

Protease Profiling Using a Fluorescent Domino Peptide Cocktail

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Supporting Information

- Data for reference fragments
- MS-traces of cocktail peptides
- HPLC traces of cocktail references and protease fingerprints

1P1 (*KDESY): Starting with 70 mg of Fmoc-Tyr_(t-bu)-Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-KDESY-OH (15.1 mg, 0.018 mmol, 41 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₈H₄₆N₆O₁₆ [M+H]⁺: 843.3048, found 843.3072.

1P2 (*KDES): Starting with 70 mg of Fmoc-Ser_(t-bu)-Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-KDES-OH (15.0 mg, 0.022 mmol, 50 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₉H₃₈N₅O₁₄ [M+H]⁺: 680.2415, found 680.2421.

1P3 (*KDE): Starting with 80 mg of Fmoc-Glu_(ot-bu)-Wang resin (0.043 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-KDE-OH (18.3 mg, 0.025 mmol, 58 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₆H₃₂N₄O₁₂ [M+H]⁺: 593.2094, found 593.2090.

1P4 (*KD): Starting with 70 mg of Fmoc-Asp_(ot-bu)-Wang resin (0.042 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-KD-OH (11.8 mg, 0.025 mmol, 60 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₁H₂₆N₃O₉ [M+H]⁺: 464.1669, found 464.1678.

1P5 (*K): Starting with 70 mg of Fmoc-Lys_(boc)-Wang resin (0.046 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-K-OH (4.2 mg, 0.012 mmol, 26 %) as colorless foamy solid. ESI⁺-MS: calc. for C₁₇H₂₁N₂O₆ [M+H]⁺: 349.1399, found 349.1393.

2P1 (*AVPER): Starting with 70 mg of Fmoc-Arg_(pbf)-Wang resin (0.046 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-AVPER-OH

(17.4 mg, 0.023 mmol, 50 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₅H₄₉N₈O₁₂ [M+H]⁺: 773.3469, found 773.3467.

2P2 (*AVPE): Starting with 70 mg of Fmoc- Glu_(ot-bu)-Wang resin (0.043 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-AVPE-OH (5.8 mg, 0.0094 mmol, 22 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₉H₃₅N₄O₁₁ (Na⁺) [M+H]⁺: 639.2278, found 639.2268.

2P3 (*AVP): Starting with 70 mg of Fmoc-Pro-Wang resin (0.063 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-AVP-OH (2 mg, 0.004 mmol, 6 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₄H₂₉N₃O₈ (Na⁺) [M+H]⁺: 510.1852, found 510.1867.

2P4 (*AV): Starting with 100 mg of Fmoc-Val-Wang resin (0.051 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-AV-OH (11.5 mg, 0.029 mmol, 57 %) as colorless foamy solid. ESI⁺-MS: calc. for C₁₉H₂₂N₂O₇ [M+H]⁺: 390.142701, found 390.143070.

2P5 (*A): Starting with 130 mg of Fmoc-Ala-Wang resin (0.042 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-A-OH (3.9 mg, 0.013 mmol, 31 %) as colorless foamy solid. ESI⁺-MS: calc. for C₁₄H₁₃N₁O₆ [M+H]⁺: 290.074287, found 290.074220.

3P1 (*EFVGS): Starting with 70 mg of Fmoc- Ser_(t-bu)-Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-EFVGS-OH (8.0 mg, 0.011 mmol, 25 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₅H₄₁N₅O₁₃ [M+H]⁺: found 739.5828.

3P2 (*EFVG): Starting with 70 mg of Fmoc- Gly-Wang resin (0.046 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-EFVG-OH (11.0 mg, 0.017 mmol, 37 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₂H₃₆N₄O₁₁Na [M+H]⁺: 675.2278, found 675.2298.

3P3 (*EFV): Starting with 100 mg of Fmoc-Val-Wang resin (0.051 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-EFV-OH (9.0 mg, 0.015 mmol, 29 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₀H₃₃N₃O₁₀ [M+H]⁺: 596.2244, found 596.2274.

3P4 (*EF): Starting with 61 mg of Fmoc-Phe-Wang resin (0.061 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-EF-OH (13.8 mg, 0.028 mmol, 46 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₅H₂₅N₂O₉ [M+H]⁺: 497.1560, found 497.1577.

3P5 (*E): Starting with 80 mg of Fmoc- Glu_(ot-bu)-Wang resin (0.043 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-E-OH (12.0 mg, 0.034 mmol, 79 %) as colorless foamy solid. ESI⁺-MS: calc. for C₁₆H₁₆N₁O₈ [M+H]⁺: 349.079767, found 349.081210.

4P1 (*YARKL): Starting with 70 mg of Fmoc- Leu -Wang resin (0.043 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-YARKL-OH (15.8 mg, 0.019 mmol, 44 %) as colorless foamy solid. ESI⁺-MS: calc. for C₄₁H₅₈N₉O₁₁ [M+H]⁺: 852.4255, found 852.4249.

4P2 (*YARK): Starting with 69 mg of Fmoc- Lys_(boc) -Wang resin (0.046 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-YARK-OH (23.5 mg, 0.032 mmol, 70 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₅H₄₇N₈O₁₀ [M+H]⁺: 739.3412, found 739.3398.

4P3 (*YAR): Starting with 79 mg of Fmoc- Arg_(pbf)-Wang resin (0.046 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-YAR-OH (16 mg, 0.026 mmol, 57 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₉H₃₅N₆O₉ [M+H]⁺: 611.2465, found 611.2471.

4P4 (*YA): Starting with 130 mg of Fmoc- Ala -Wang resin (0.042 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-YA-OH (2.5 mg, 0.0055 mmol, 12 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₃H₂₂N₂O₈Na [M+H]⁺: 477.1273, found 477.1271.

4P5 (*Y): Starting with 70 mg of Fmoc- Tyr_(t-bu)-Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-Y-OH (5.8 mg, 0.015 mmol, 34 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₀H₁₈NO₇ [M+H]⁺: 384.1083, found 384.1087.

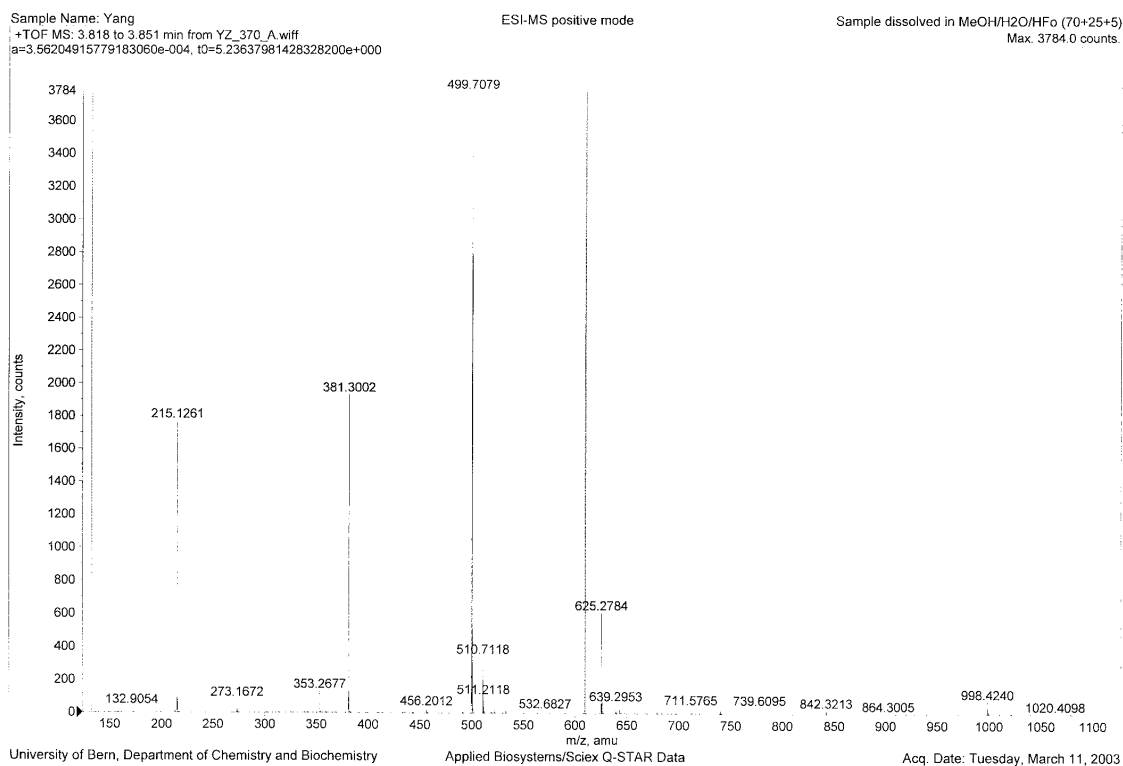
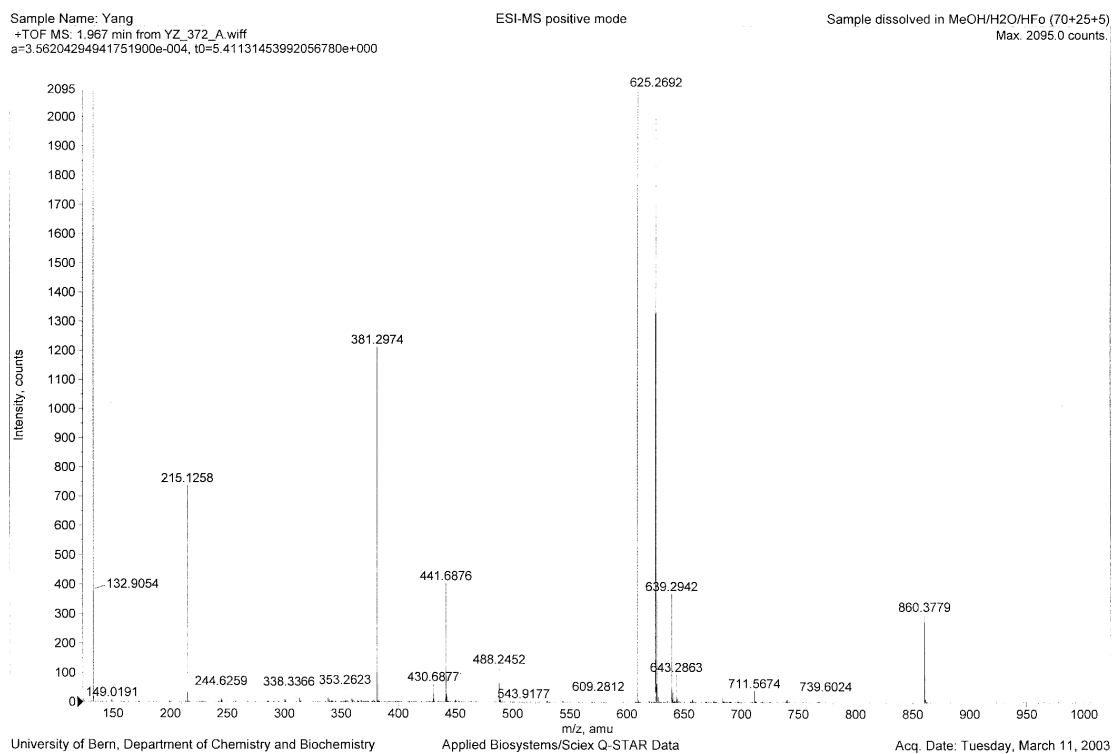
5P1 (*LKYFD): Starting with 70 mg of Fmoc- Asp_(ot-bu)-Wang resin (0.042 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-LKYFD-OH (18.4 mg, 0.021 mmol, 50 %) as colorless foamy solid. ESI⁺-MS: calc. for C₄₅H₅₅N₆O₁₃ [M+H]⁺: 887.3827, found 887.3865.

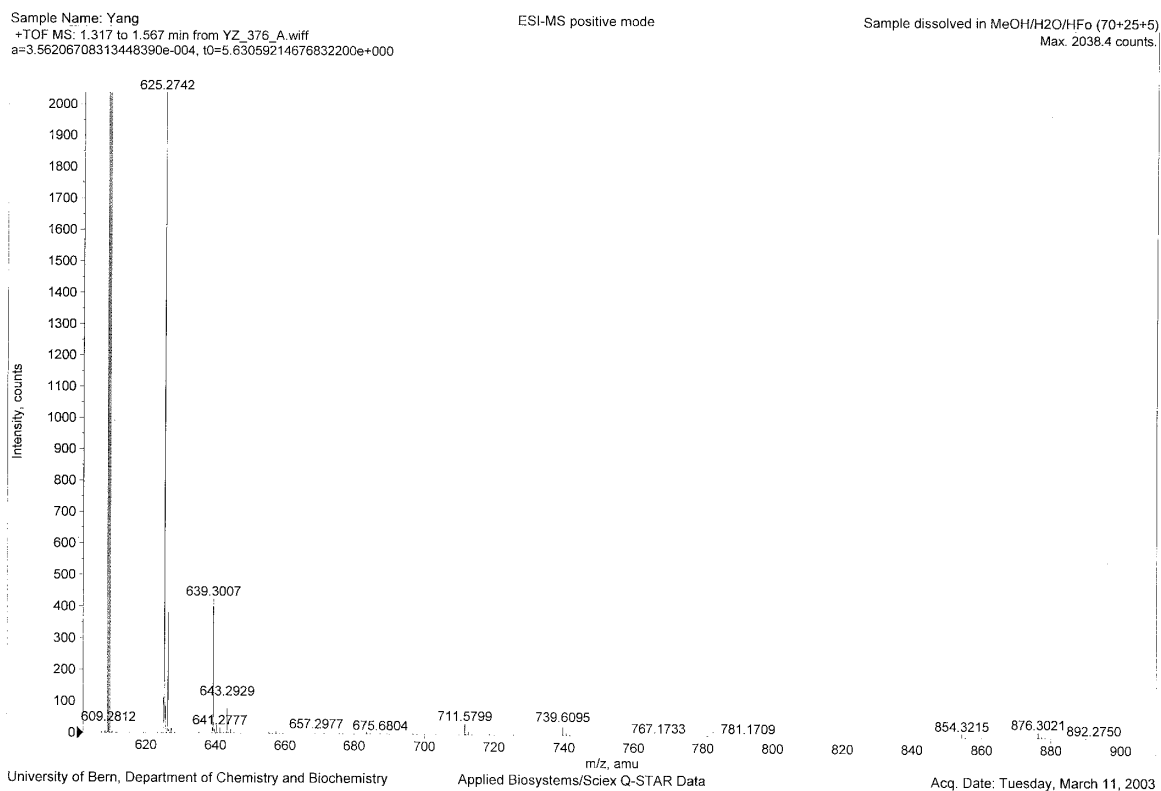
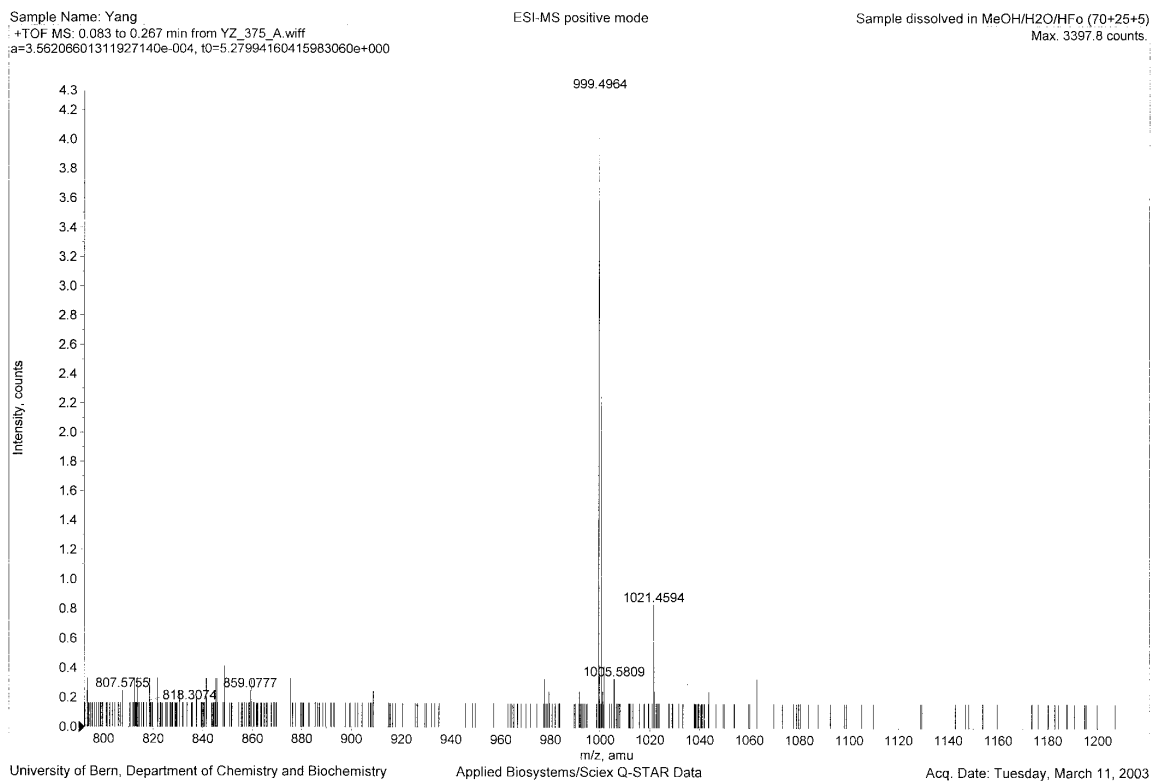
5P2 (*LKYF): Starting with 70 mg of Fmoc- Phe -Wang resin (0.061 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-LKYF-OH (20.1 mg, 0.026 mmol, 43 %) as colorless foamy solid. ESI⁺-MS: calc. for C₄₁H₅₀N₅O₁₀ [M+H]⁺: 772.3557, found 772.3538.

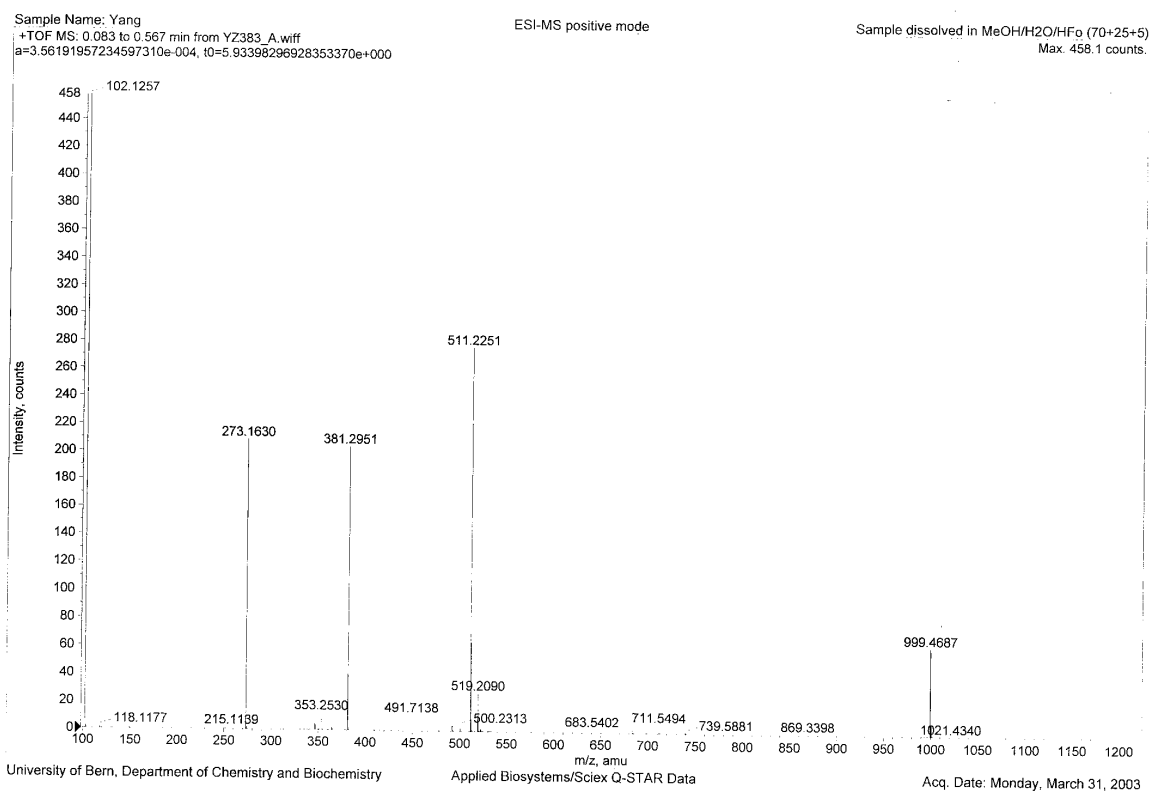
5P3 (*LKY): Starting with 70 mg of Fmoc- Tyr_(t-bu)-Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-LKY-OH (11.4 mg, 0.018 mmol, 41 %) as colorless foamy solid. ESI⁺-MS: calc. for C₃₂H₄₁N₄O₉ [M+H]⁺: 625.2873, found 625.2897.

5P4 (*LK): Starting with 69 mg of Fmoc- Lys_(boc)-Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-LK-OH (11.4 mg, 0.025 mmol, 57 %) as colorless foamy solid. ESI⁺-MS: calc. for C₂₃H₃₂N₃O₇ [M+H]⁺: 462.2260, found 462.2262.

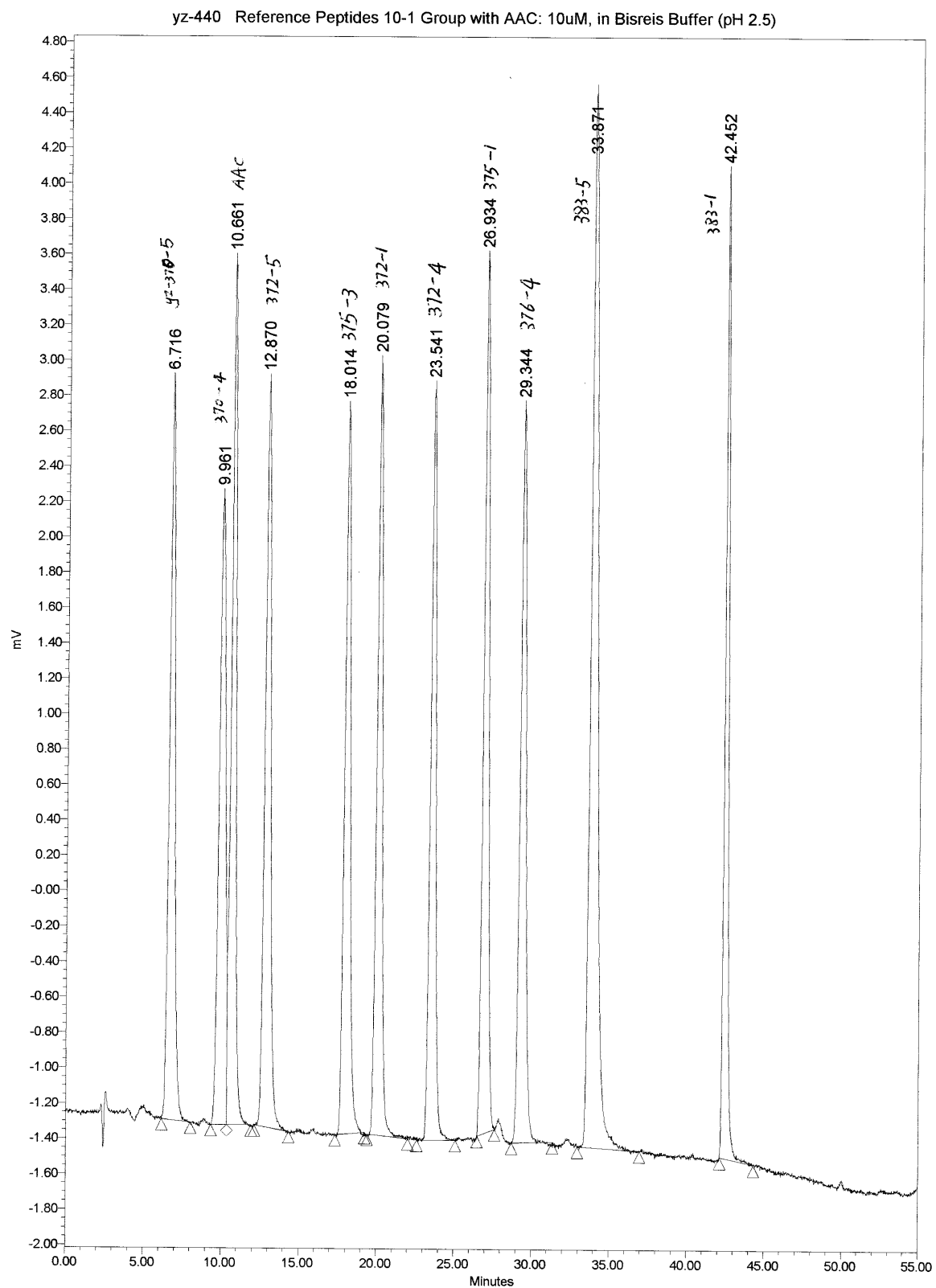
5P5 (*L): Starting with 69 mg of Fmoc- Leu -Wang resin (0.044 mmol), Fmoc-type spps followed by cleavage and purification by preparative RP-HPLC gave AAC-L-OH (2.3 mg, 0.007 mmol, 16 %) as colorless foamy solid. ESI⁺-MS: calc. for C₁₇H₂₀NO₆ [M+H]⁺: 333.121238, found 333.121190.

Peptide 1. ESI⁺-MS: calc. for C₄₄H₆₀N₁₁O₁₆ [M+H]⁺: 998.4219, found 998.4240.**Peptide 2.** ESI⁺-MS: calc. for C₃₈H₅₄N₉O₁₄ [M+H]⁺: 860.3790, found 860.3779.

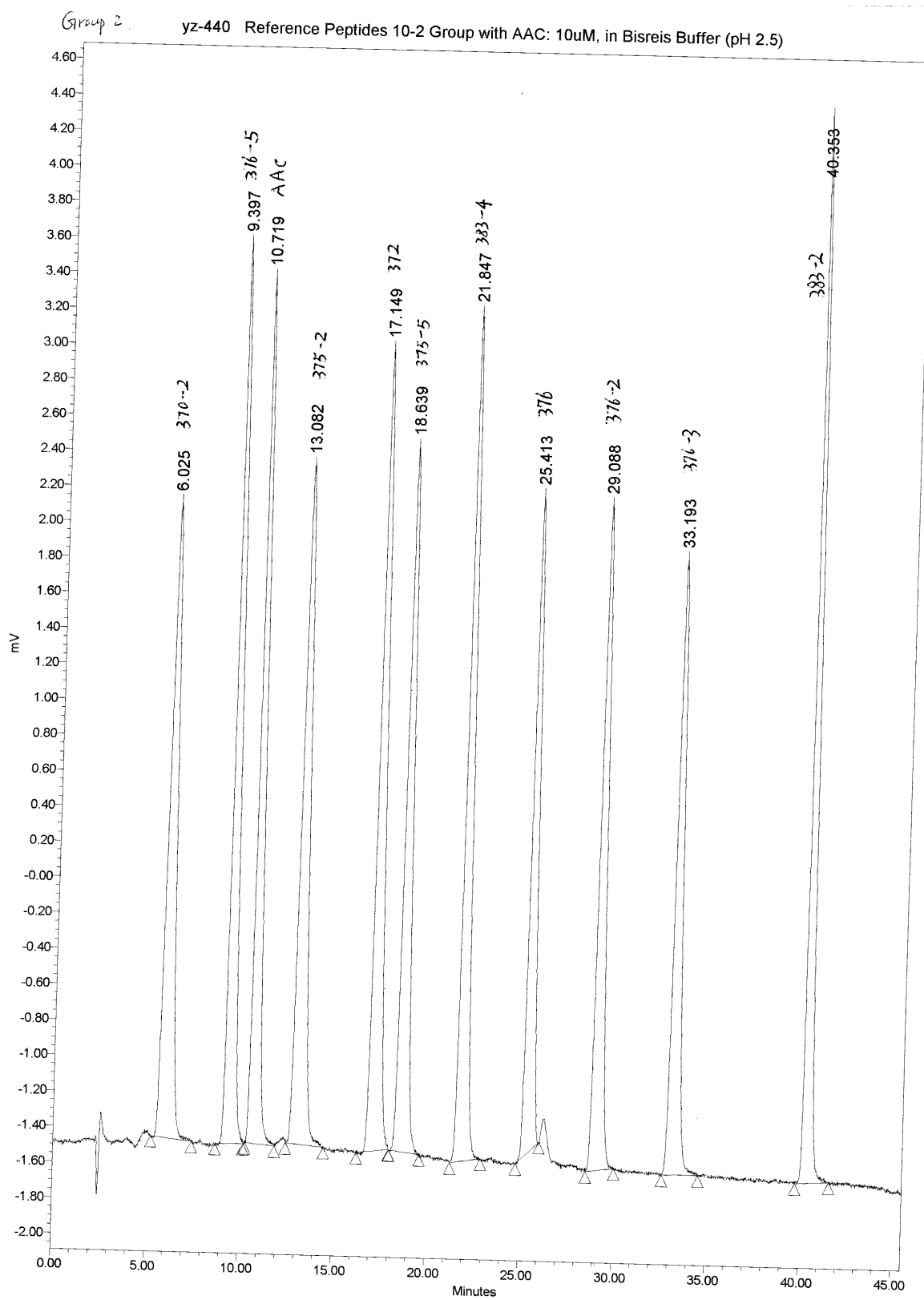
Peptide 3. ESI⁺-MS: calc. for C₃₉H₄₇N₇O₁₅ [M+H]⁺: 854.3208, found 854.3215.**Peptide 4.** ESI⁺-MS: calc. for C₅₀H₆₇N₁₀O₁₂ [M+H]⁺: 999.4939, found 999.4964.

Peptide 5. ESI⁺-MS: calc. for C₅₁H₆₇N₈O₁₃ [M+H]⁺: 999.4827, found 999.4796.

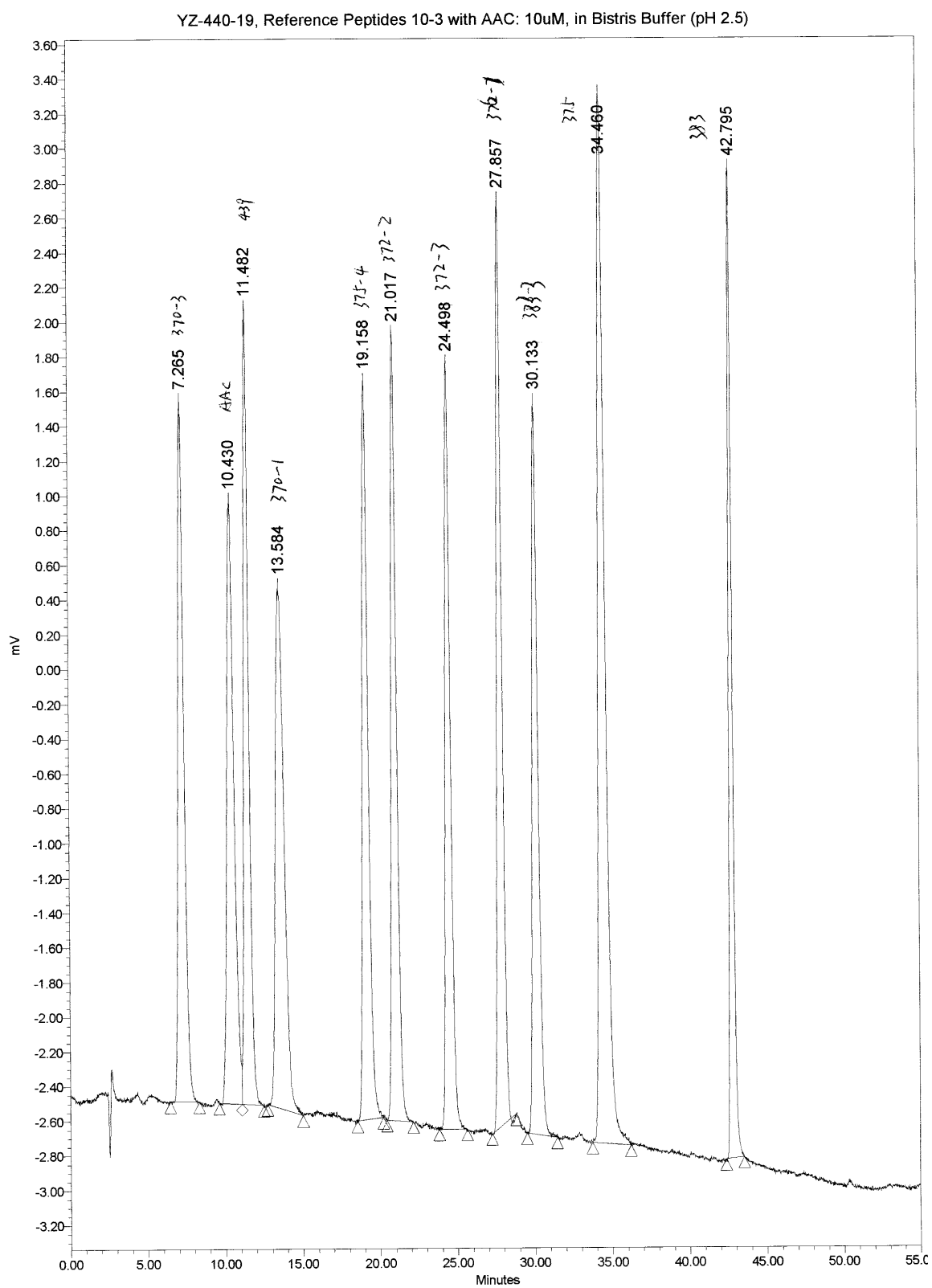
1. HPLC Traces for Reference Group 1



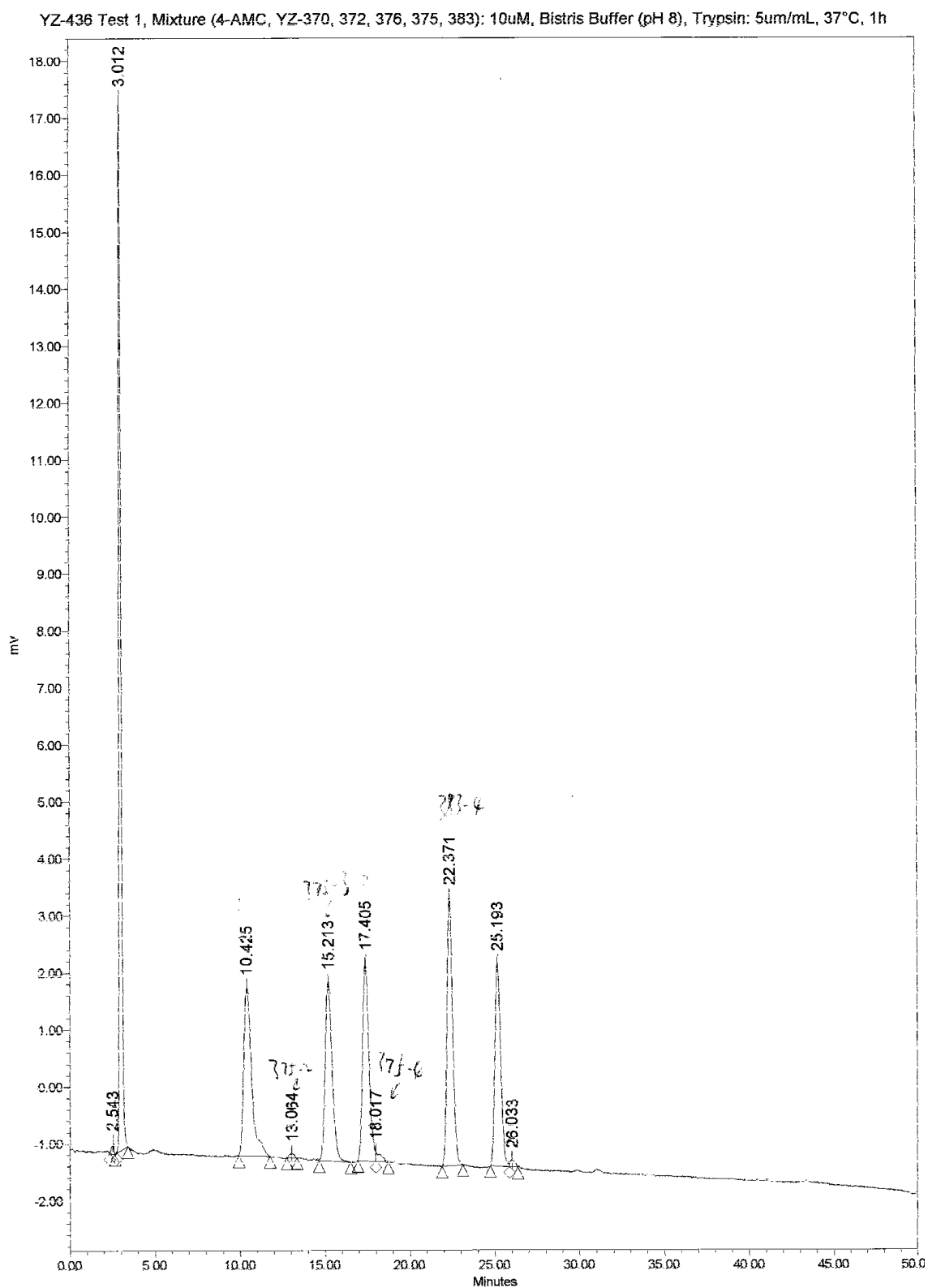
2. HPLC Traces for Reference Group 2



3. HPLC Traces for Reference Group 3

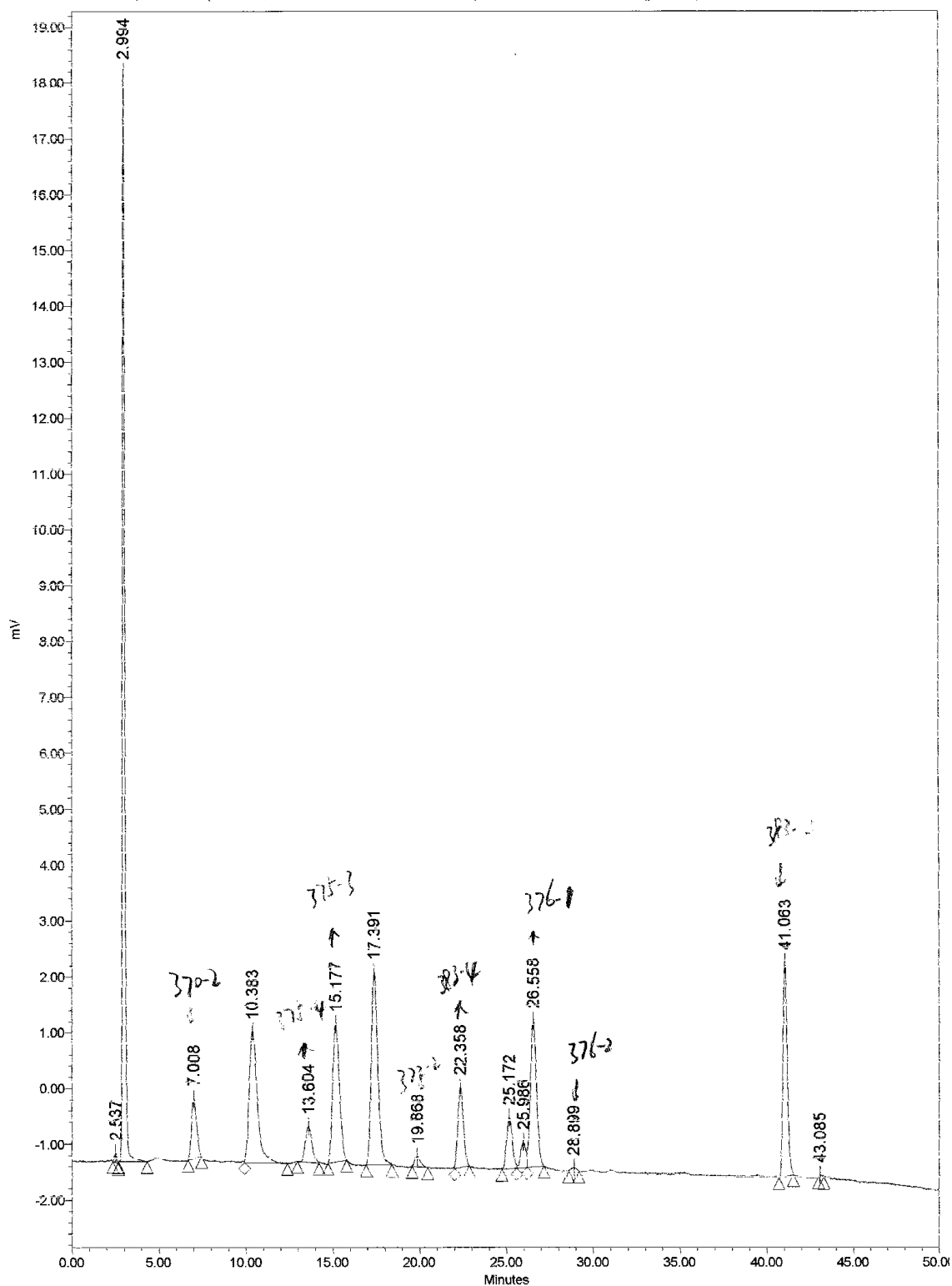


4. HPLC Traces for Trypsin



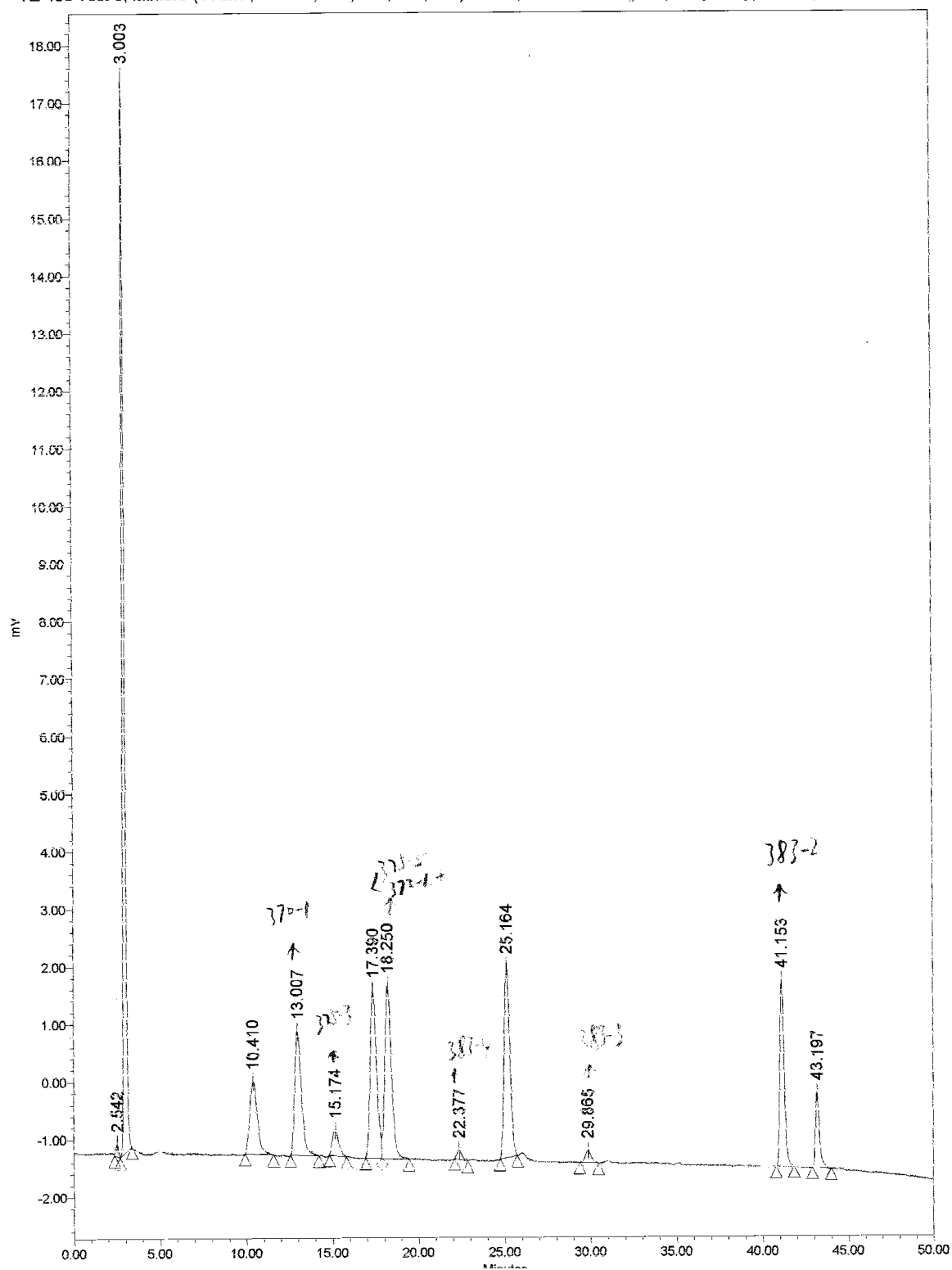
5. HPLC Traces for Subtilisin

YZ-436 Test 2, Mixture (4-AMC, YZ-370, 372, 376, 375, 383): 10uM, Bistris Buffer (pH 6.5), Subtilisin: 5um/mL, 37°C, 1h



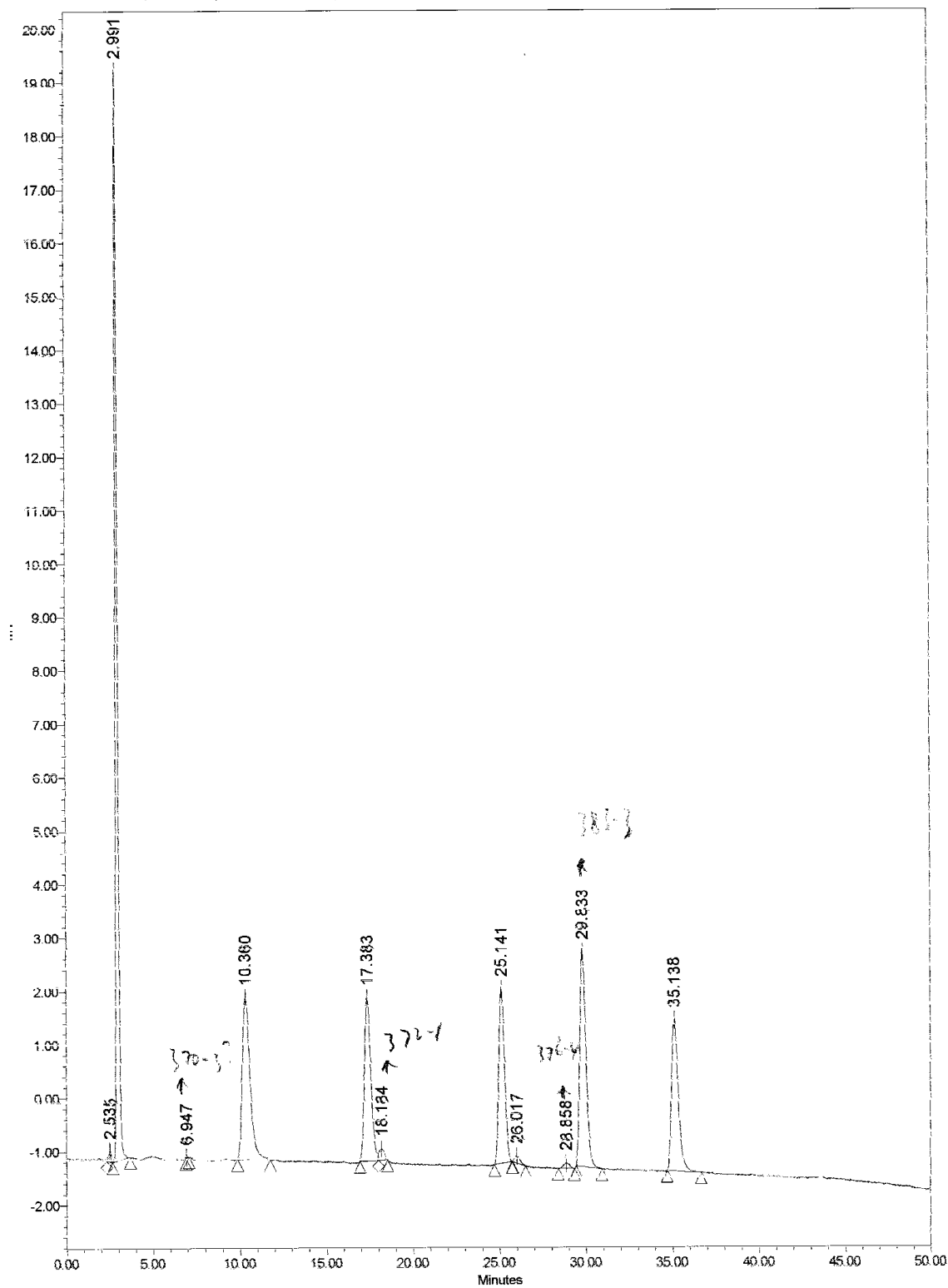
6. HPLC Traces for Chmotrypsin

YZ-436 Test 3, Mixture (4-AMC, YZ-370, 372, 376, 375, 383): 10uM, Bistris Buffer (pH 8), Chymotrypsin: 5ug/mL, 37°C, 1h

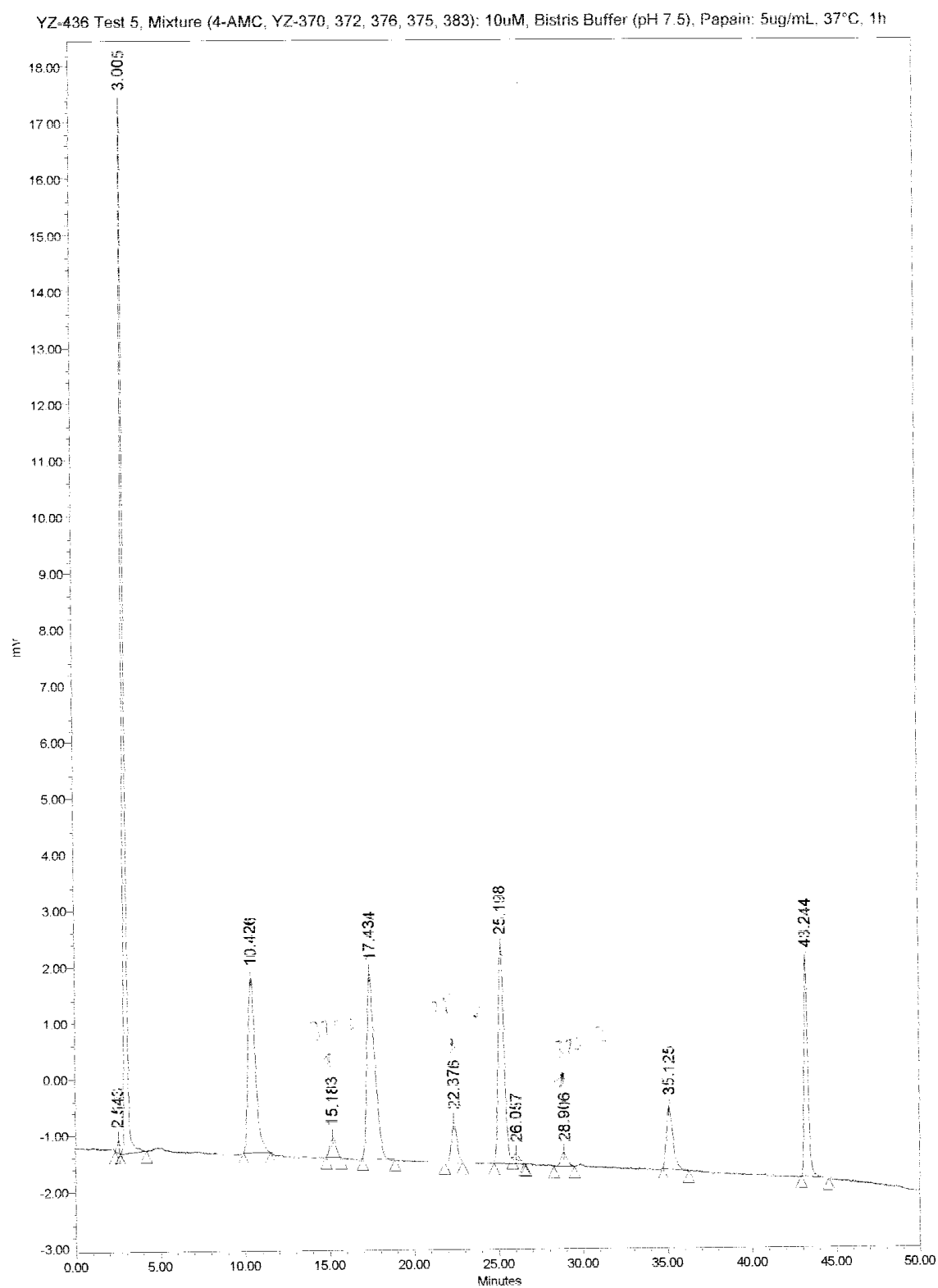


7. HPLC Traces for Pepsin

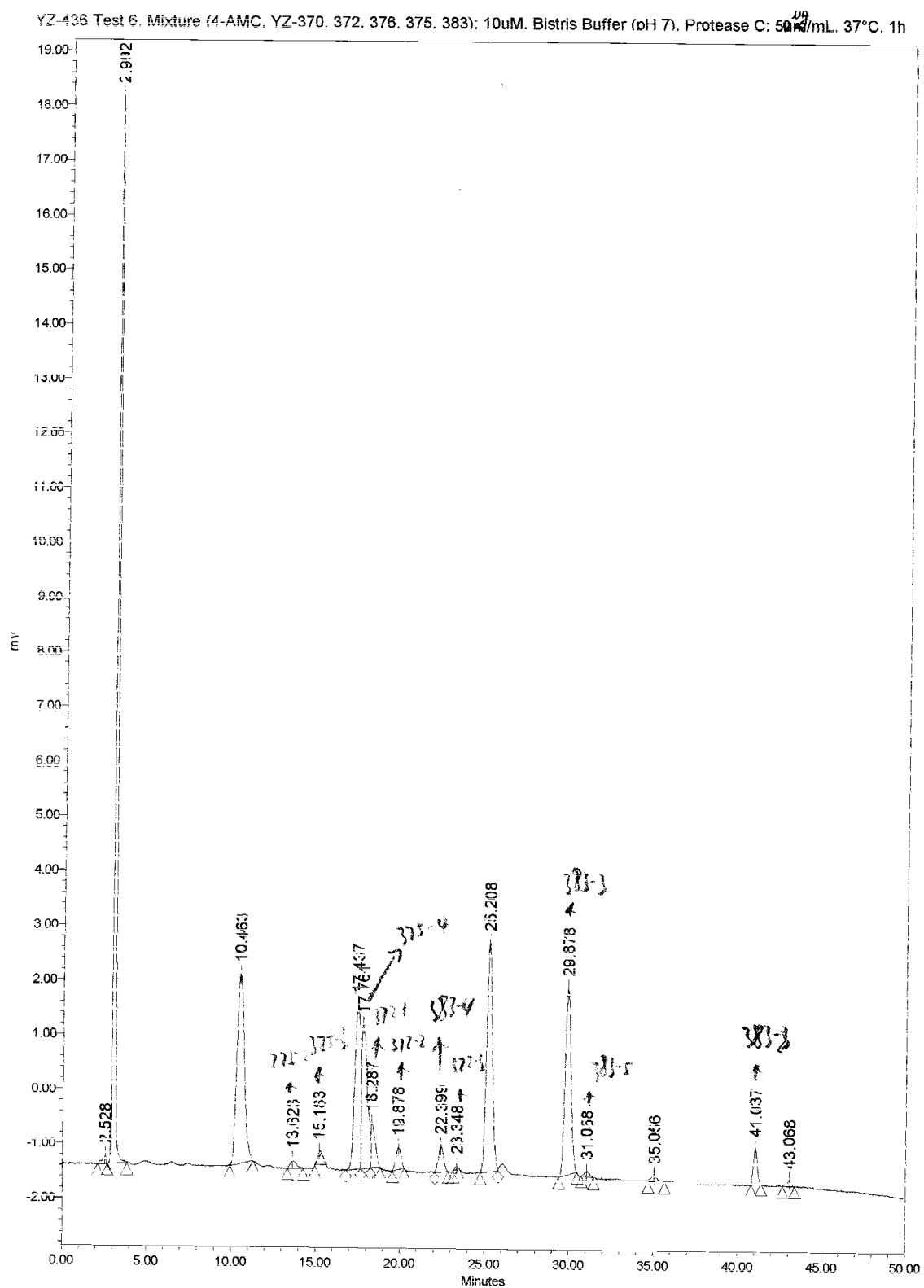
YZ-436 Test 4, Mixture (4-AMC, YZ-370, 372, 376, 375, 383): 10uM, Bistris Buffer (pH 4), Pepsin: 5ug/mL, 37°C, 1h



8. HPLC Traces for Papain

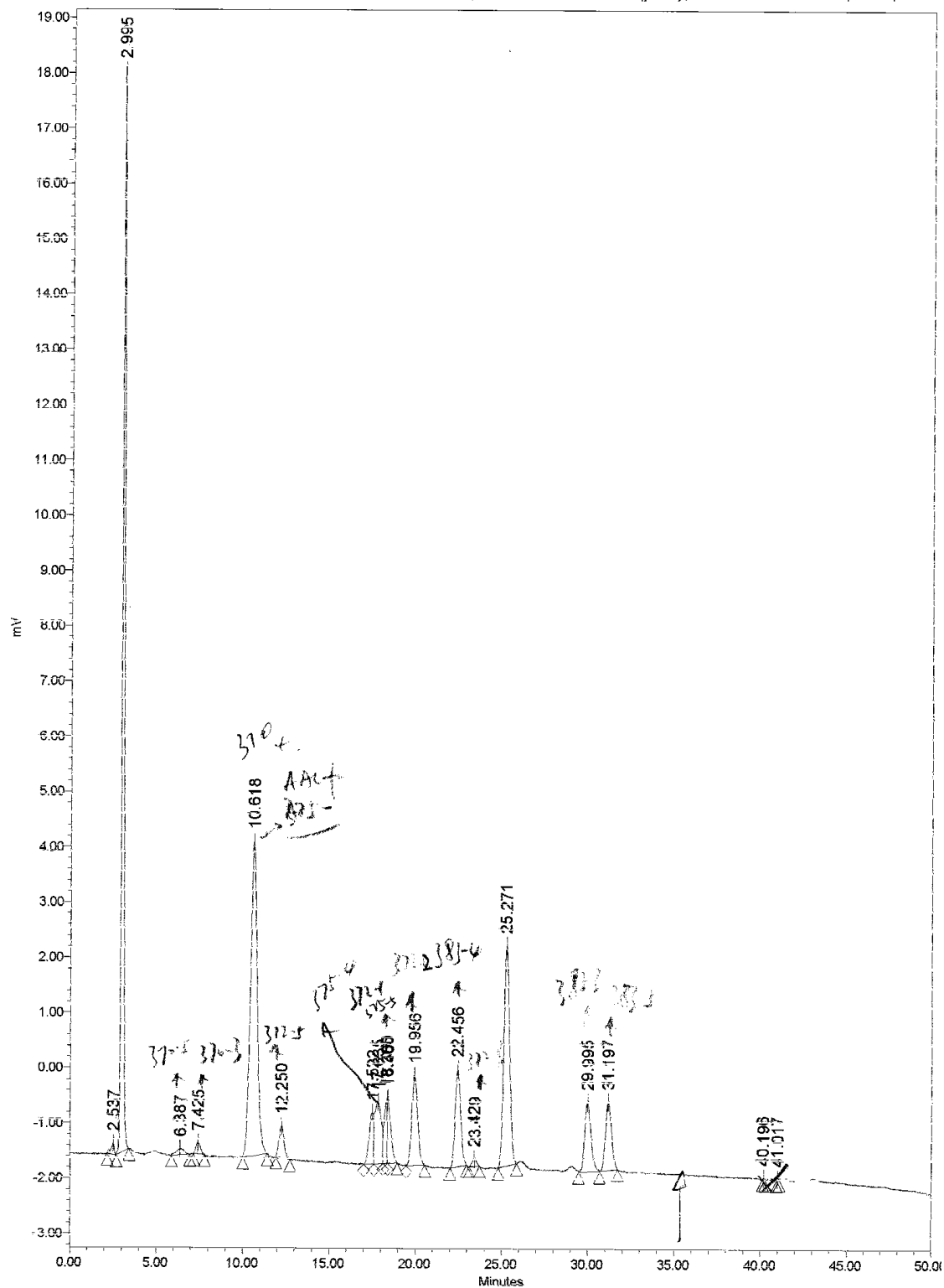


9. HPLC Traces for Purified Protease C Wild Type (1h)



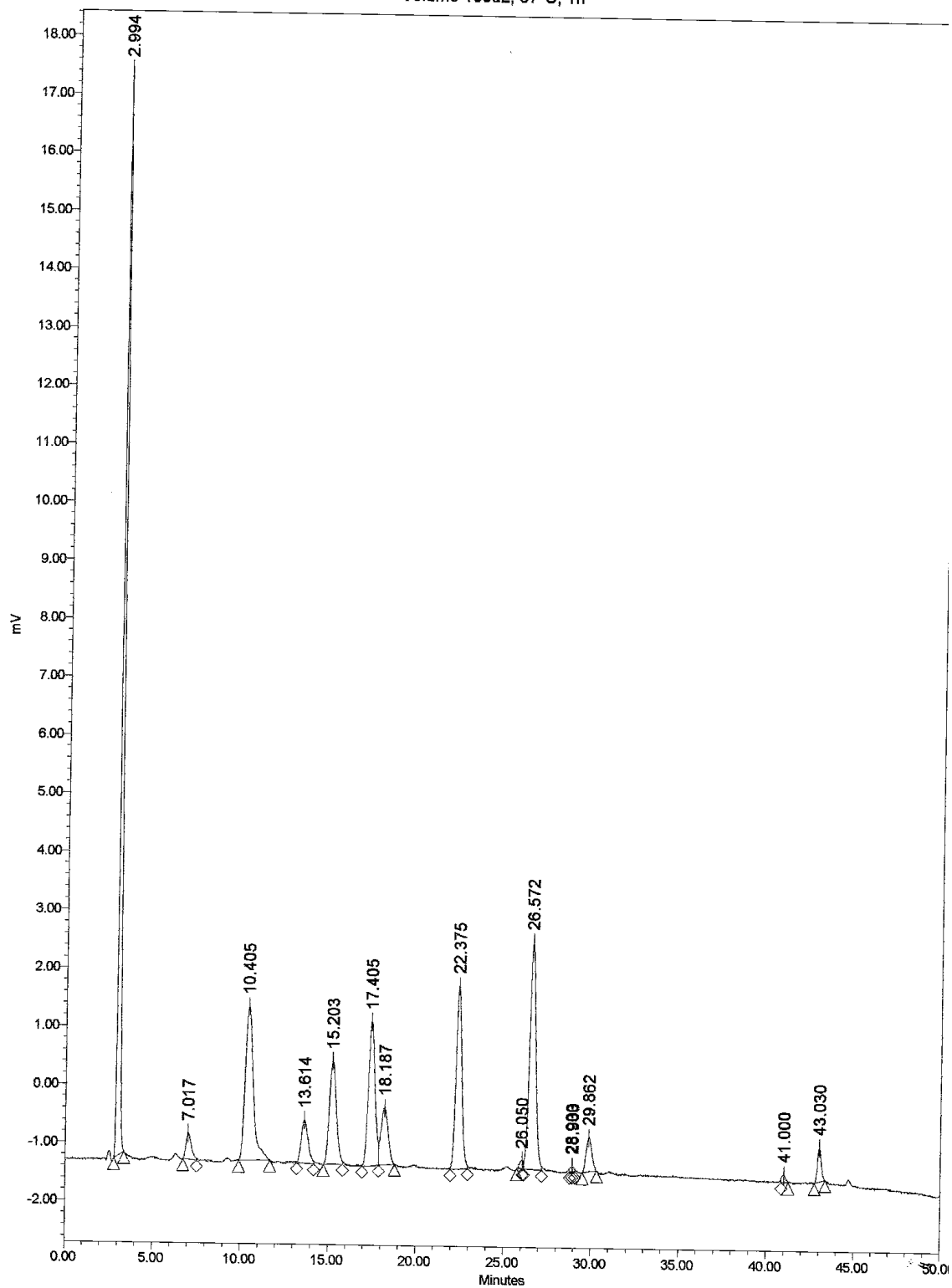
10 HPLC Traces for Protrase C Wild Type (10h)

YZ-436 Test 6, Mixture (4-AMC, YZ-370, 372, 376, 375, 383): 10uM, Bistris Buffer (pH 7), Protease C: 50 ^{mg}/mL, 37°C, 10h

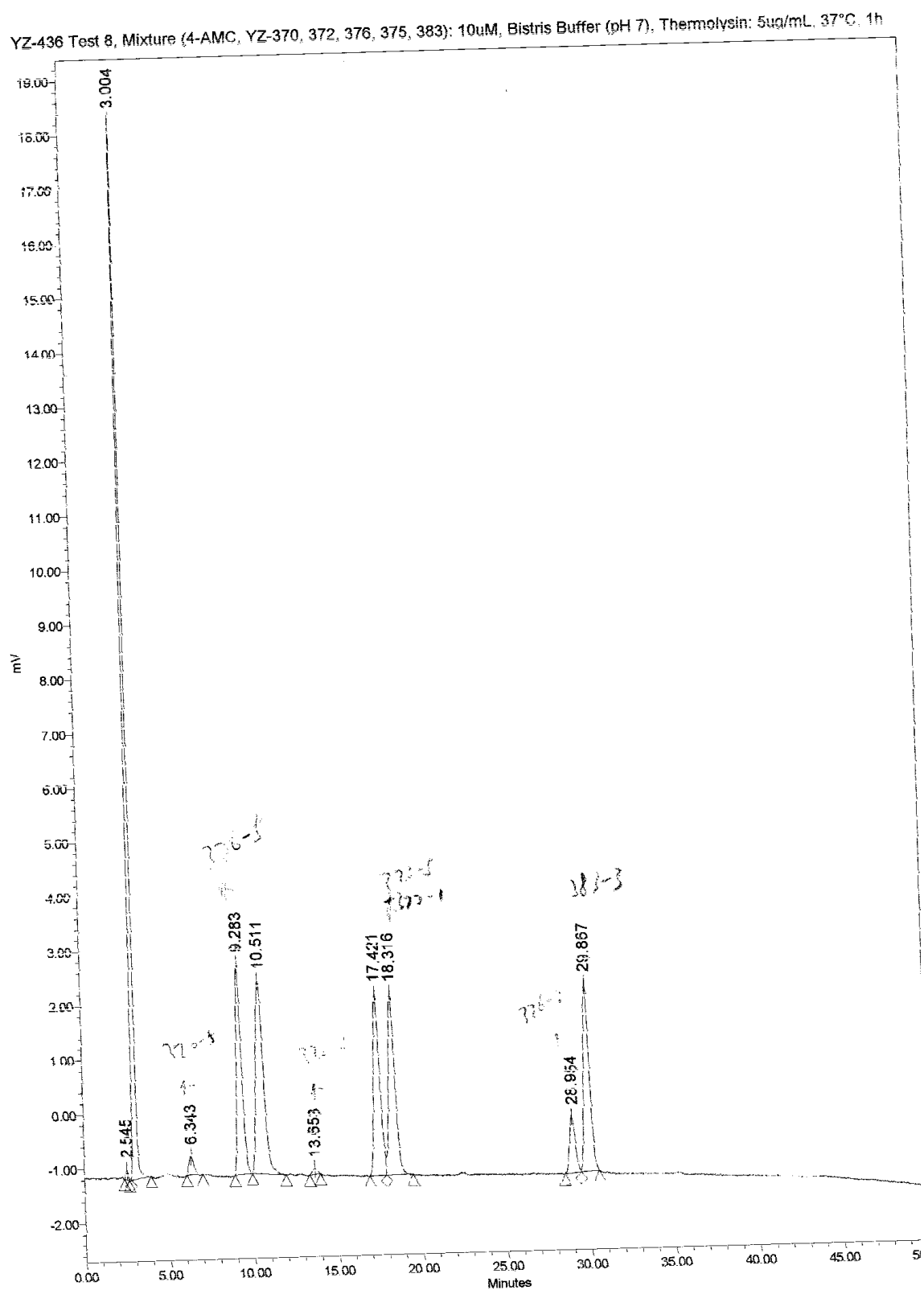


11. HPLC Traces for Poteinase K

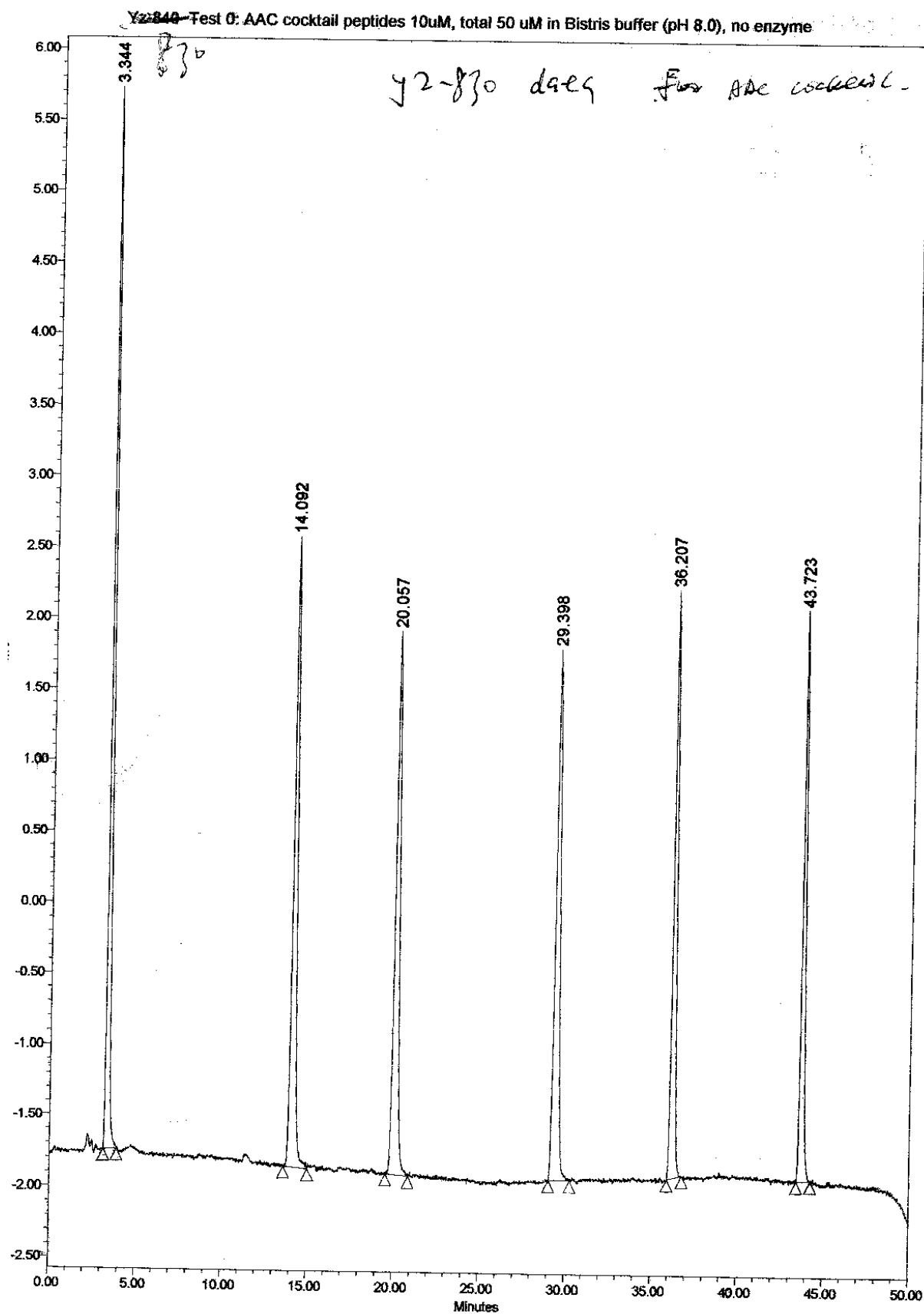
Measurement Condition: Peptide Mixture Concentration, 10uM; 20 mM Bistris Buffer (pH 9.0); Proteinase K, 50ug/mL; in Tot Volume 100uL; 37°C; 1h



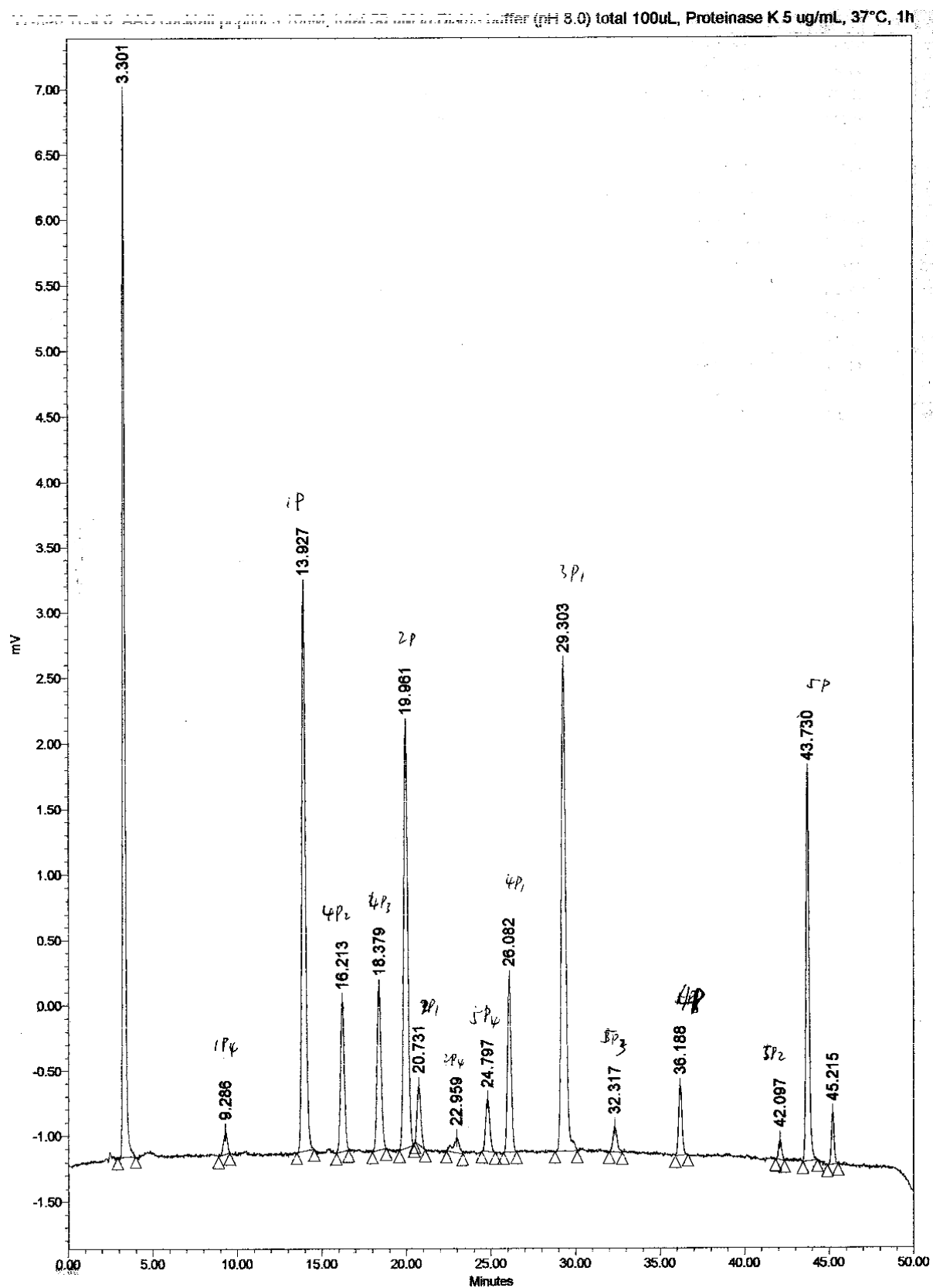
12. HPLC Traces for Thermolysin



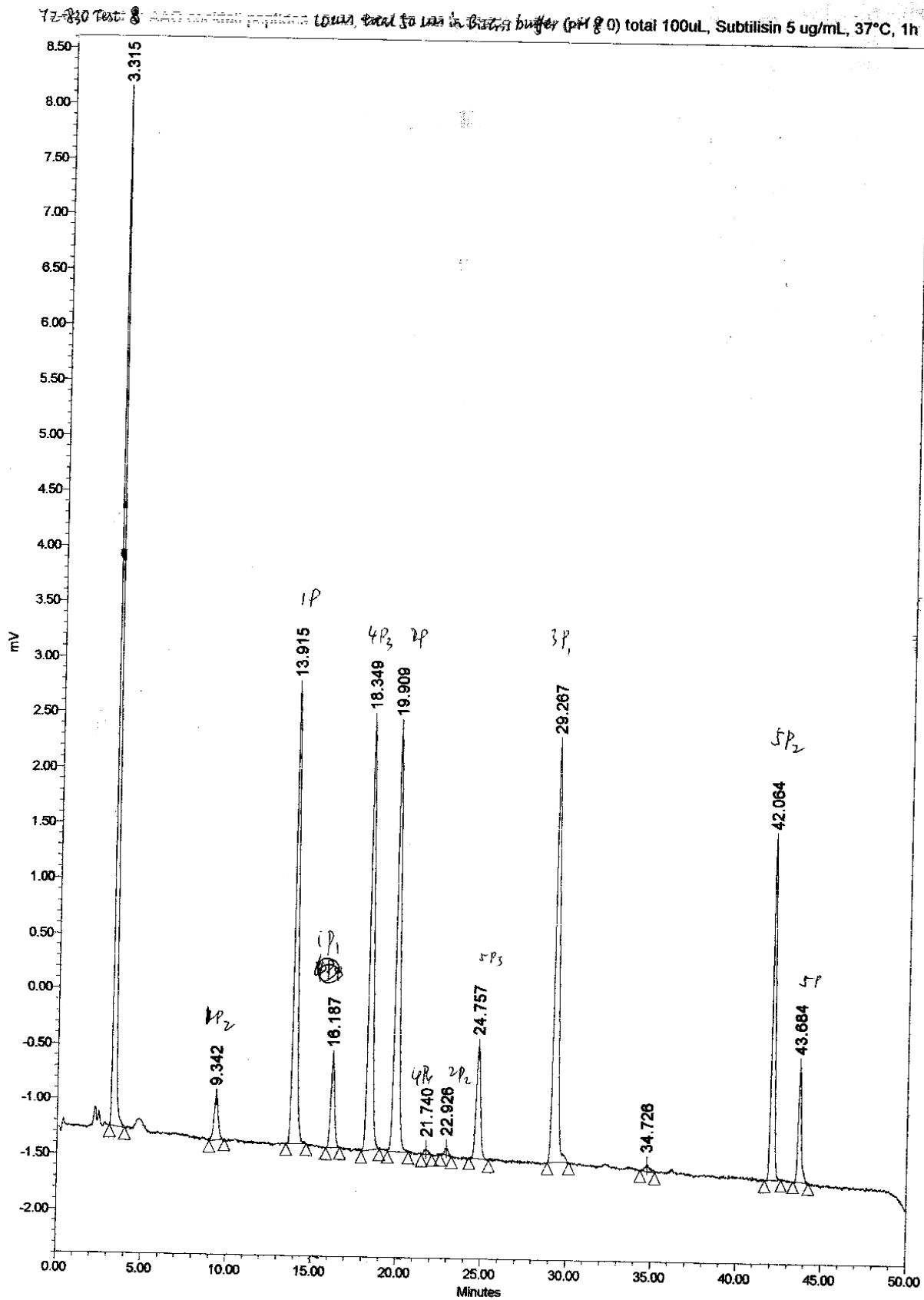
13. HPLC Traces for blank



14. HPLC Traces for Proteinase K-1 -1h

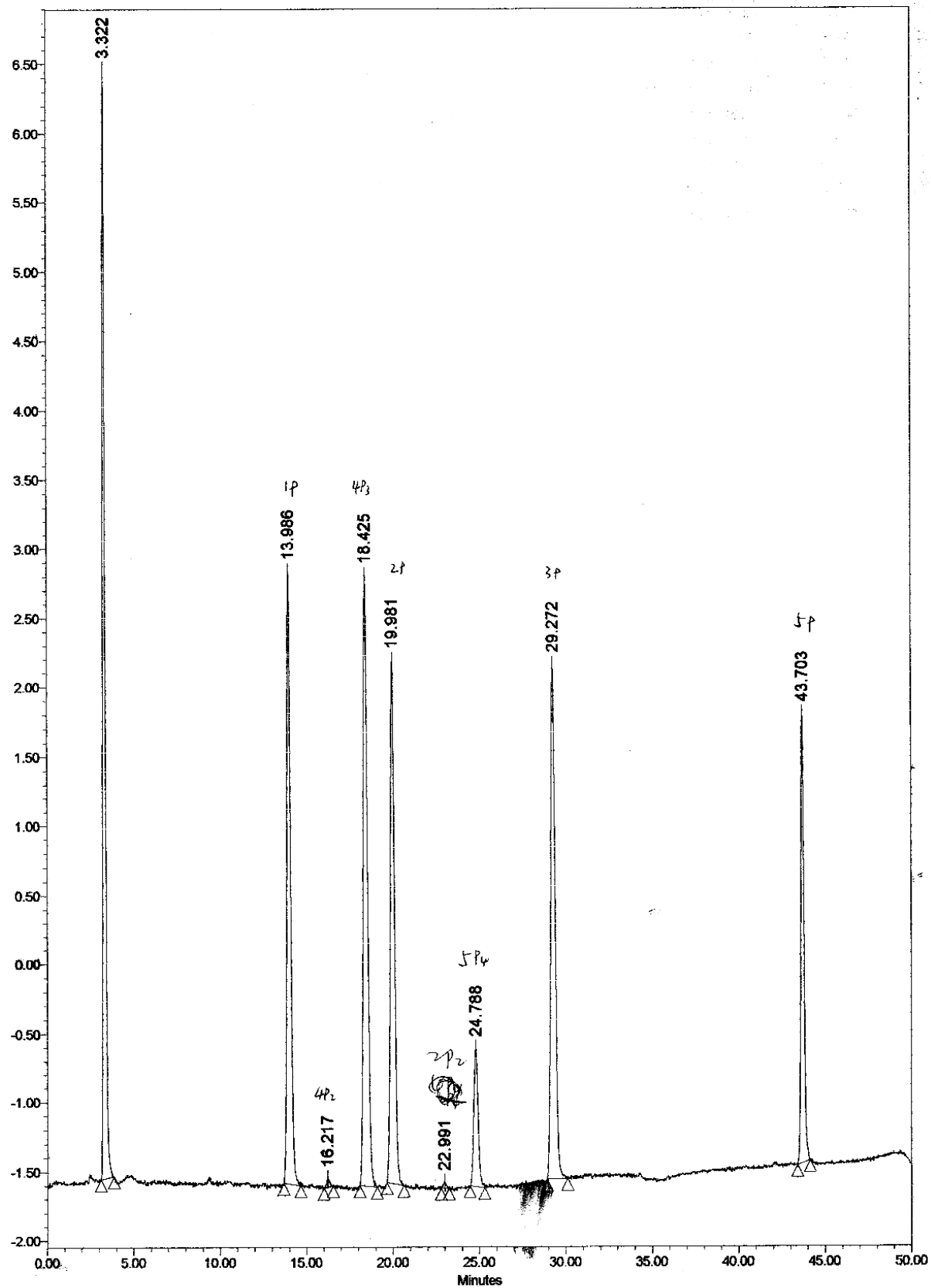


15. HPLC Traces for Subtilisin-1 -1h



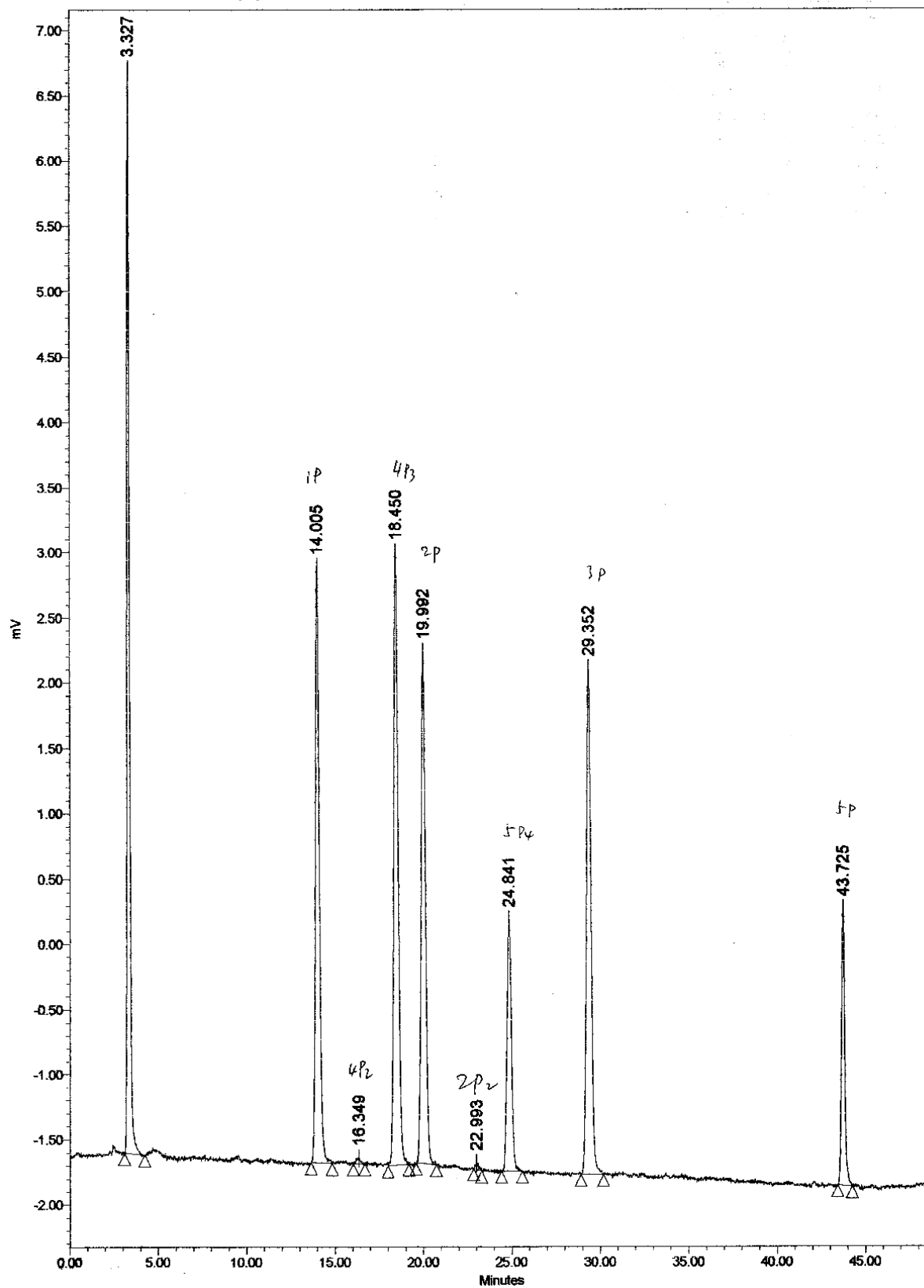
17. HPLC Traces for Trypsin-1 -10 min

z-340 Test 1: AAC cocktail peptides 10uM, total 50 uM in Bistris buffer (pH 8.0) total 100uL, Trypsin 5 ug/mL, 37°C, 10 min



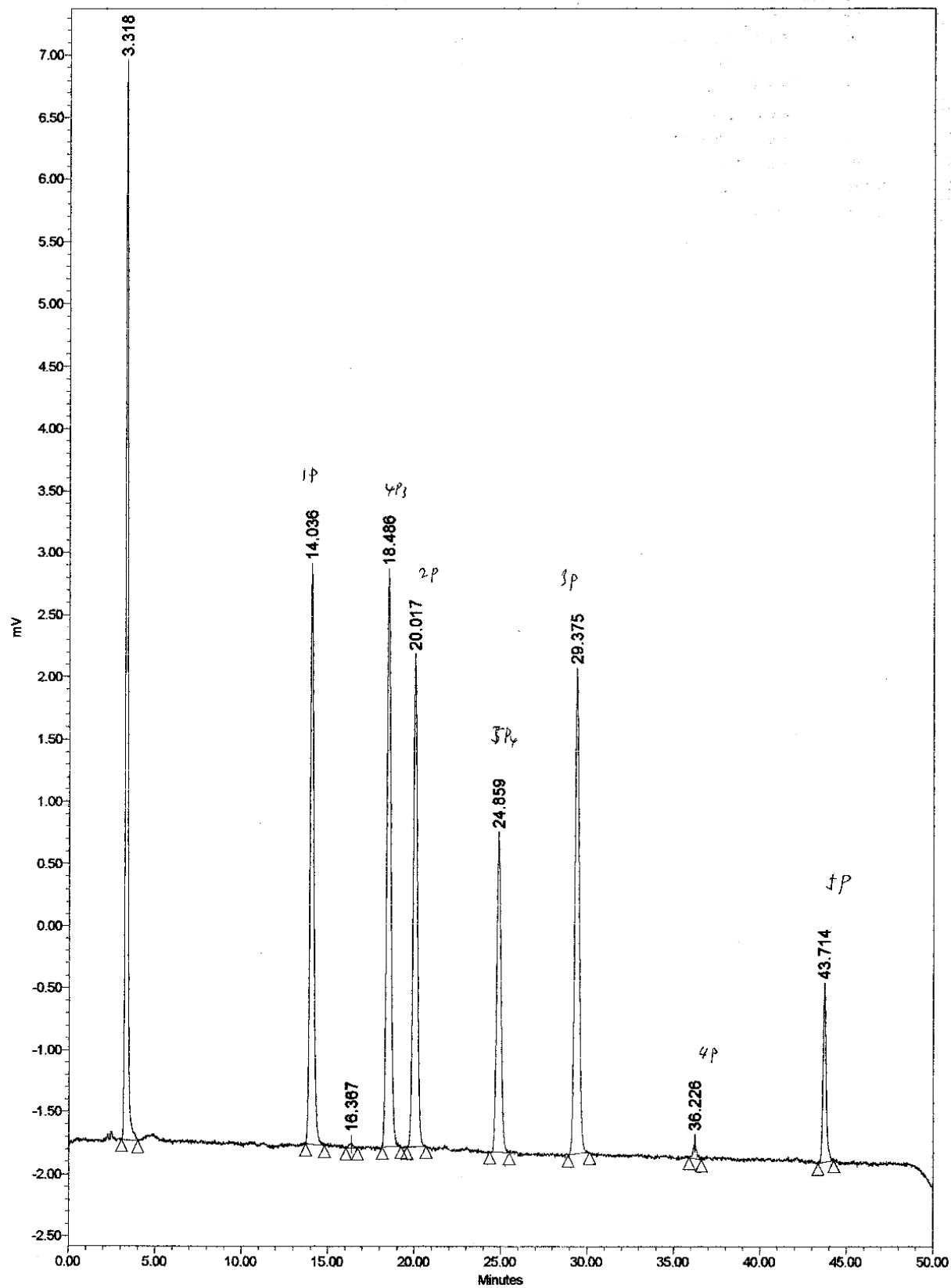
18. HPLC Traces for Trypsin-1 -30 min

Yz-840 Test 2: AAC cocktail peptides 10uM, total 50 uM in Bistris buffer (pH 8.0) total 100uL, Trypsin 5 ug/mL, 37°C, 0.5h



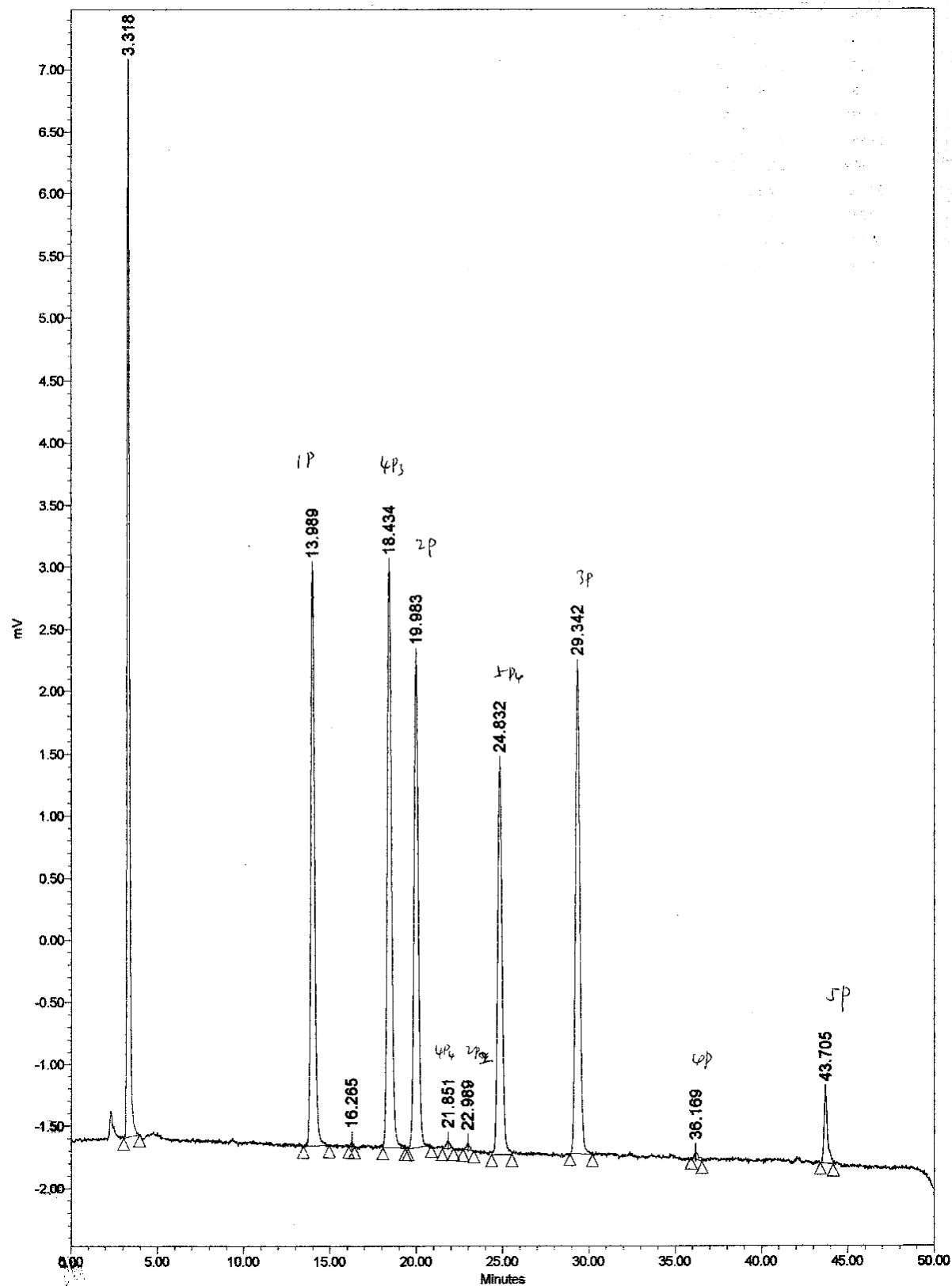
19. HPLC Traces for Trypsin-1 -1h

Yz-840 Test 3: AAC cocktail peptides 10uM, total 50 uM in Bistris buffer (pH 8.0) total 100uL, Trypsin 5 ug/mL, 37°C, 1h



20. HPLC Traces for Trypsin-1 -2h

Yz-840 Test 4: AAC cocktail peptides 10uM, total 50 uM in Bistris buffer (pH 8.0) total 100uL, Trypsin 5 ug/mL, 37°C, 2h



List of labeling of reference fragments (transfer from lab label)

Fragments	Sequence	Lab label	Ret.T (min)
1P5	*K	370-5	6.86
1P2	*KDES	370-2	6.89
1P3	*KDE	370-3	7.23
1P4	*KD	370-4	7.63
3P5	*E	376-5	9.15
P6	*	AAC	11.44
1P	*KDESYR	370	11.67
2P5	*A	372-5	12.17
4P2	*YARK	375-2	13.67
1P1	*KDESYR	370-1	14.08
4P3	*YAR	375-3	15.33
2P	*AVPERS	372	17.46
4P4	*YA	375-4	18.01
2P1	*AVPERS	372-1	18.14
4P5	*Y	375-5	18.63
2P2	*AVPE	372-2	19.87
2P4	*AV	372-4	20.19
5P2	*LKYF	383-2	22.52
2P3	*AVP	372-3	23.39
4P1	*YARKL	375-1	24.14
3P	*EFVGS	376	25.14
3P1	*EFVGS	376-1	26.64
3P4	*EF	376-4	28.7
3P2	*EFVG	376-2	28.72
5P3	*LKY	383-3	30.12
5P5	*L	383-5	31.01
3P3	*EFV	376-3	32.83
4P	*YARKLF	375	34.97
5P1	*LKYFD	383-1	39.02
5P4	*LK	383-4	41.05
5P	*LKYFDI	383	43.02