) is 511.44614 Da.

| \(z\) | Calculated m/z | Measured m/z |
|---|---|---|
| 1 | 512.45 | 512.63 |
| 2 | 256.73 | - |

Measured value of 1025.01 m/z refers to dimer – [2M+2H]\(^{2+}\) (calculated value is 1024.91).
A3: Pal-RR-NH₂
Monoisotopic mass of the Pal-RR-NH₂ is 567.458 Da.

Table 3. Pal-RR-NH₂.

| z | Calculated m/z | Measured m/z |
|---|----------------|--------------|
| 1 | 568.47         | 568.63       |
| 2 | 284.74         | 285.02       |

A4: Pexiganan
Monoisotopic mass of the Pexiganan is 2475.630 Da.

Table 4. Pexiganan.

| z  | Calculated m/z | Measured m/z |
|----|----------------|--------------|
| 1  | 2476.64        | -            |
| 2  | 1238.82        | 1239.48      |
| 3  | 826.22         | 826.85       |
| 4  | 619.92         | 620.70       |
| 5  | 496.13         | 496.70       |
| 6  | 413.61         | 414.23       |
| 7  | 354.67         | -            |
| 8  | 310.46         | -            |
| 9  | 276.08         | -            |
| 10 | 248.57         | -            |

A5: Temporin A
Monoisotopic mass of the Temporin A is 1395.900 Da.

Table 5. Temporin A.

| z  | Calculated m/z | Measured m/z |
|----|----------------|--------------|
| 1  | 1396.90        | -            |
| 2  | 698.96         | 699.51       |

B \ HPLC analysis

B1: Citropin 1.1
B2: Pal-KK-NH2

B3: Pal-RR-NH2

B4: Pexiganan

B5: Temporin A