

Small lipopeptides possess anti-biofilm capability comparable to daptomycin and vancomycin

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Supporting information:

Table S1;

Table S2

Fig. S1;

Fig. S2.

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Table S1. Minimal inhibitory concentration (MIC) of selected lipopeptides against fungi

| Peptide name | MIC (μ M) | | |
|--------------|--------------------|--------------------|----------------------|
| | <i>C. albicans</i> | <i>C. glabrata</i> | <i>C. tropicalis</i> |
| C14-KK | >25 | 25 | 6.25 |
| C14-RRR | >25 | 25 | 6.25 |
| C14-LK | 6.25 | 1.5 | 1.56 |
| C14-RW | >25 | >25 | >25 |
| C14-WR | >25 | 25-50 | 12.5-25 |
| C14-WWK | >25 | >25 | >25 |
| C14-KWI | >25 | >25 | >25 |
| C14-LKK | >25 | 6.25 | 1.56 |
| C14-EE* | >25 | >25 | >25 |
| C14-LE* | >25 | >25 | >25 |
| C14-WE* | >25 | >25 | >25 |
| C14-EW* | >25 | >25 | >25 |

*The antifungal assay of these anionic peptides was done in the presence or absence of 2 mM Ca^{2+}

Table S2. Influence of acyl chain length on the antimicrobial activity of lipopeptides against bacteria

| Peptide name | Purity | MIC (μM) | | | |
|--------------|--------|----------------------------|------------------------------|--------------------|--------------------|
| | | <i>S. aureus</i> USA300 | <i>P. aeruginosa</i> PAO1 | <i>E. coli</i> K12 | <i>B. subtilis</i> |
| C14-KK | 97.44% | 6.25 | 50 | ≤ 6.25 | ≤ 6.25 |
| C12-KK | 95.82% | 100 | > 200 | 100 | 100 |
| C10-KK | 96.43% | > 200 | > 200 | > 200 | > 200 |
| C8-KK | 98.19% | > 200 | > 200 | > 200 | > 200 |
| C6-KK | 98.44% | > 200 | > 200 | > 200 | > 200 |

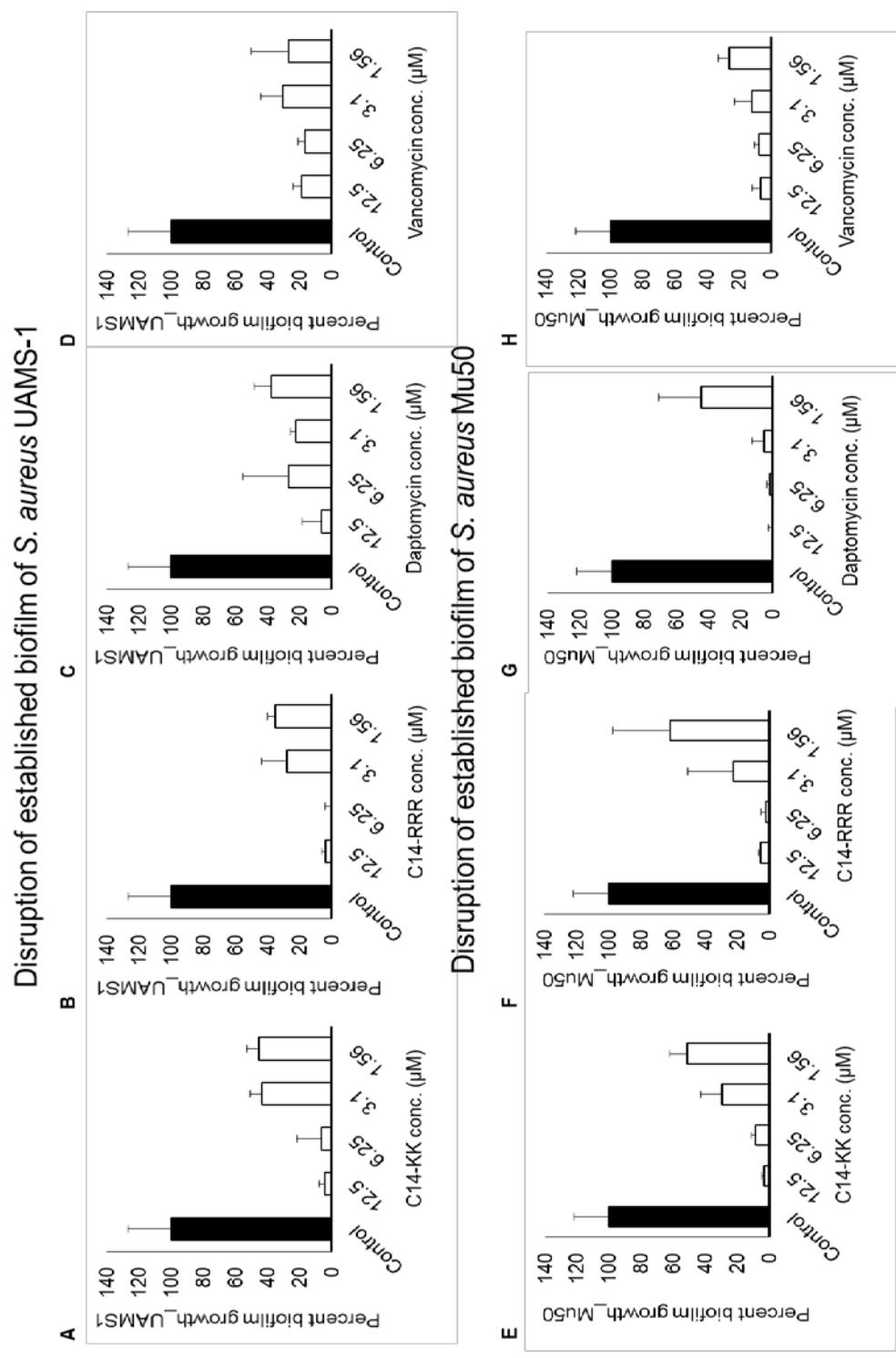


Figure S1. Disruption of the 24 hour established biofilms of UAMS-1 (A, B, C and D) and Mu50 (E, F, G and H) by C14-KK, C14-RRR, Daptomycin and Vancomycin respectively.

Figure S2. HPLC chromatograms and MS of the lipopeptides

| Peptide name | Peptide code | Purity (%)* |
|--------------|--------------|-------------|
| C14-KK | G701 | 97.44 |
| C14-RRR | G714 | 95.77 |
| C14-LK | G702 | 96.84 |
| C14-RW | G703 | 97.91 |
| C14-WR | G704 | 95.26 |
| C14-EE | G705 | 96.30 |
| C14-LE | G706 | 96.27 |
| C14-WE | G707 | 96.81 |
| C14-EW | G708 | 95.85 |
| C14-WWK | G709 | 95.72 |
| C14-LWE | G710 | 98.03 |
| C14-KWI | G711 | 95.29 |
| C14-LKW | G712 | 96.58 |
| C14-LKK | G713 | 95.91 |
| C14-LLK | G715 | 96.77 |



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PEPTIDE DATA SHEET

7/11 ordered

LOT NO: 95868

NAME: G701

SEQUENCE: Myr-KK-amide

C14

R7/30/14 OK

EXPECTED MASS: 483.4 ± 1.0

AVERAGE MASS: 483.4

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 97.44%

COMMENTS: white to off-white powder or lumps

DATE: 07/28/2014

VERIFIED BY: Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95868

Analyzed:07/24/14 12:00 Vail Number: 95868

Reported:07/28/14 09:53 Volume:20.0ul

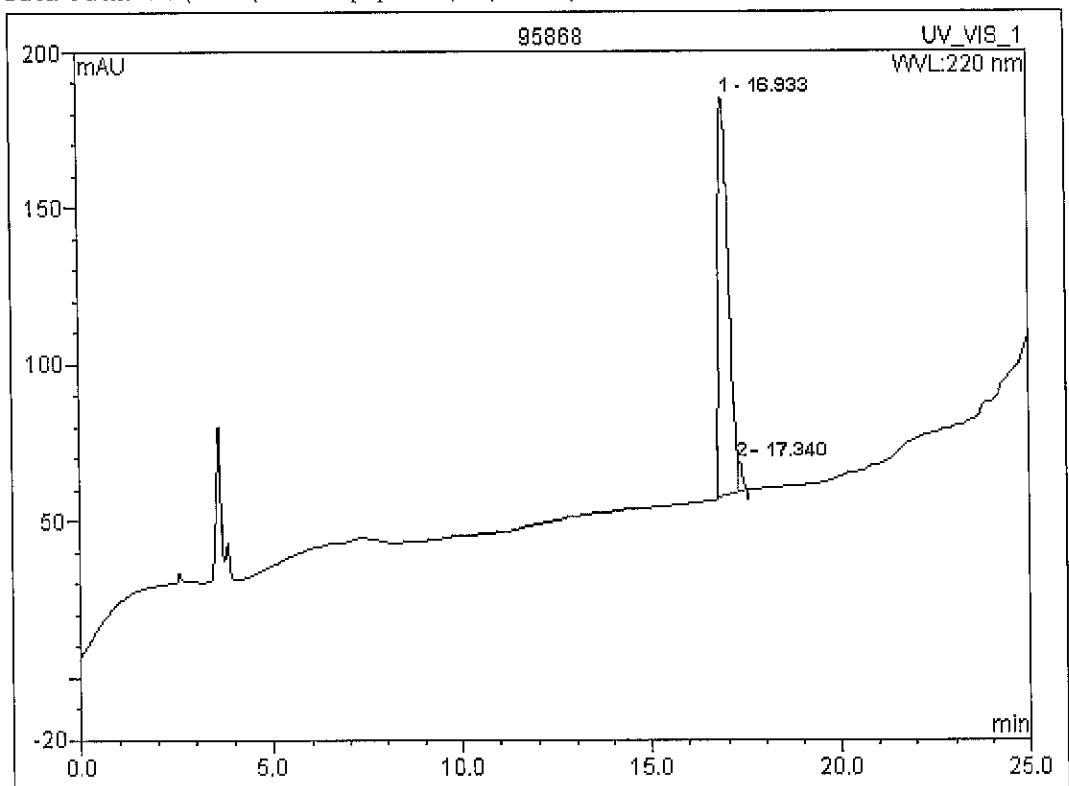
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 05:95 t=25.0mins 100:0

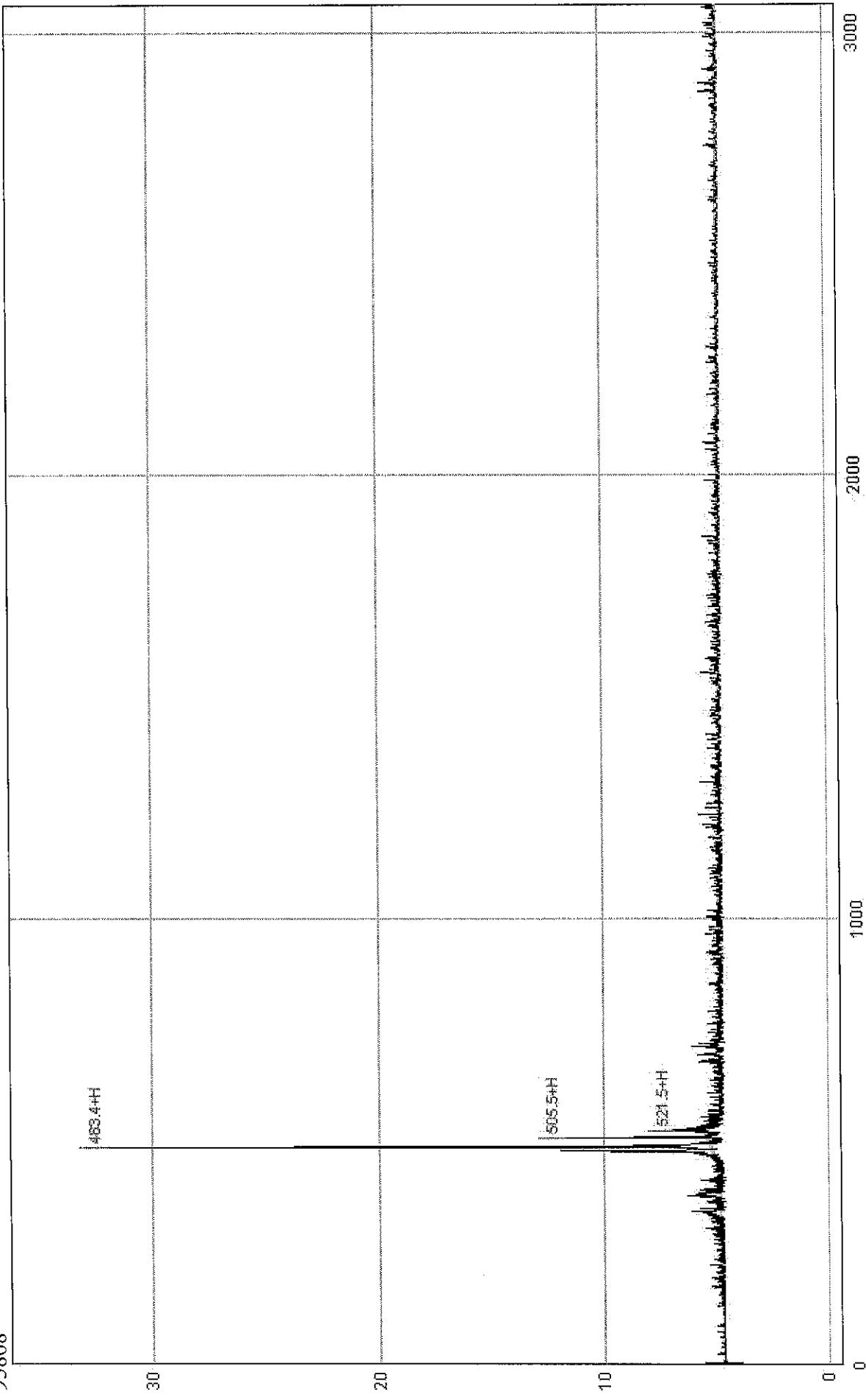
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95868\95868.hw:



| No. | Ret.Time min | Height mAU | Area mAU*min | Rel.Area % |
|---------------|-----------------|---------------|-----------------|----------------|
| 1 | 16.93 | 127.381 | 39.656 | 97.44 |
| 2 | 17.34 | 10.003 | 1.040 | 2.56 |
| Total: | | | 40.696 | 100.000 |

95868





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PEPTIDE DATA SHEET

LOT NO: 95869

NAME: G702

SEQUENCE: Myr-LK-amide

EXPECTED MASS: 468.3±1.0

AVERAGE MASS: 468.6

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 96.84%

COMMENTS: white to off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY: Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95869

Analyzed:07/17/14 14:18 Vail Number: 95869

Reported:07/23/14 13:03 Volume:20.0ul

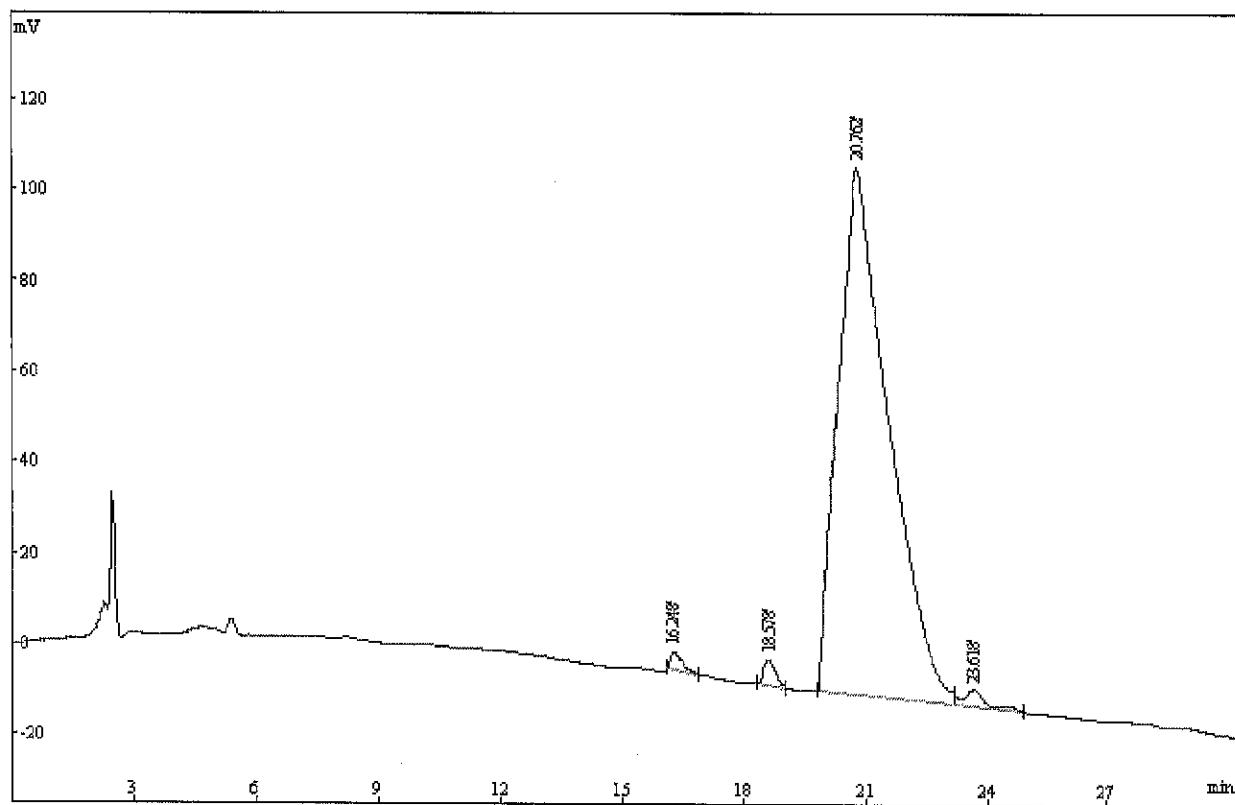
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 35:65 t=30.0mins 75:25

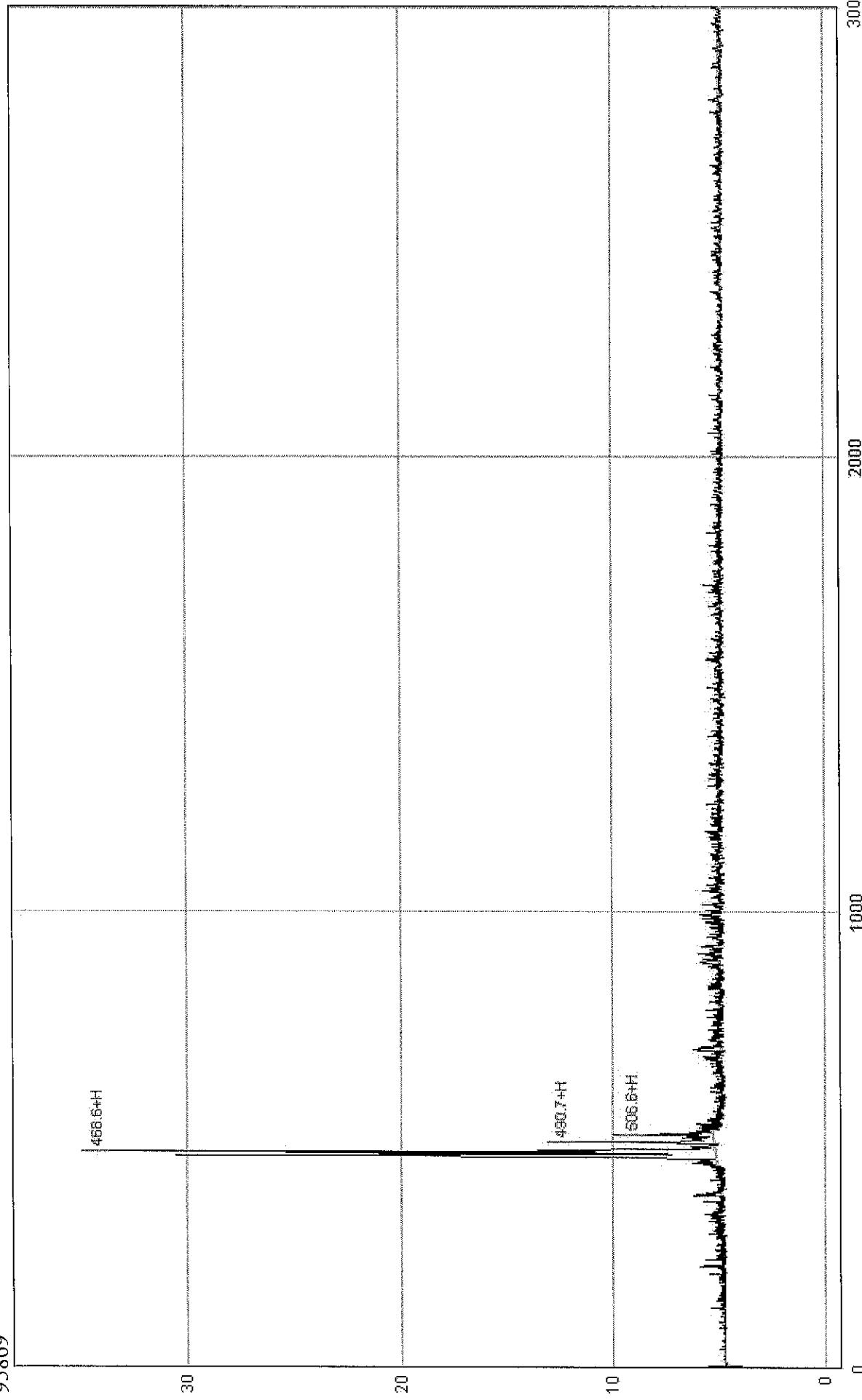
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95869\95869.hw:



| No. | RT | Area% | Area |
|-------|--------|-------|----------|
| 1 | 16.248 | 0.753 | 77668 |
| 2 | 18.578 | 1.072 | 110591 |
| 3 | 20.762 | 96.84 | 9988882 |
| 4 | 23.618 | 1.341 | 138302 |
| Total | | 100 | 10315443 |

95869





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PEPTIDE DATA SHEET

LOT NO: 95870

NAME: G703

SEQUENCE: Myr-RW-amide

EXPECTED MASS: 569.4 ± 1.0

AVERAGE MASS: 569.7

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 97.91%

COMMENTS: white to off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY:

Zhang -ZQ

Customer Peptide HPLC Report

Sample name: 95870

Analyzed:07/21/14 15:49 Vail Number: 95870

Reported:07/23/14 10:02 Volume:20.0ul

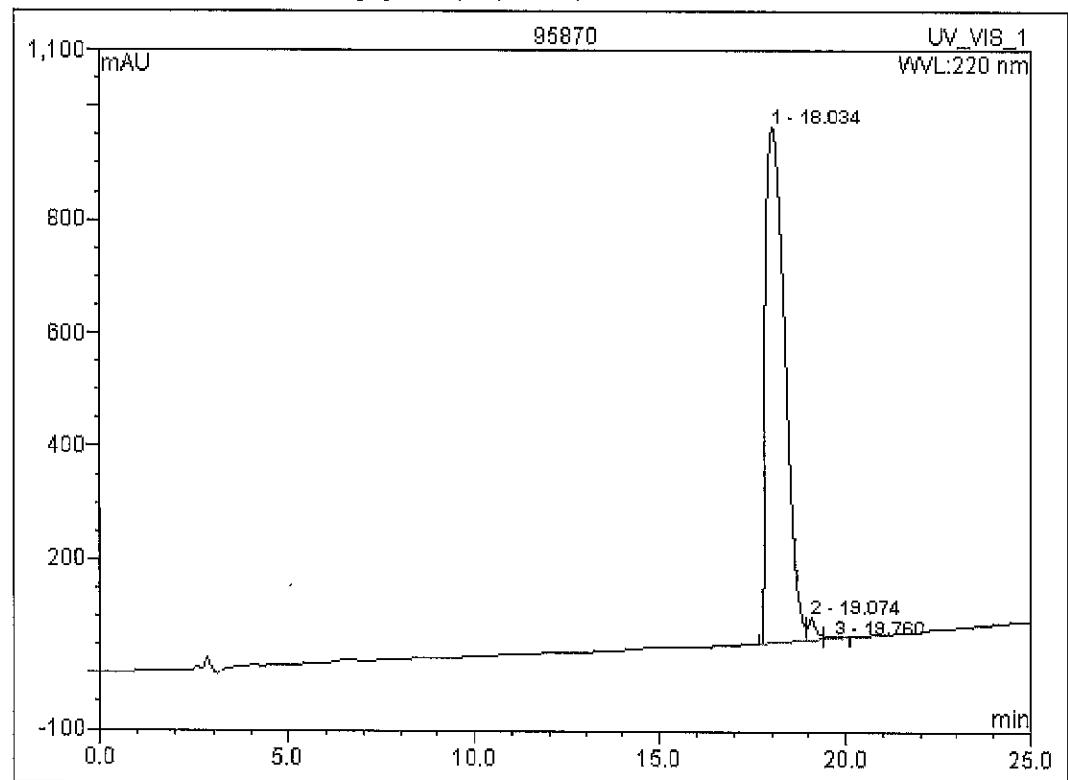
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 30:70 t=25.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95870\95870.hw:



| No. | Ret.Time min | Height mAU | Area mAU*min | Rel.Area % |
|---------------|-----------------|---------------|-----------------|----------------|
| 1 | 18.03 | 908.087 | 535.419 | 97.91 |
| 2 | 19.07 | 39.443 | 9.568 | 1.75 |
| 3 | 19.76 | 0.732 | 1.879 | 0.34 |
| Total: | | | 546.866 | 100.000 |

95870

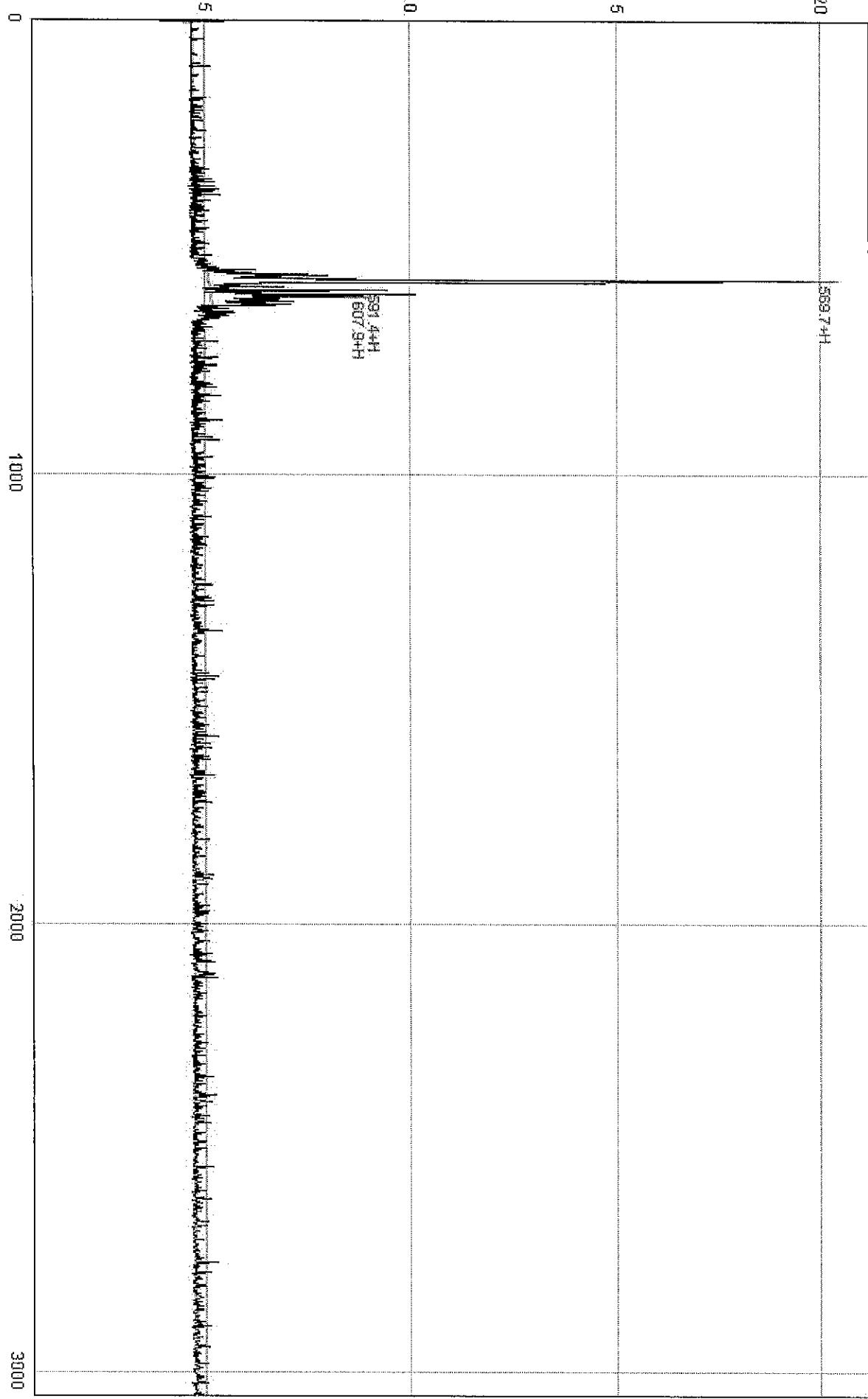
20

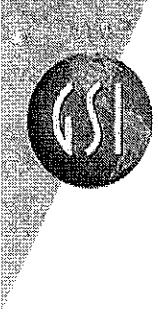
559.74†

15

10

591.4+H
607.9+H





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PEPTIDE DATA SHEET

LOT NO: 95871

NAME: G704

SEQUENCE: Myr-WR-amide

EXPECTED MASS: 569.4 ± 1.0

AVERAGE MASS: 569.8

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 95.26%

COMMENTS: white to off-white powder or lumps

DATE: 07/28/2014

VERIFIED BY:

Zhang .zq

Customer Peptide HPLC Report

Sample name: 95871

Analyzed:07/25/14 12:49 Vail Number: 95871

Reported:07/28/14 13:32 Volume:20.0ul

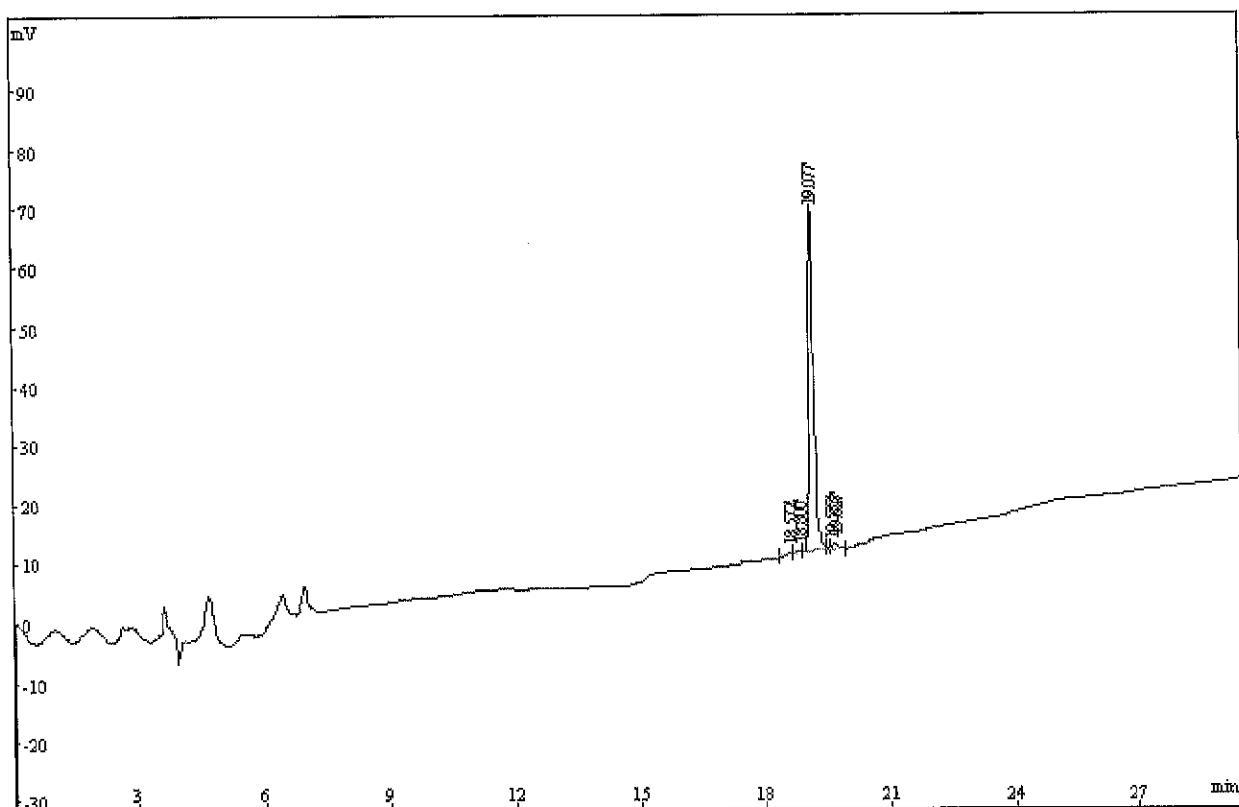
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0. 1%TFA acetonitrile Solvent B:0. 1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95871\95871.hw:



| No. | RT | Area% | Area |
|-------|--------|--------|--------|
| 1 | 18.572 | 0.8366 | 4428 |
| 2 | 18.800 | 1.599 | 8463 |
| 3 | 19.077 | 95.26 | 504176 |
| 4 | 19.533 | 0.4836 | 2559 |
| 5 | 19.627 | 1.82 | 9633 |
| Total | | 100 | 529259 |

95871

100

75

50

25

0

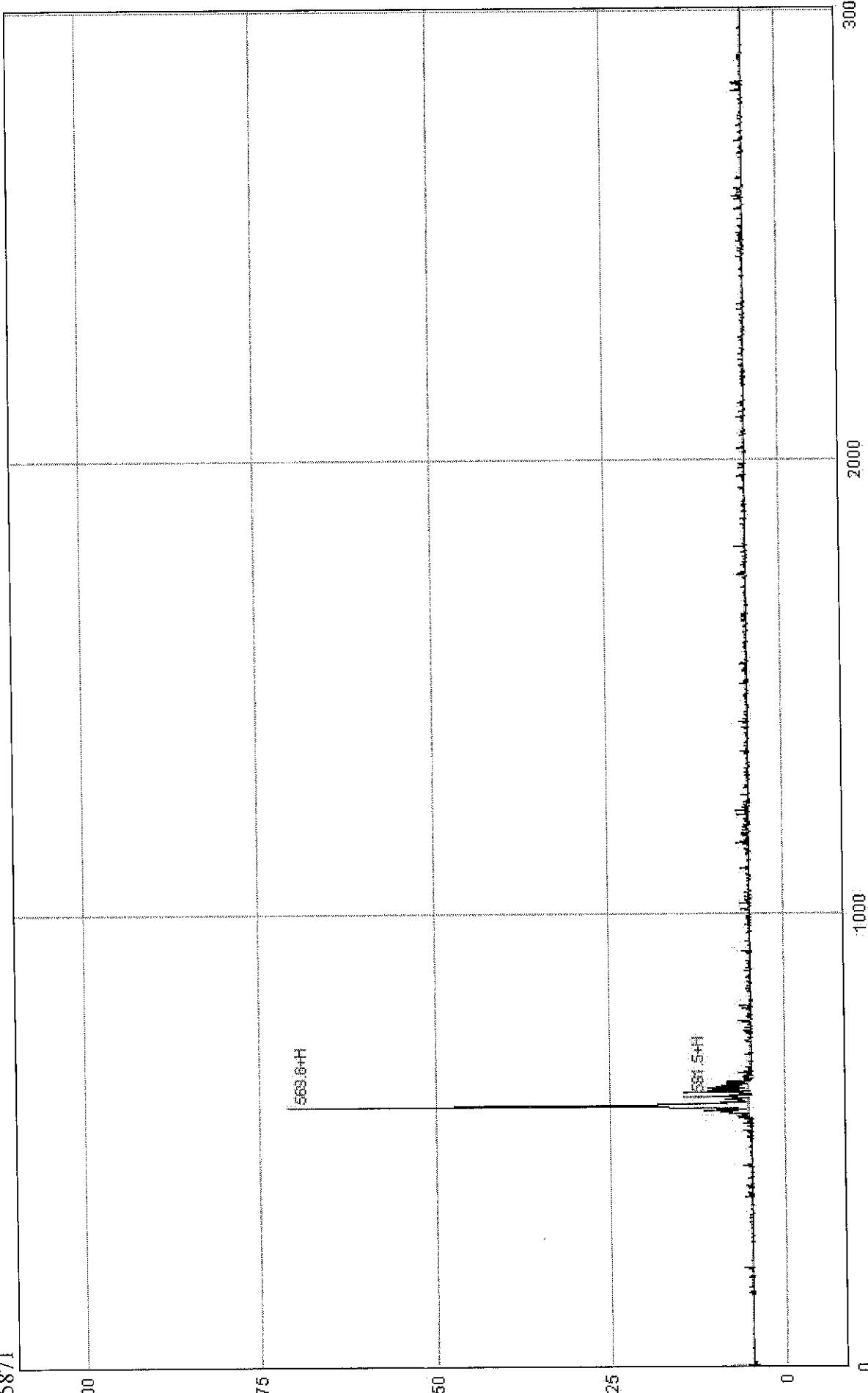
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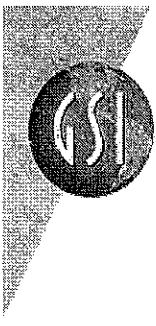
2000

3000

553.8+H

581.5+I





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PEPTIDE DATA SHEET

LOT NO: 95876

NAME: G709

SEQUENCE: Myr-WWK-amide

EXPECTED MASS: 727.6±1.0

AVERAGE MASS: 727.9

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 95.72%

COMMENTS: off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY:

Zhang Zq

Customer Peptide HPLC Report

Sample name: 95876

Analyzed:07/18/14 14:42 Vail Number: 95876

Reported:07/23/14 13:09 Volume:20.0ul

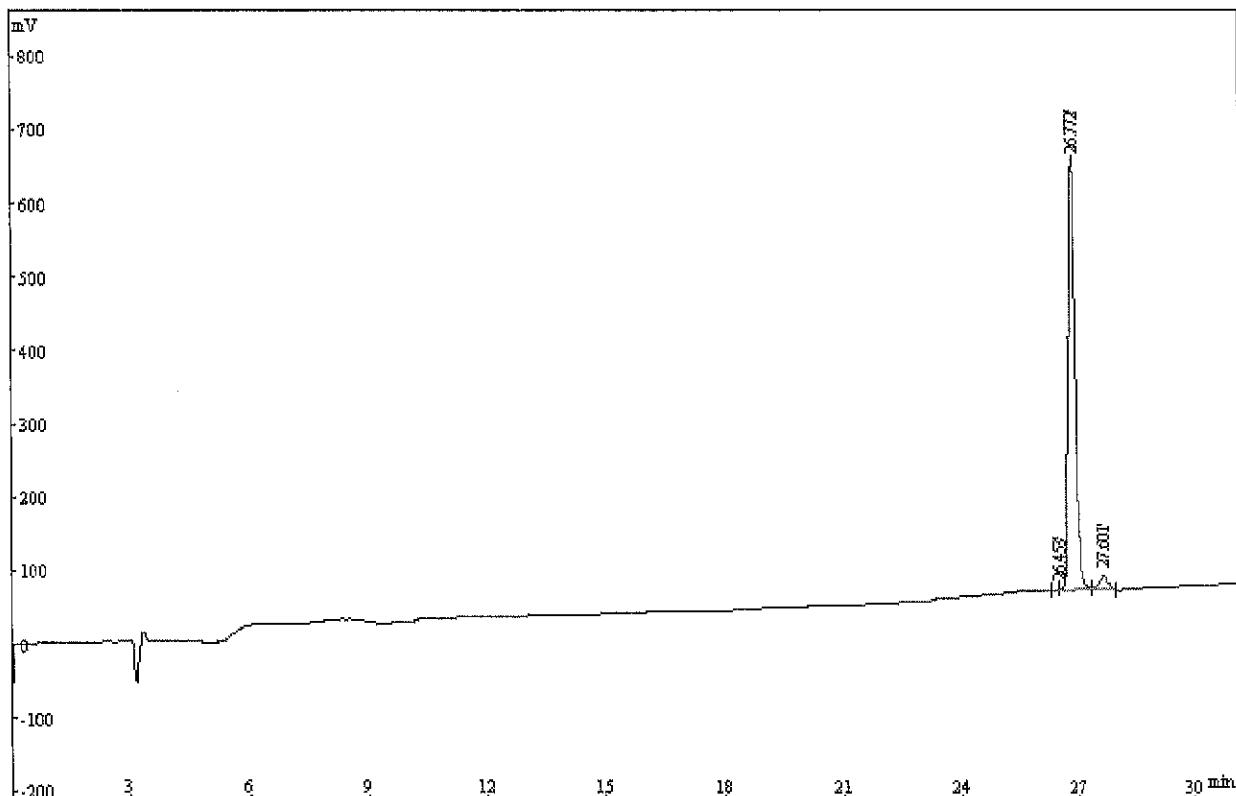
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95876\95876.hw:



| No. | RT | Area% | Area |
|-------|--------|--------|---------|
| 1 | 26.453 | 0.2981 | 23537 |
| 2 | 26.772 | 95.72 | 7558726 |
| 3 | 27.601 | 3.981 | 314371 |
| Total | | 100 | 7896634 |

95876

60

722.9+H

749.9+H

765.9+H

20

40





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PEPTIDE DATA SHEET

LOT NO: 95877

NAME: G710

SEQUENCE: Myr-LWE-amide

R 8/22/14 6/C

EXPECTED MASS: 655.5 ± 1.0

AVERAGE MASS: 655.6

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 98.03%

COMMENTS: white to off-white powder or lumps

DATE: 08/13/2014

VERIFIED BY: Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95877

Analyzed:08/08/14 15:46 Vail Number: 95877

Reported:08/13/14 10:33 Volume:20.0ul

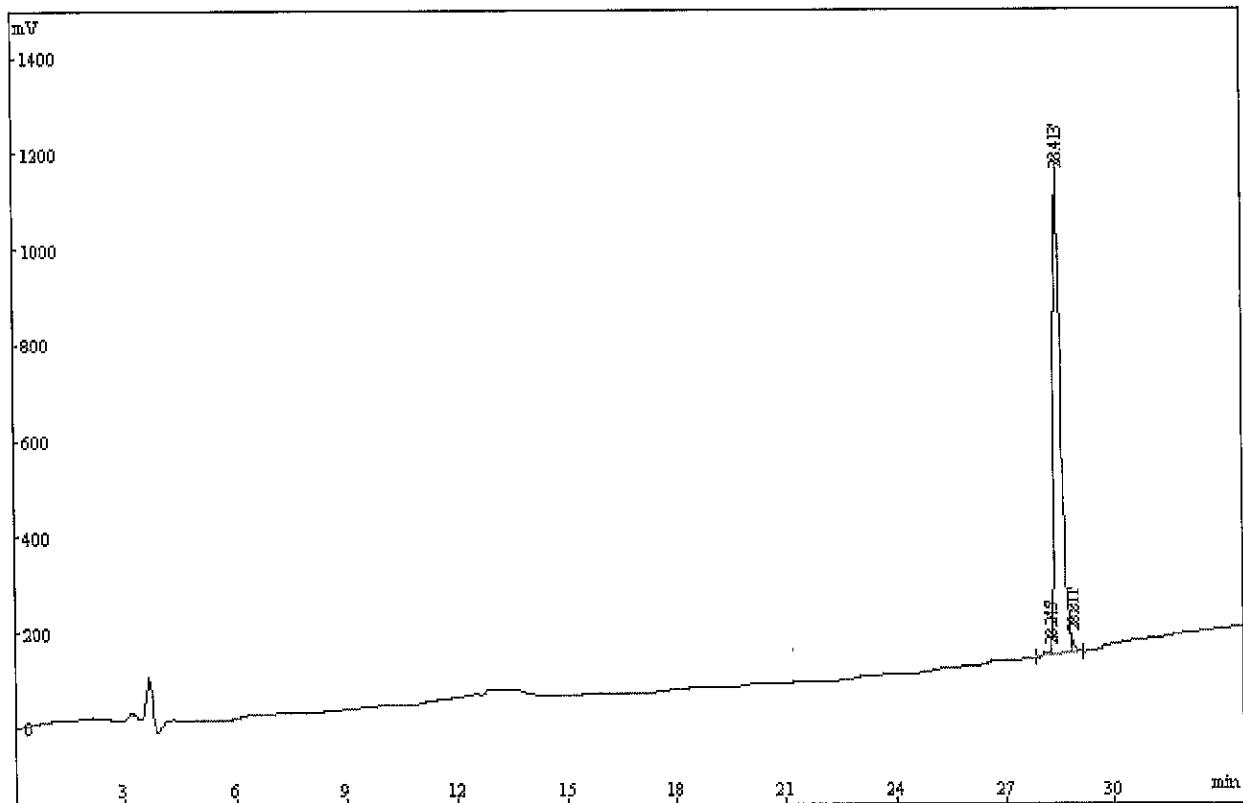
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

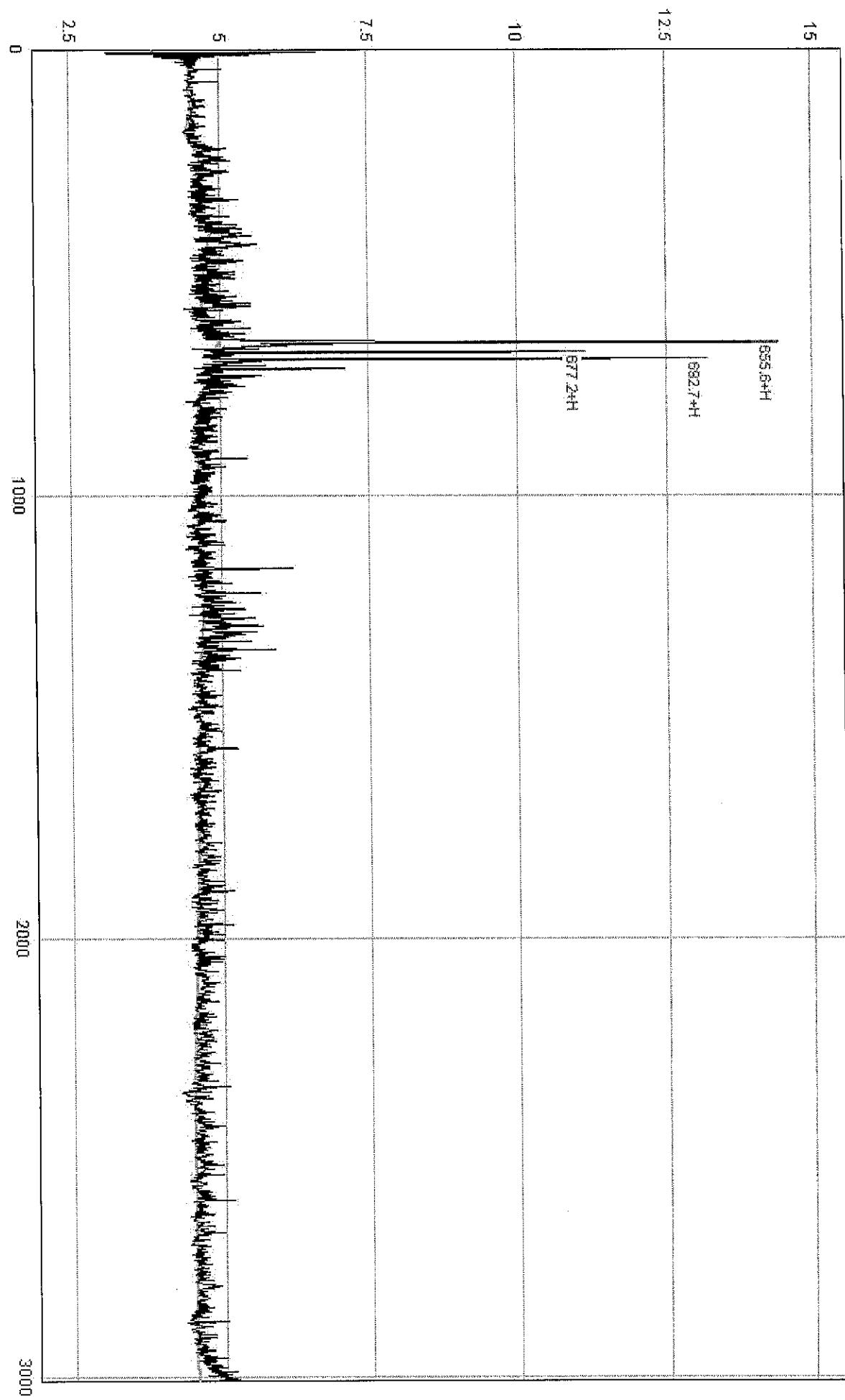
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95877\95877.hw:



| No. | RT | Area% | Area |
|-------|--------|--------|----------|
| 1 | 28.245 | 0.8207 | 113851 |
| 2 | 28.413 | 98.03 | 13597930 |
| 3 | 28.811 | 1.151 | 159604 |
| Total | | 100 | 13871385 |

95877





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PEPTIDE DATA SHEET

LOT NO: 95878

NAME: G711

SEQUENCE: Myr-KWI-amide

EXPECTED MASS: 654.4±1.0

AVERAGE MASS: 654.6

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 95.29%

COMMENTS: yellow powder or lumps

DATE: 07/23/2014

VERIFIED BY: Zhang -Zq

Customer Peptide HPLC Report

Sample name: 95878

Analyzed:07/18/14 15:18 Vail Number: 95878

Reported:07/23/14 13:07 Volume:20.0ul

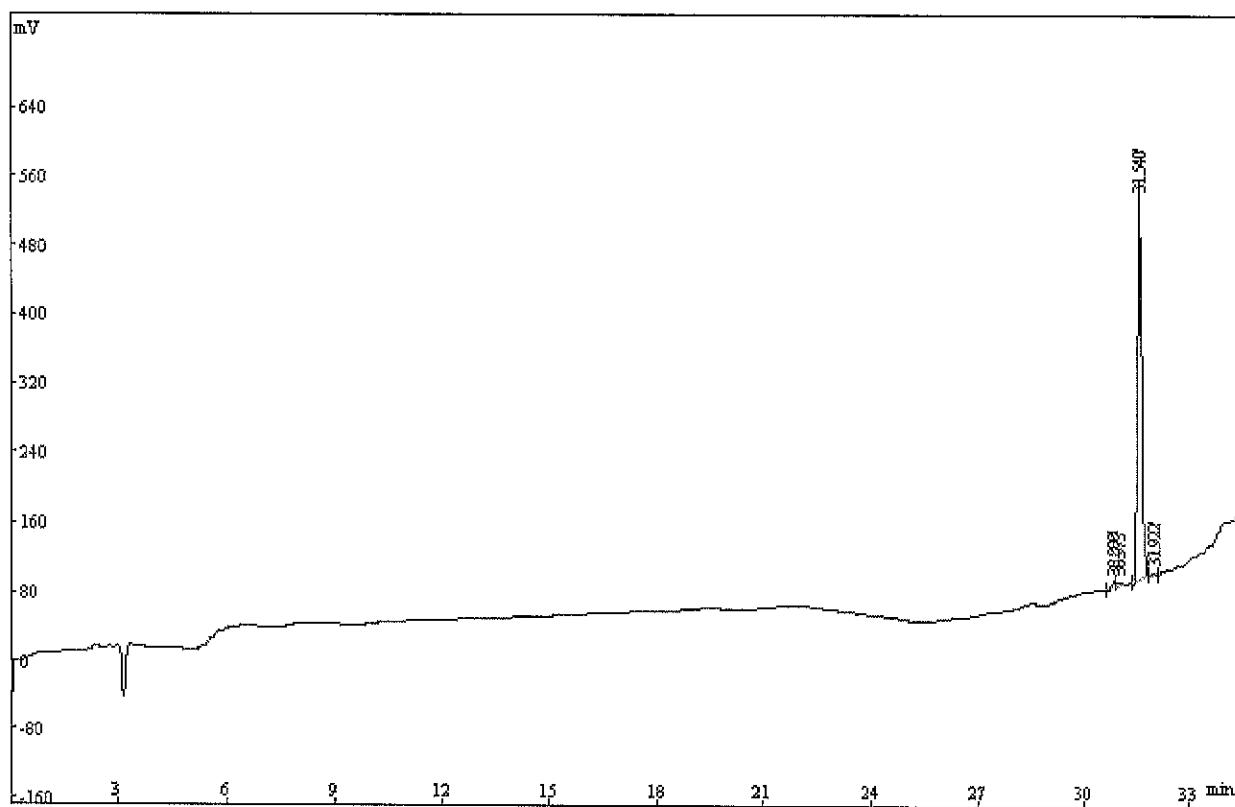
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

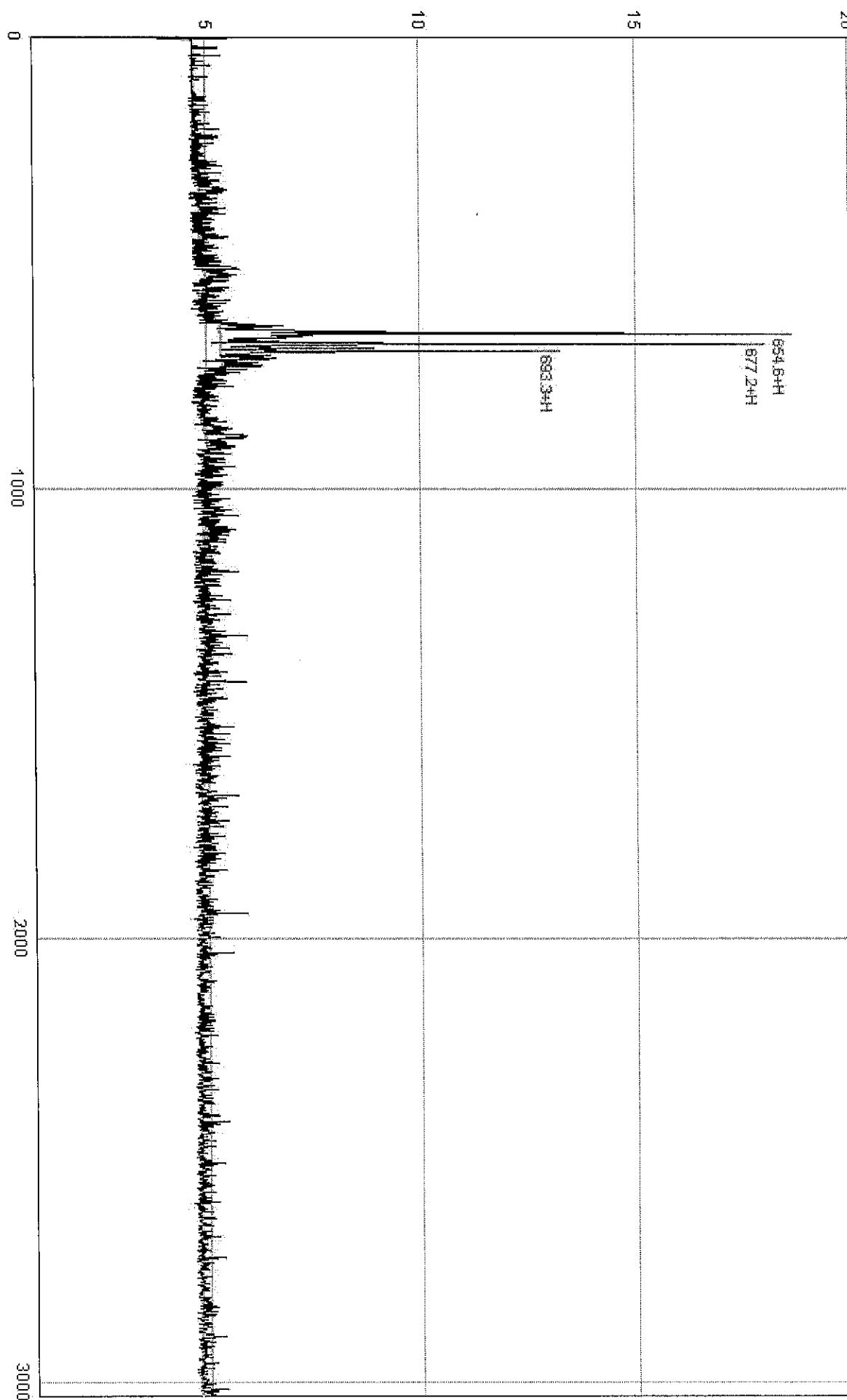
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95878\95878.hw:



| No. | RT | Area% | Area |
|-------|--------|-------|---------|
| 1 | 30.808 | 1.868 | 70809 |
| 2 | 30.973 | 1.667 | 63201 |
| 3 | 31.540 | 95.29 | 3612554 |
| 4 | 31.922 | 1.181 | 44764 |
| Total | | 100 | 3791328 |

95878





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PEPTIDE DATA SHEET

LOT NO: 95879

NAME: G712

R 8/22/14

SEQUENCE: Myr-LKW-amide

EXPECTED MASS: 654.5±1.0

AVERAGE MASS: 654.7

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 96.58%

COMMENTS: off-white powder or lumps

DATE: 08/13/2014

VERIFIED BY: Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95879

Analyzed:08/08/14 15:09 Vail Number: 95879

Reported:08/13/14 10:31 Volume:20.0ul

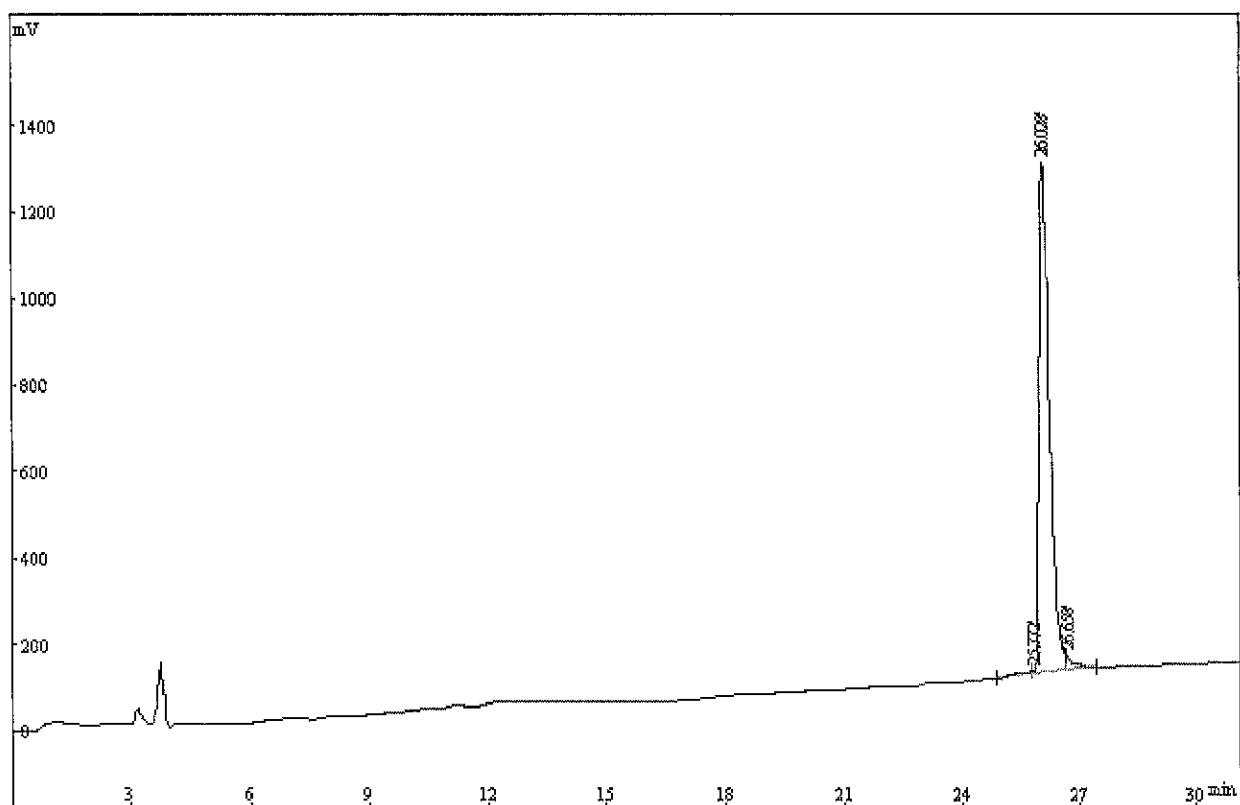
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

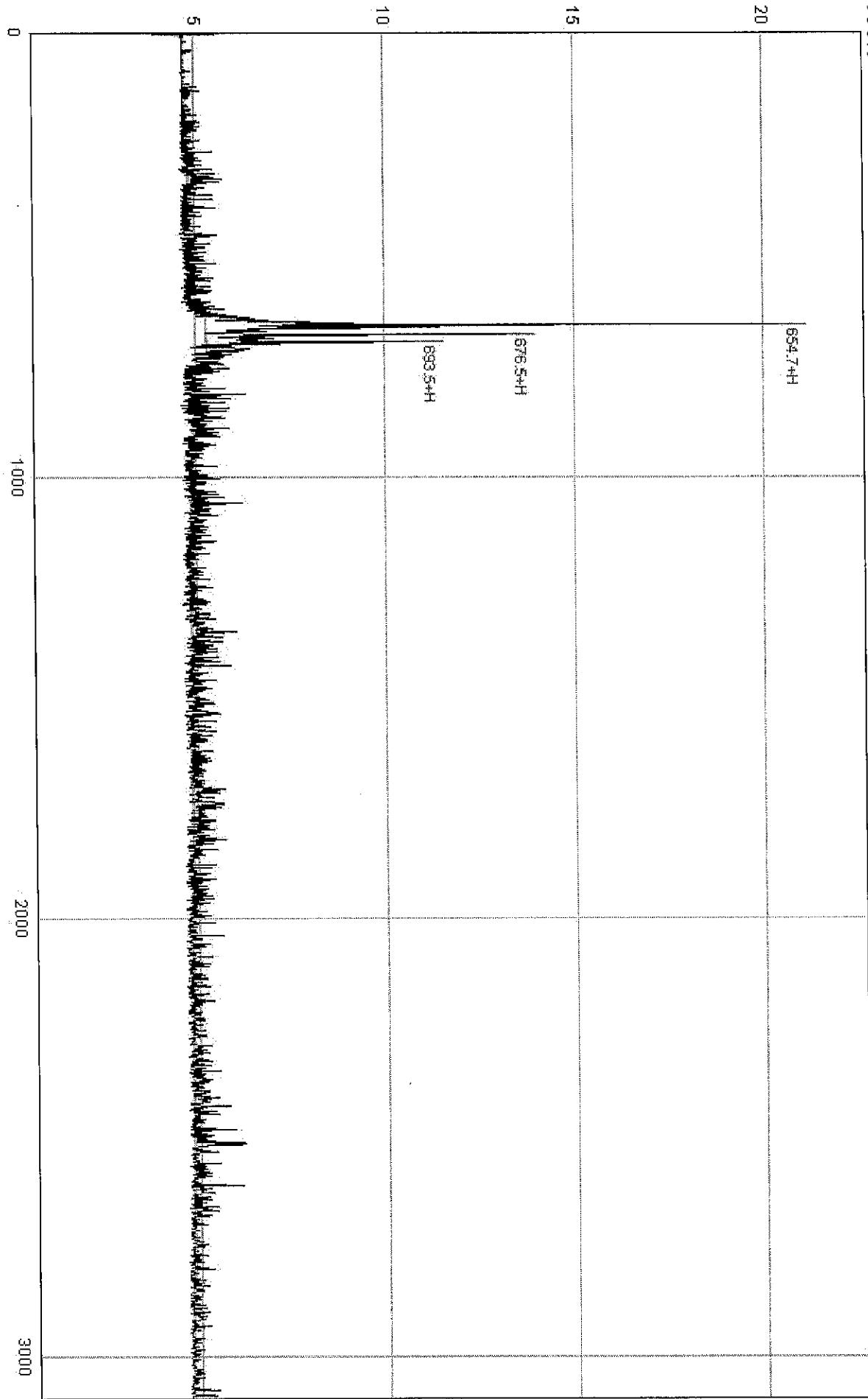
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95879\95879.hw:



| No. | RT | Area% | Area |
|-------|--------|-------|----------|
| 1 | 25.772 | 1.016 | 227375 |
| 2 | 26.028 | 96.58 | 21612608 |
| 3 | 26.658 | 2.41 | 539218 |
| Total | | 100 | 22379201 |

95879





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PEPTIDE DATA SHEET

LOT NO: 95880

NAME: G713

SEQUENCE: Myr-LKK-amide

EXPECTED MASS: 596.5±1.0

AVERAGE MASS: 596.7

SOLUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 95.91%

COMMENTS: white to off-white powder or lumps

DATE: 07/28/2014

VERIFIED BY: Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95880

Analyzed:07/25/14 15:17 Vail Number: 95880

Reported:07/28/14 13:34 Volume:20.0ul

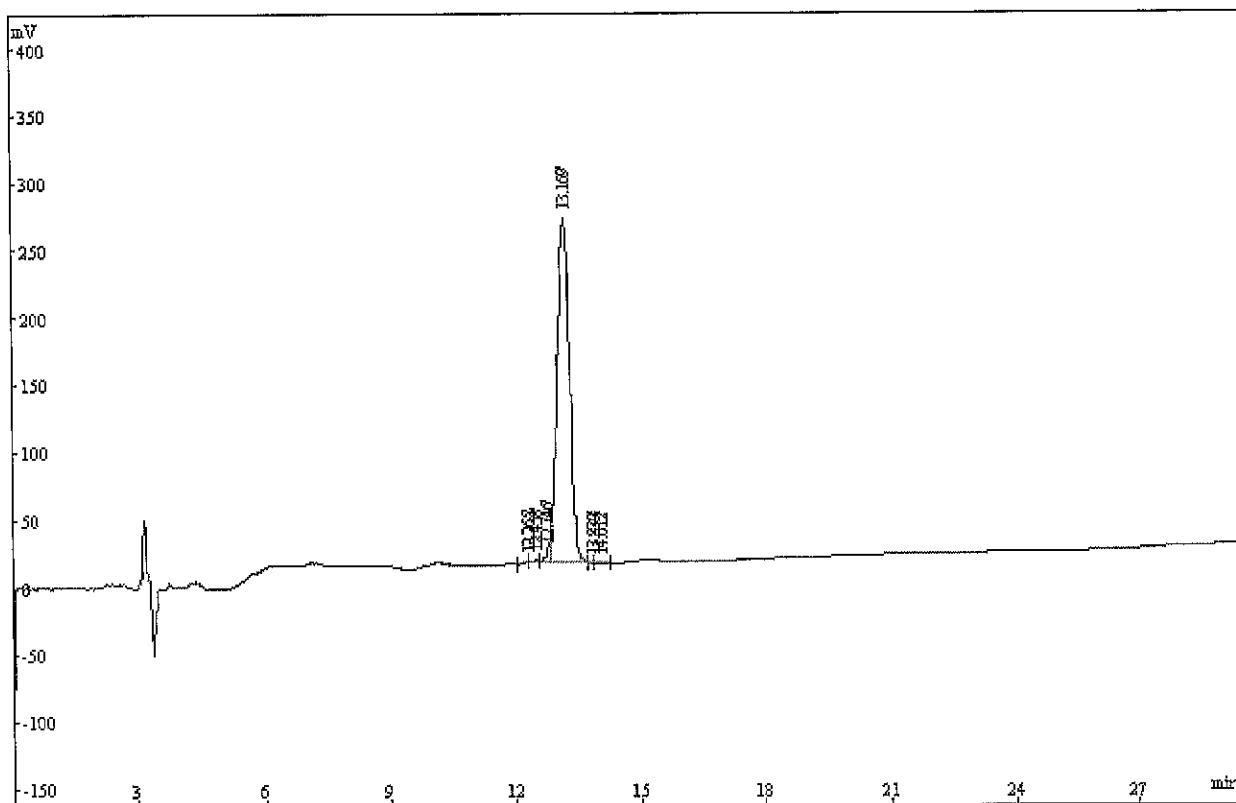
Column Type: 250*4.6mm Kromasil C4 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95880\95880.hw:



| No. | RT | Area% | Area |
|-------|--------|---------|---------|
| 1 | 12.265 | 0.2164 | 11853 |
| 2 | 12.458 | 0.5815 | 31860 |
| 3 | 12.746 | 3.104 | 170051 |
| 4 | 13.169 | 95.91 | 5254659 |
| 5 | 13.839 | 0.05101 | 2795 |
| 6 | 14.012 | 0.1306 | 7156 |
| Total | | 100 | 5478374 |

95880

60

40

20

0

1000

2000

3000

596.7+/-

616.8544

624.7+/-

0 1000 2000 3000



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PEPTIDE DATA SHEET

LOT NO: 95881

NAME: G714

SEQUENCE: Myr-RRR-amide

EXPECTED MASS: 695.5 ± 1.0

AVERAGE MASS: 695.5

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 95.77%

COMMENTS: white to off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY: Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95881

Analyzed:07/19/14 09:55 Vail Number: 95881

Reported:07/23/14 12:59 Volume:20.0ul

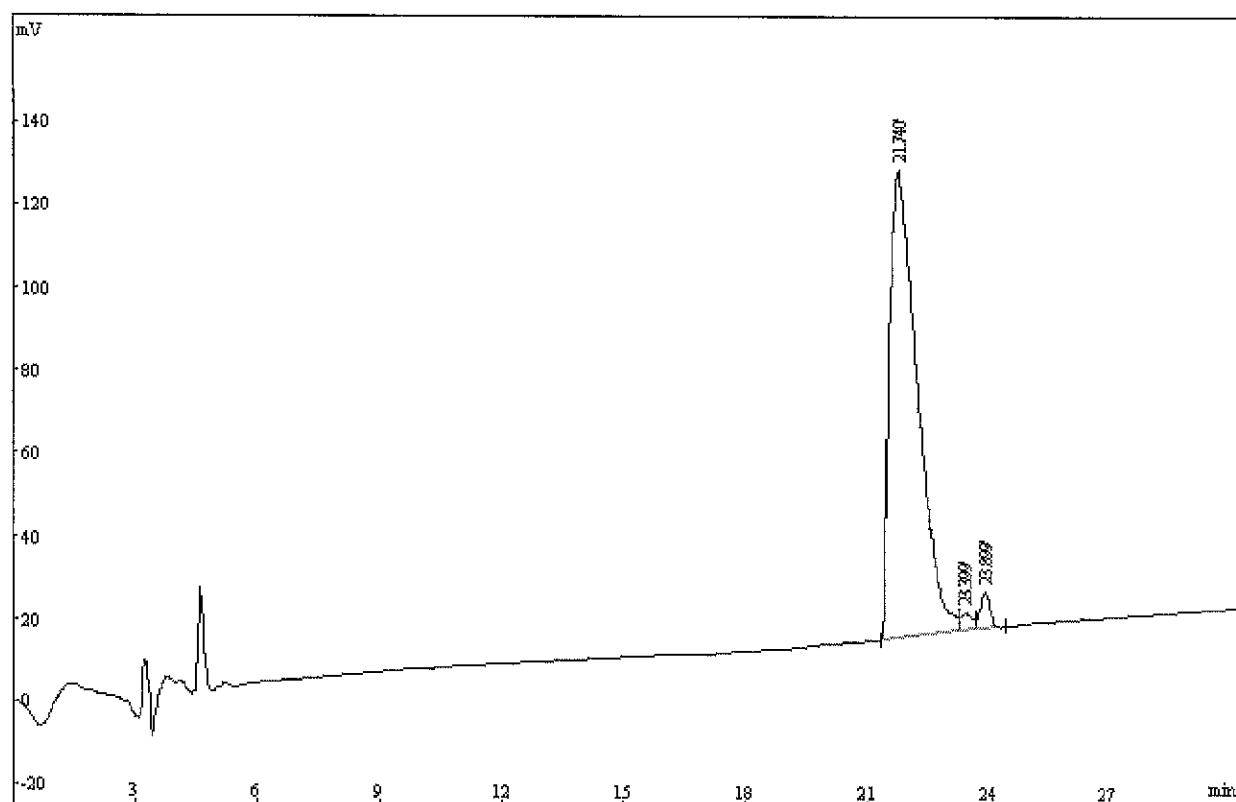
Column Type: 250*4.6mm Kromasil C18 5 μ

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 25:75 t=30.0mins 65:35

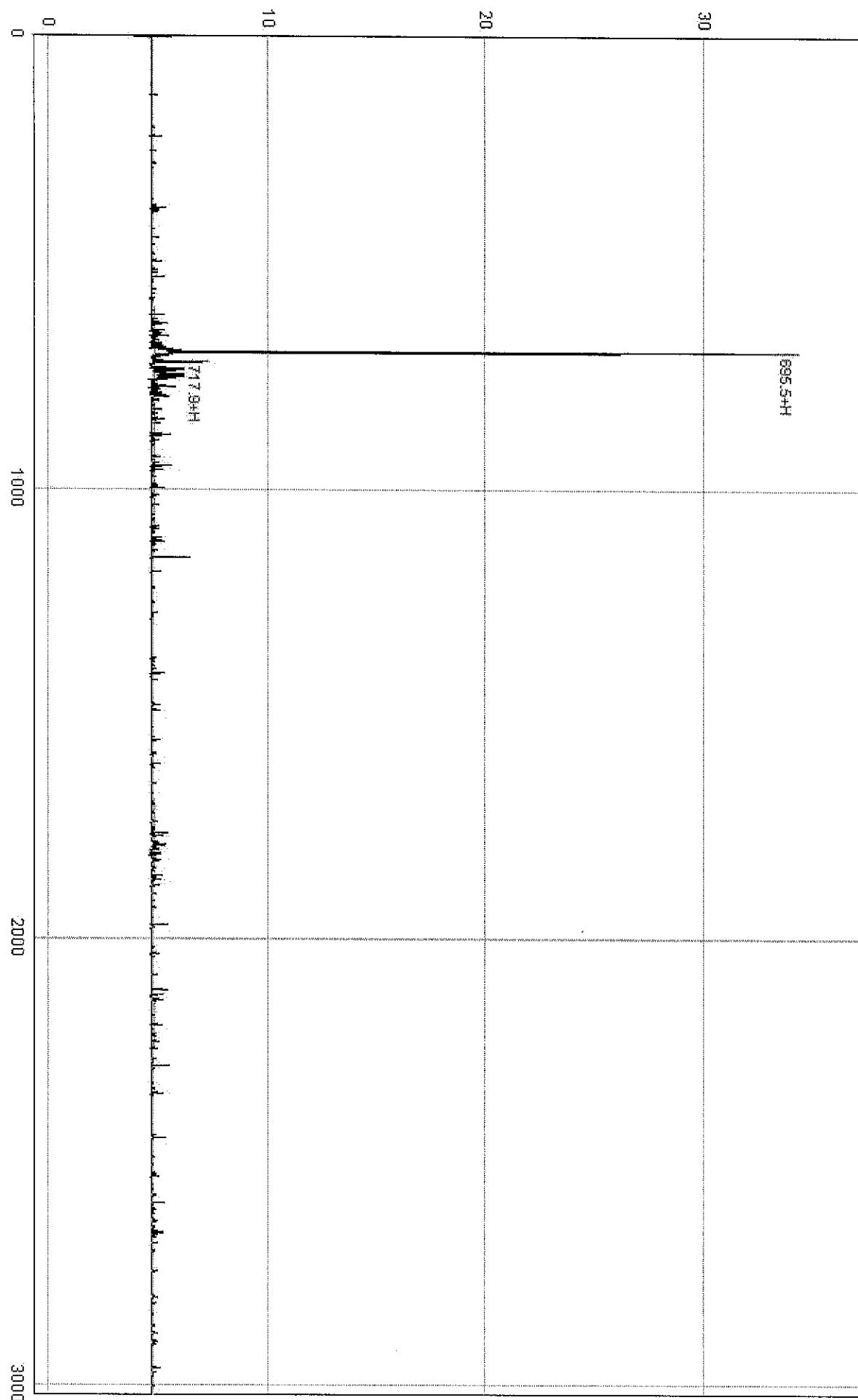
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95881\95881.hw;



| No. | RT | Area% | Area |
|-------|--------|-------|---------|
| 1 | 21.740 | 95.77 | 5412808 |
| 2 | 23.399 | 1.516 | 85673 |
| 3 | 23.899 | 2.712 | 153262 |
| Total | | 100 | 5651743 |

95881





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PEPTIDE DATA SHEET

LOT NO: 95882

NAME: G715

SEQUENCE: Myr-LLK-amide

not soluble

EXPECTED MASS: 581.5±1.0

AVERAGE MASS: 581.4

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 96.77%

COMMENTS: white to off-white powder or lumps

DATE: 07/28/2014

VERIFIED BY:

Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95882

Analyzed:07/25/14 15:53 Vail Number: 95882

Reported:07/28/14 13:36 Volume:20.0ul

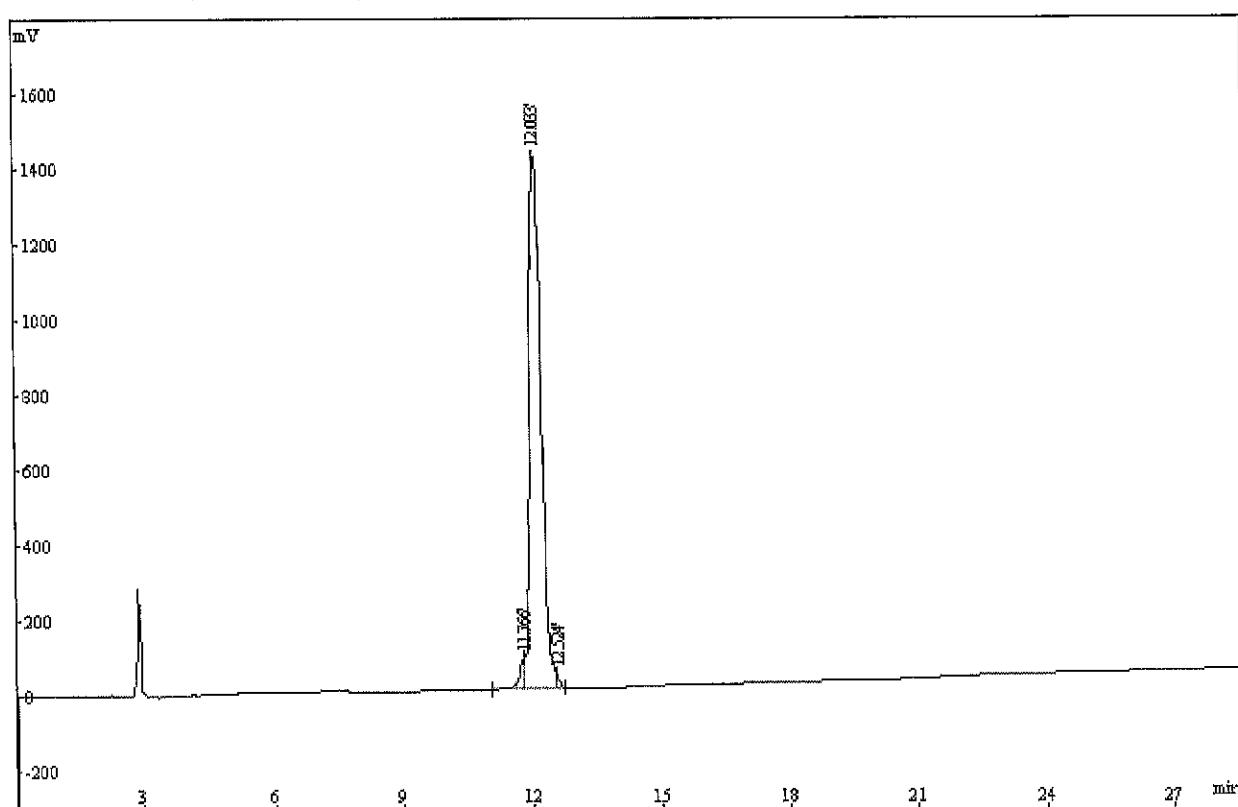
Column Type: 250*4.6mm Kromasil C4 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95882\95882.hw:



| No. | RT | Area% | Area |
|-------|--------|--------|----------|
| 1 | 11.766 | 2.648 | 735123 |
| 2 | 12.033 | 96.77 | 26862848 |
| 3 | 12.524 | 0.5836 | 162008 |
| Total | | 100 | 27759979 |

95882

60

40

20

0

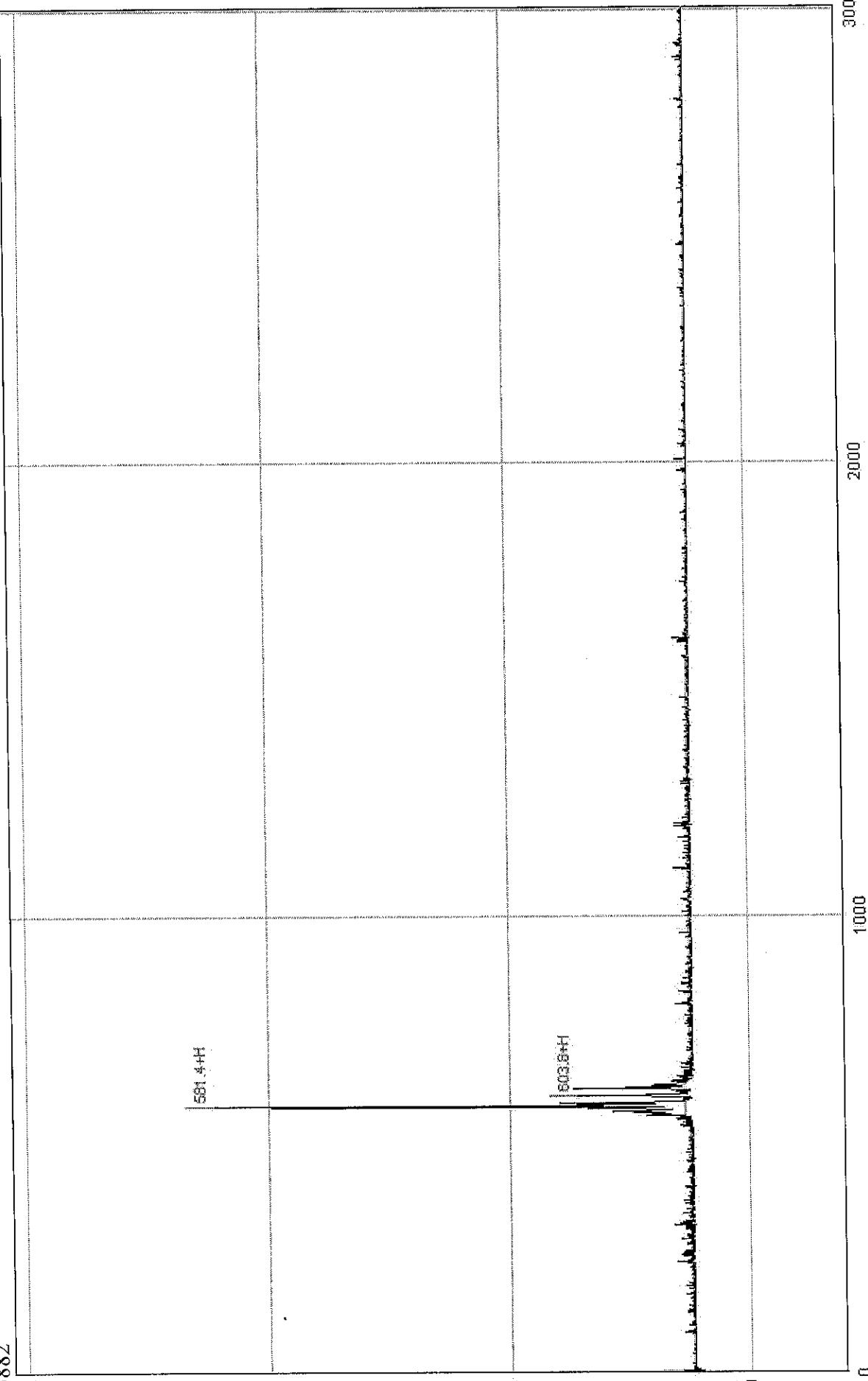
1000

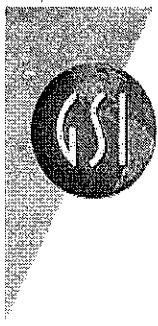
2000

3000

503.641

551.341





Genemed Synthesis, Inc.
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Toll free (800) 344-5337; **Phone:** (210) 745-5988; **Fax** (210) 745-5992
Email: info@genemedsyn.com, **Website:** www.genemedsyn.com

PEPTIDE DATA SHEET

LOT NO: 95872

NAME: G705

SEQUENCE: Myr-EE-amide

EXPECTED MASS: 485.2 ± 1.0

AVERAGE MASS: 485.5

SOLUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 96.30%

COMMENTS: white to off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY:

Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95872

Analyzed:07/21/14 14:24 Vail Number: 95872

Reported:07/23/14 13:11 Volume:20.0ul

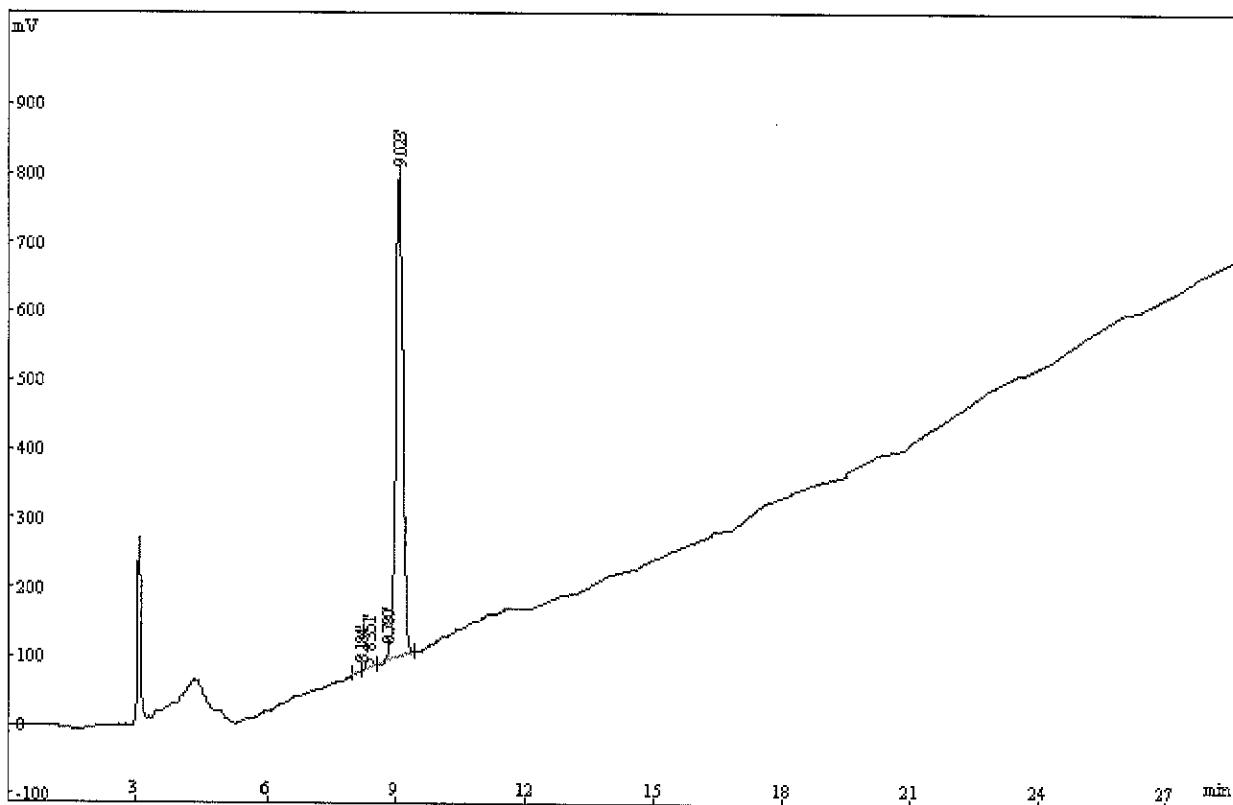
Column Type: 250*4.6mm Kromasil C4 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 10:90 t=30.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95872\95872.hw:



| No. | RT | Area% | Area |
|-------|-------|--------|---------|
| 1 | 8.184 | 0.2637 | 22337 |
| 2 | 8.351 | 2.156 | 182625 |
| 3 | 8.780 | 1.282 | 108588 |
| 4 | 9.023 | 96.3 | 8155470 |
| Total | | 100 | 8469020 |

95872

25

20

15

485.5+H

10

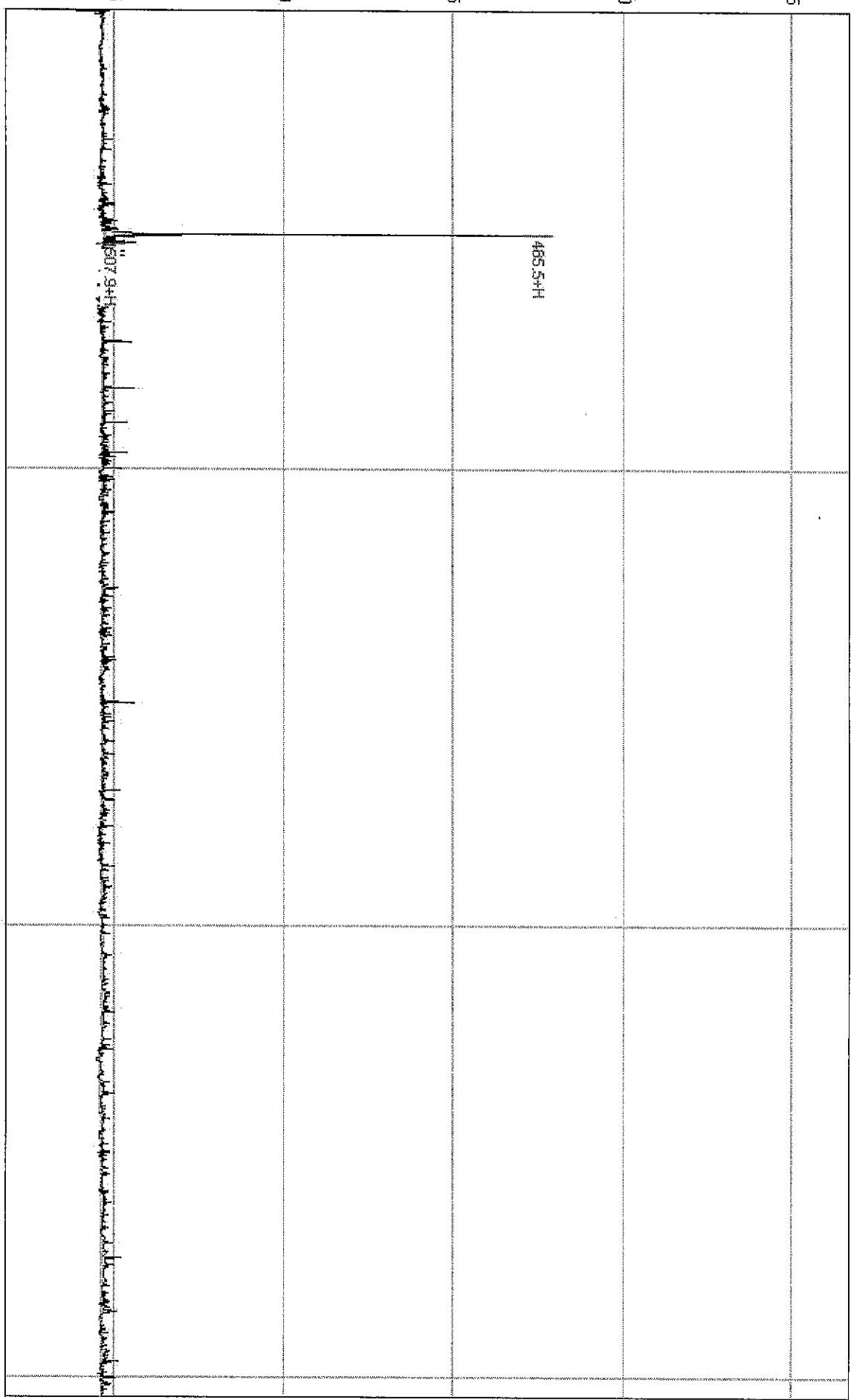
5

1000

2000

3000

0





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PEPTIDE DATA SHEET

LOT NO: 95873

NAME: G706

SEQUENCE: Myr-LE-amide

EXPECTED MASS: 469.3 ± 1.0

AVERAGE MASS: 469.4

SOLUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 96.27%

COMMENTS: white to off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY: Zhang -4-q

Customer Peptide HPLC Report

Sample name: 95873

Analyzed:07/21/14 17:36 Vail Number: 95873

Reported:07/23/14 10:04 Volume:20.0ul

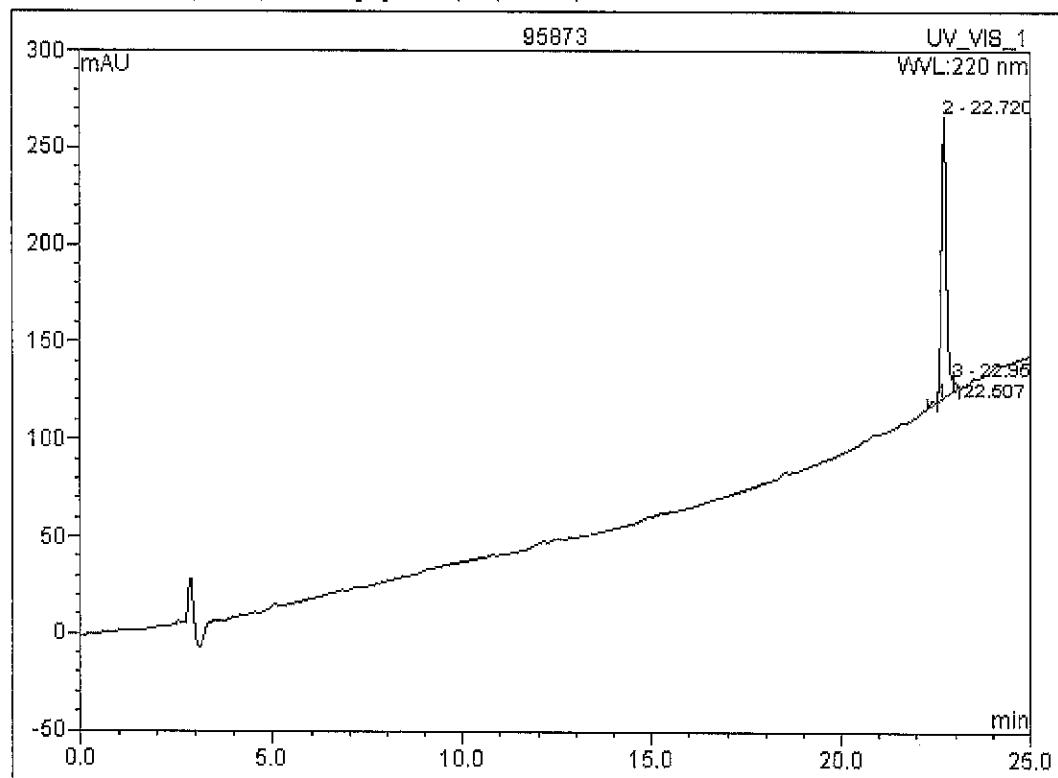
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 30:70 t=25.0mins 100:0

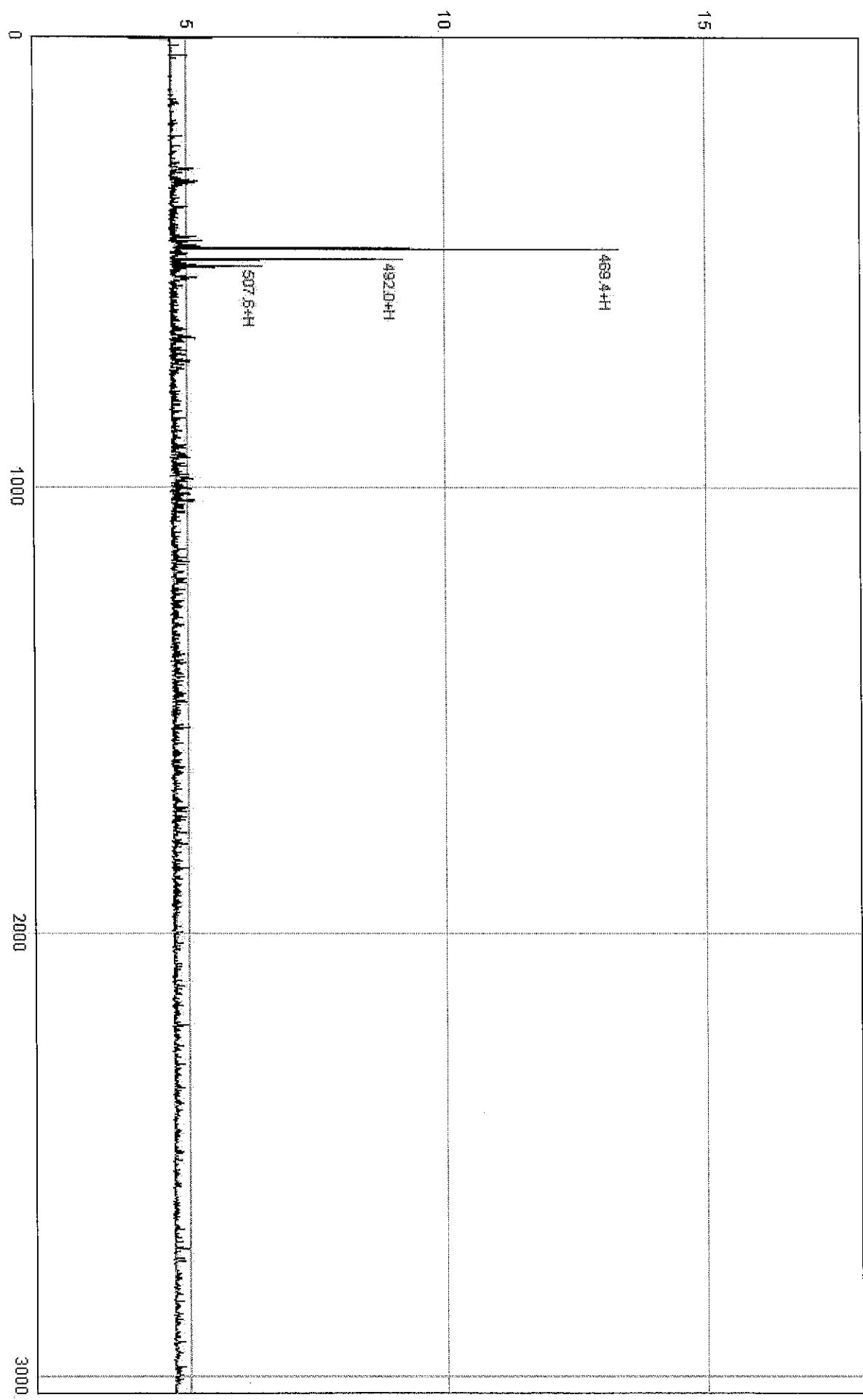
Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95873\95873.hw:



| No. | Ref.Time min | Height mAU | Area mAU*min | Rel.Area % |
|---------------|-----------------|---------------|-----------------|----------------|
| 1 | 22.51 | 0.707 | 0.293 | 1.26 |
| 2 | 22.72 | 143.953 | 22.467 | 96.27 |
| 3 | 22.95 | 5.777 | 0.576 | 2.47 |
| Total: | | | 23.336 | 100.000 |

95873





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PEPTIDE DATA SHEET

LOT NO: 95874

NAME: G707

SEQUENCE: Myr-WE-amide

EXPECTED MASS: 542.3±1.0

AVERAGE MASS: 542.3

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 96.81%

COMMENTS: yellow powder or lumps

DATE: 07/23/2014

VERIFIED BY:

Zhang Z.Q.

Customer Peptide HPLC Report

Sample name: 95874

Analyzed:07/21/14 17:30 Vail Number: 95874

Reported:07/23/14 13:13 Volume:20.0ul

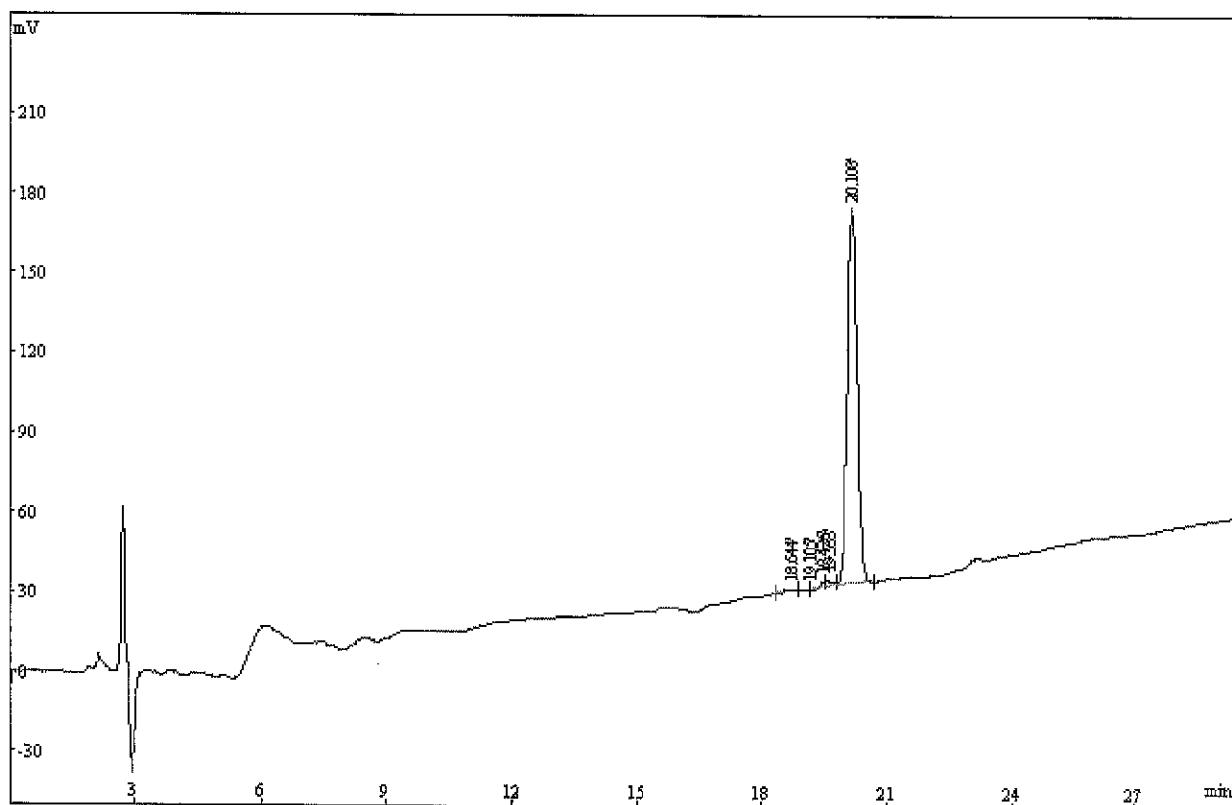
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 05:95 t=30.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95874\95874.hw:



| No. | RT | Area% | Area |
|-------|--------|---------|---------|
| 1 | 18.644 | 0.7883 | 19939 |
| 2 | 19.105 | 0.05414 | 1369 |
| 3 | 19.426 | 0.8967 | 22680 |
| 4 | 19.533 | 1.45 | 36675 |
| 5 | 20.108 | 96.81 | 2448707 |
| Total | | 100 | 2529370 |

95874

100

75

50

25

0

1000

2000

3000

565.7+1

542.3+1

581.7+1





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PEPTIDE DATA SHEET

LOT NO: 95875

NAME: G708

SEQUENCE: Myr-EW-amide

EXPECTED MASS: 542.3±1.0

AVERAGE MASS: 542.4

SOULUBILITY: Not tested

AMOUNT DELIVERED: 19mg

PURITY: 95.85%

COMMENTS: white to off-white powder or lumps

DATE: 07/23/2014

VERIFIED BY: Zhang -Z.Q.

Customer Peptide HPLC Report

Sample name: 95875

Analyzed:07/21/14 16:25 Vail Number: 95875

Reported:07/23/14 10:06 Volume:20.0ul

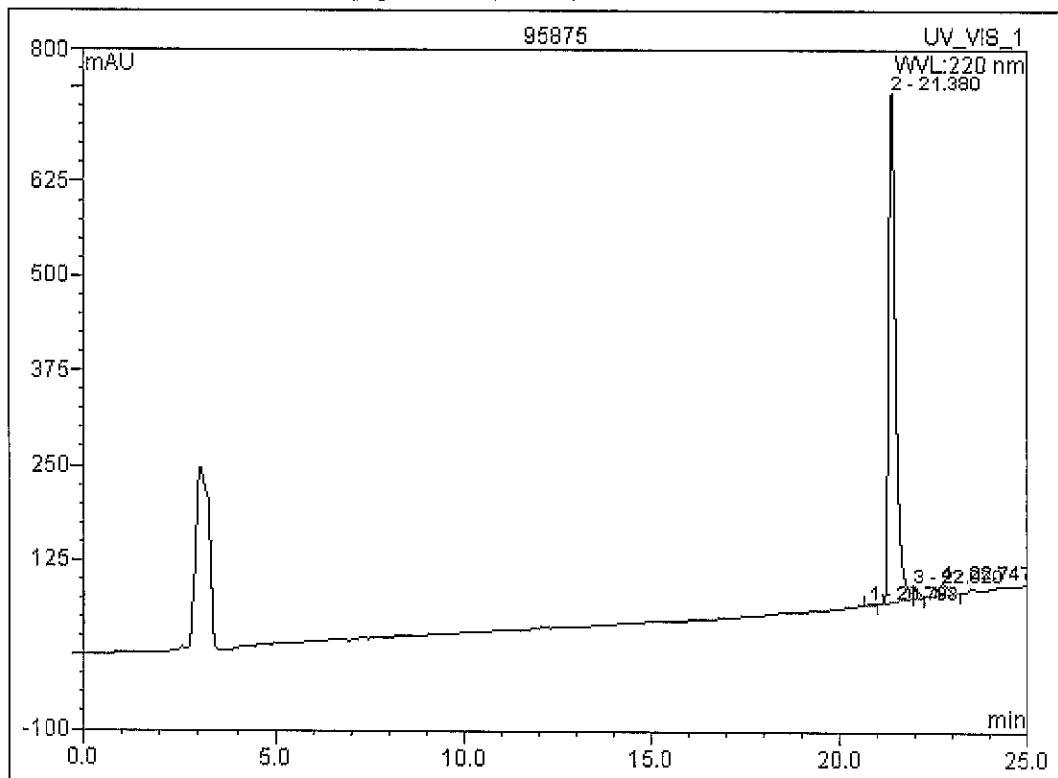
Column Type: 250*4.6mm Kromasil C18 5u

Solvent A:0.1%TFA acetonitrile Solvent B:0.1%TFA Water

Method: Gradient A:B, t=0.0mins 30:70 t=25.0mins 100:0

Wavelength: 220nm

Data Path: D:\HPLC\custom peptide\GS\95875\95875.hw;



| No. | Ret.Time min | Height mAU | Area mAU*min | Rel.Area % |
|---------------|-----------------|---------------|-----------------|----------------|
| 1 | 20.79 | 1.956 | 0.314 | 0.21 |
| 2 | 21.38 | 671.885 | 142.897 | 95.85 |
| 3 | 22.02 | 13.916 | 2.058 | 1.38 |
| 4 | 22.75 | 15.713 | 3.810 | 2.56 |
| Total: | | | 149.078 | 100.000 |

95875

