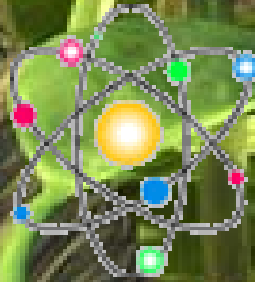


Pharmaceutical Biotechnology

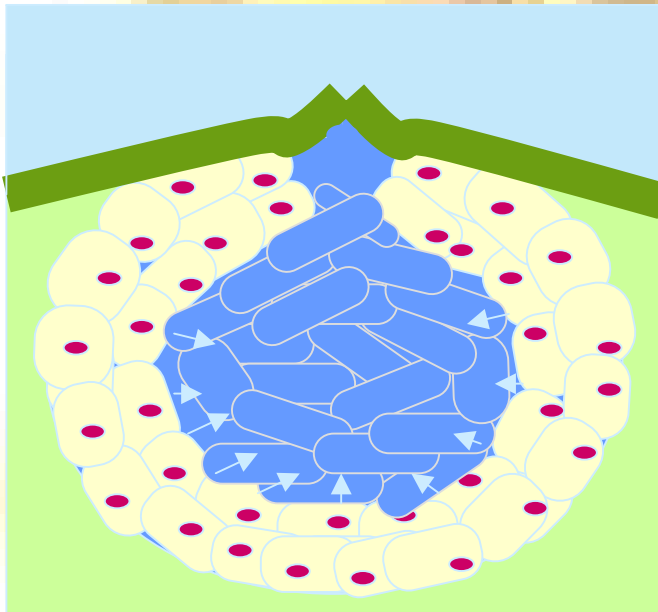


FUNCTIONAL PEPTIDOMICS OF AMPHIBIAN VENOMS

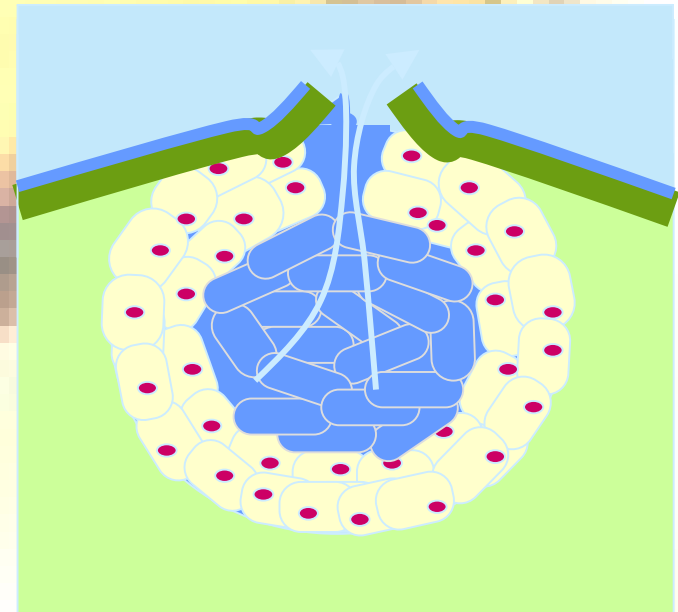




- The dermal granular (venom) gland



The venom producing cells of the granular gland produce secretions, which are stored in the cytoplasmic continuity of the syncytium



Stimulation/injury causes opening of the granular gland and emptying of the syncytium (containing cellular constituents) onto the dermal surface of the frog; this includes poly A+ mRNA





Focused interests

Ω- antimicrobials

Ω- anticancer

Ω- novel neurohormones

Ω- vasoactives

Ω- cell growth regulators

Ω- anthelmintics

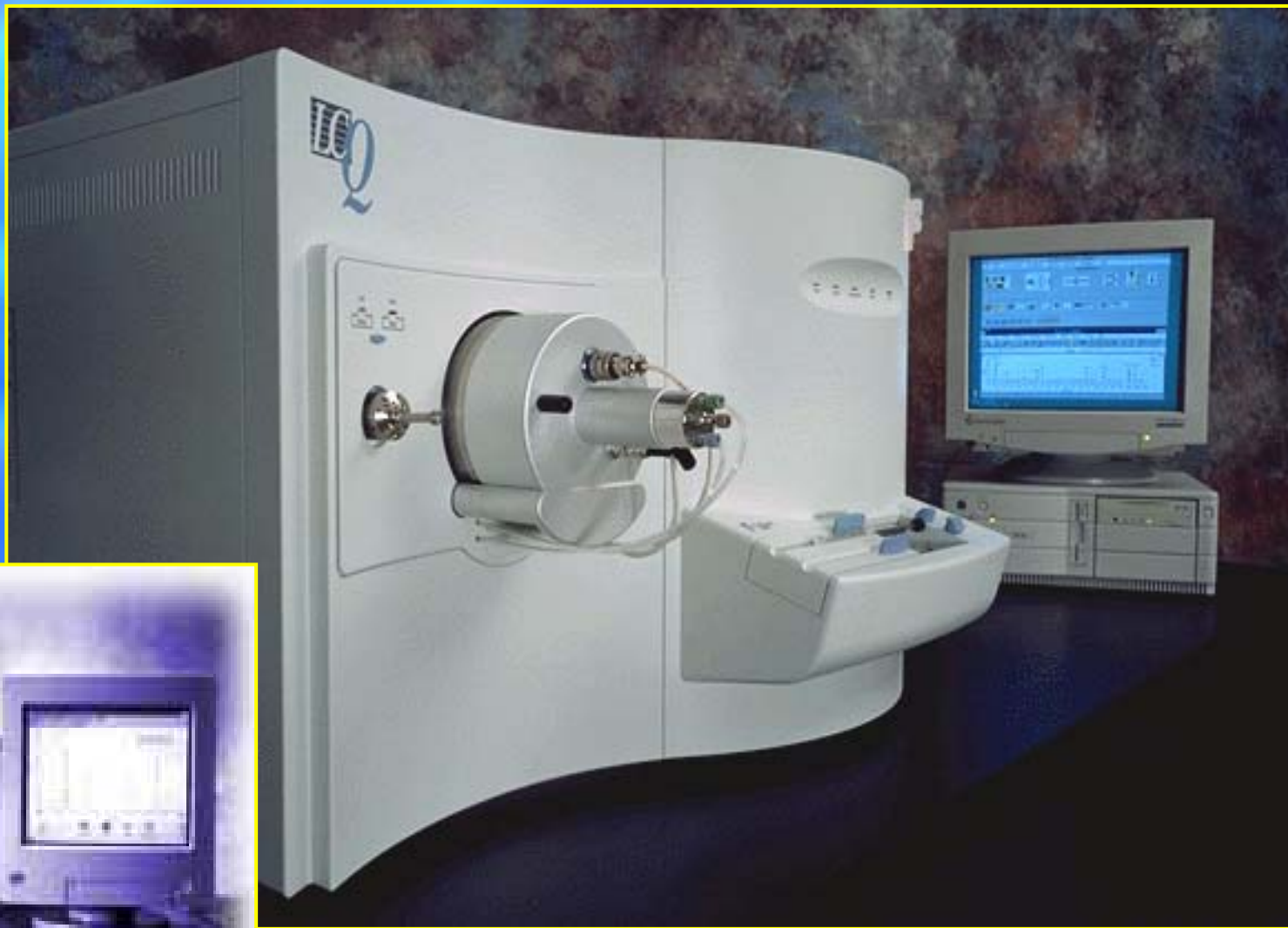
Ω- insecticides

Ω- molecular targets in pathology

Pharmaceutical Biotechnology Research Group, UUC

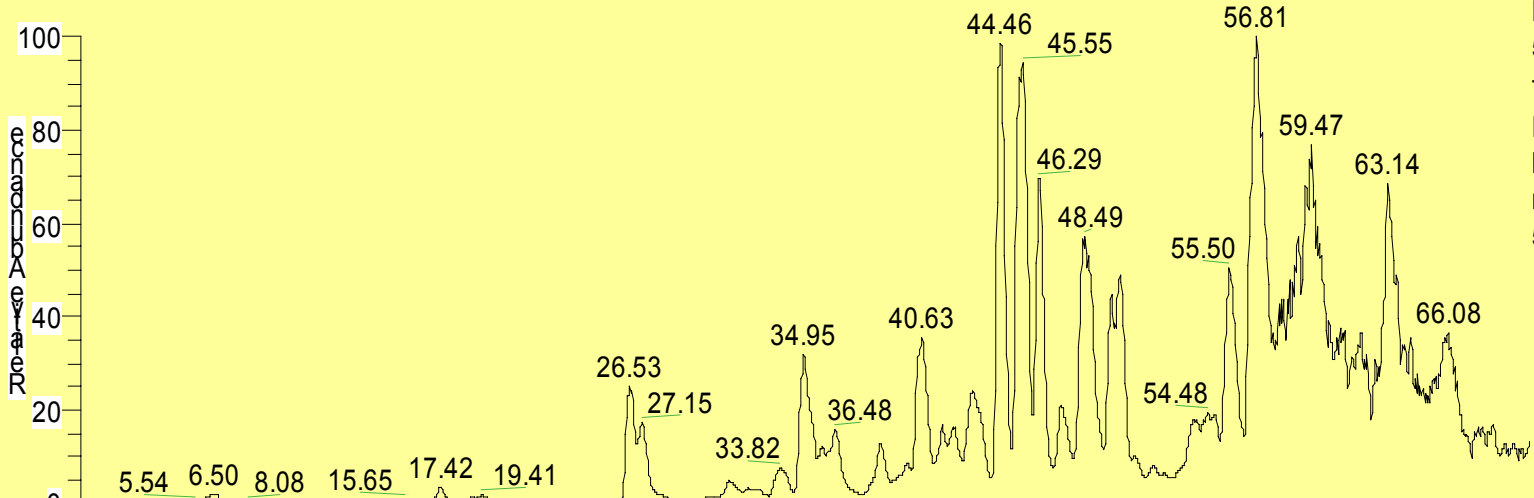
- 1 x LCQ™ “Classic” Ion Trap
- 1 x Voyager DE MALDI - TOF
- 1 x QToF Ultima

LCQ Ion Trap

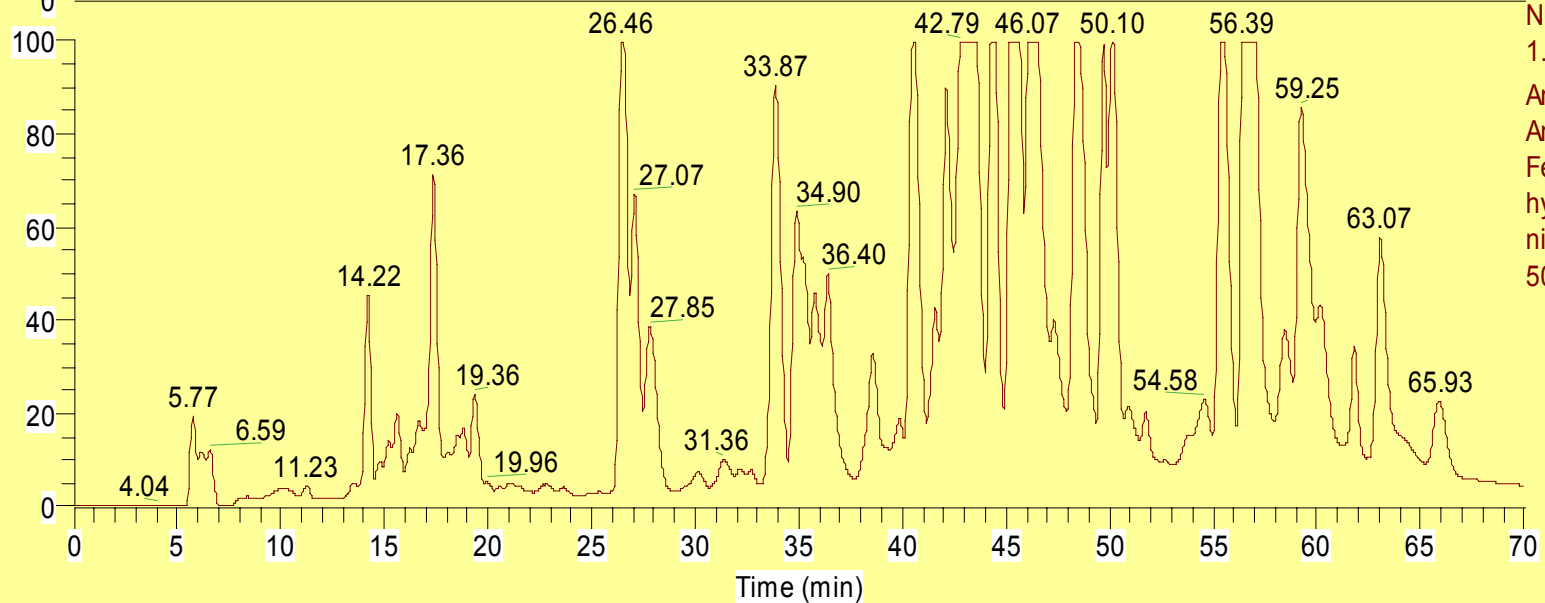


Fractionation of venom

RT: 0.00 - 70.02 SM: 7B

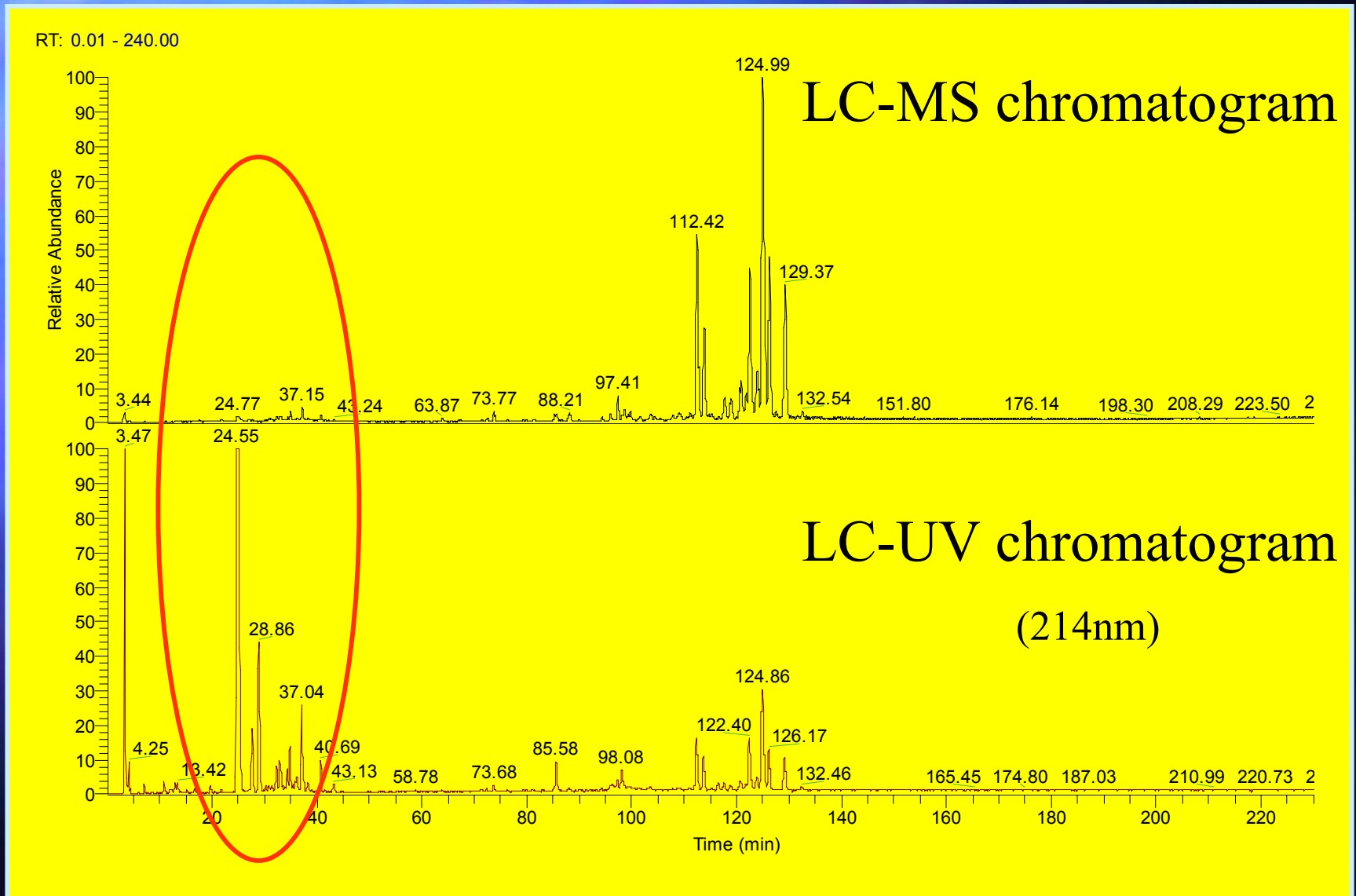


NL:
5.43E8
TIC MS
Feb25_Pac
hymed_dac
nic_5mg-
500ul



NL:
1.00
Analog 1
Analog
Feb25_Pac
hymed_dac
nic_5mg-
500ul

LC-UV-MS Analysis of *Litoria aurea* skin secretion

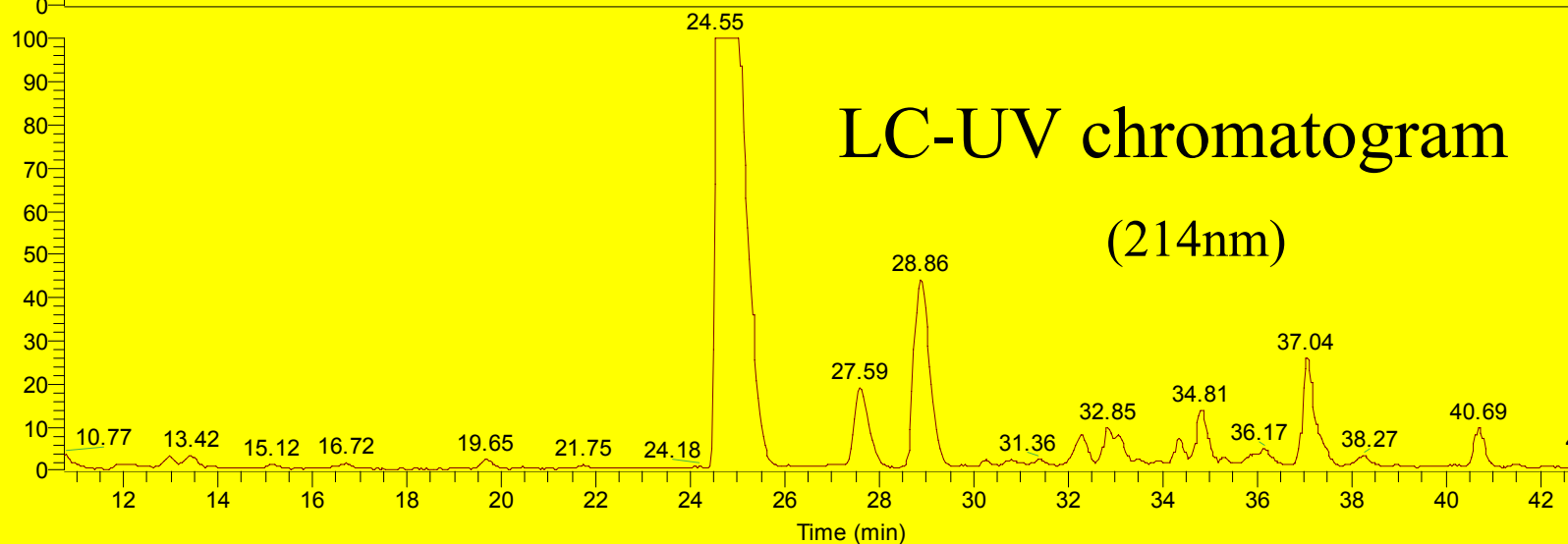
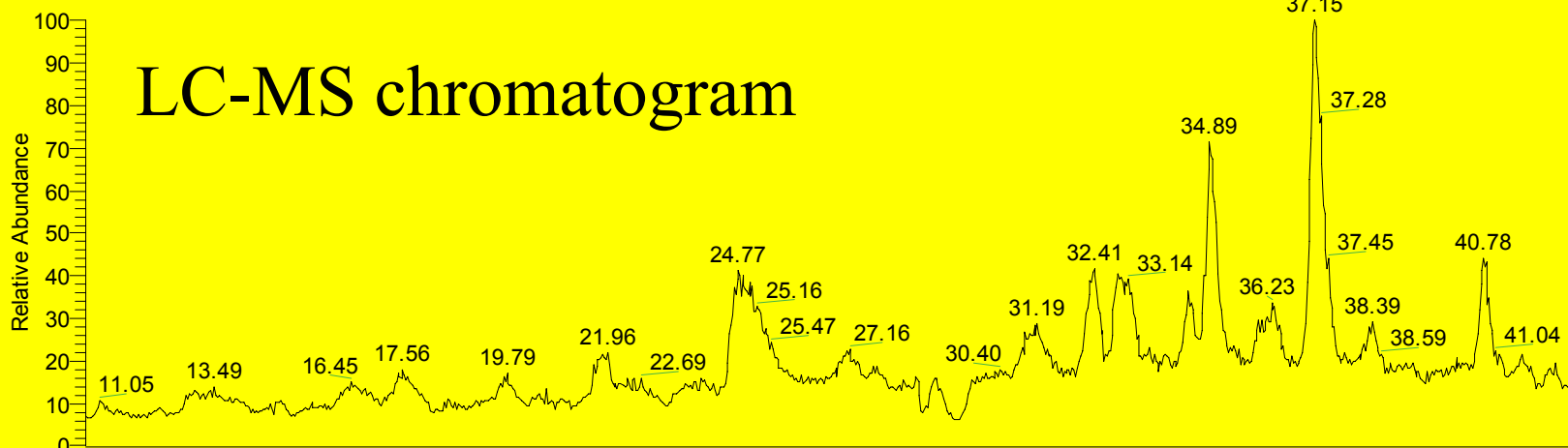


200ul injection. 0-80% ACN in 0.05% TFA water in 240 minutes.

LC-UV-MS Analysis of *Litoria aurea* skin secretion

RT: 10.73 - 44.08

Expanded area



200ul injection. 0-80% ACN in 0.05% TFA water in 240 minutes.

Serotonin-based Compounds

Serotonin



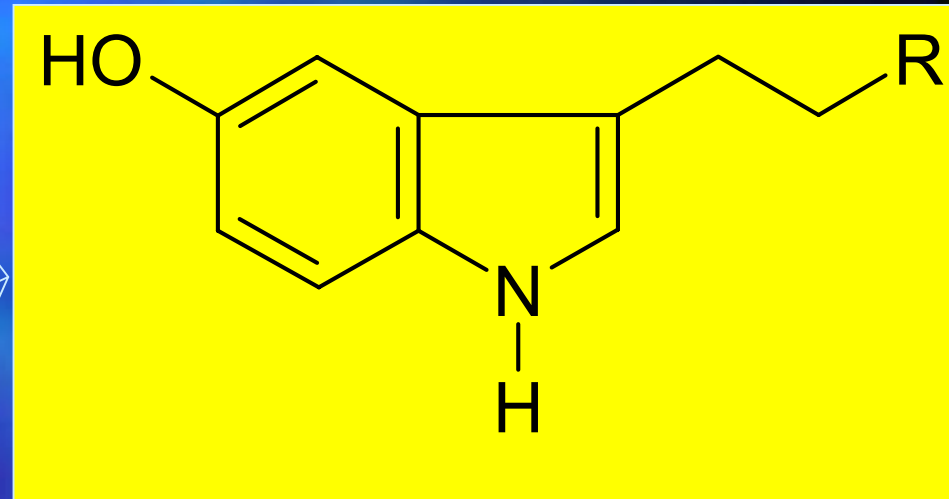
***N'* Methyl serotonin**



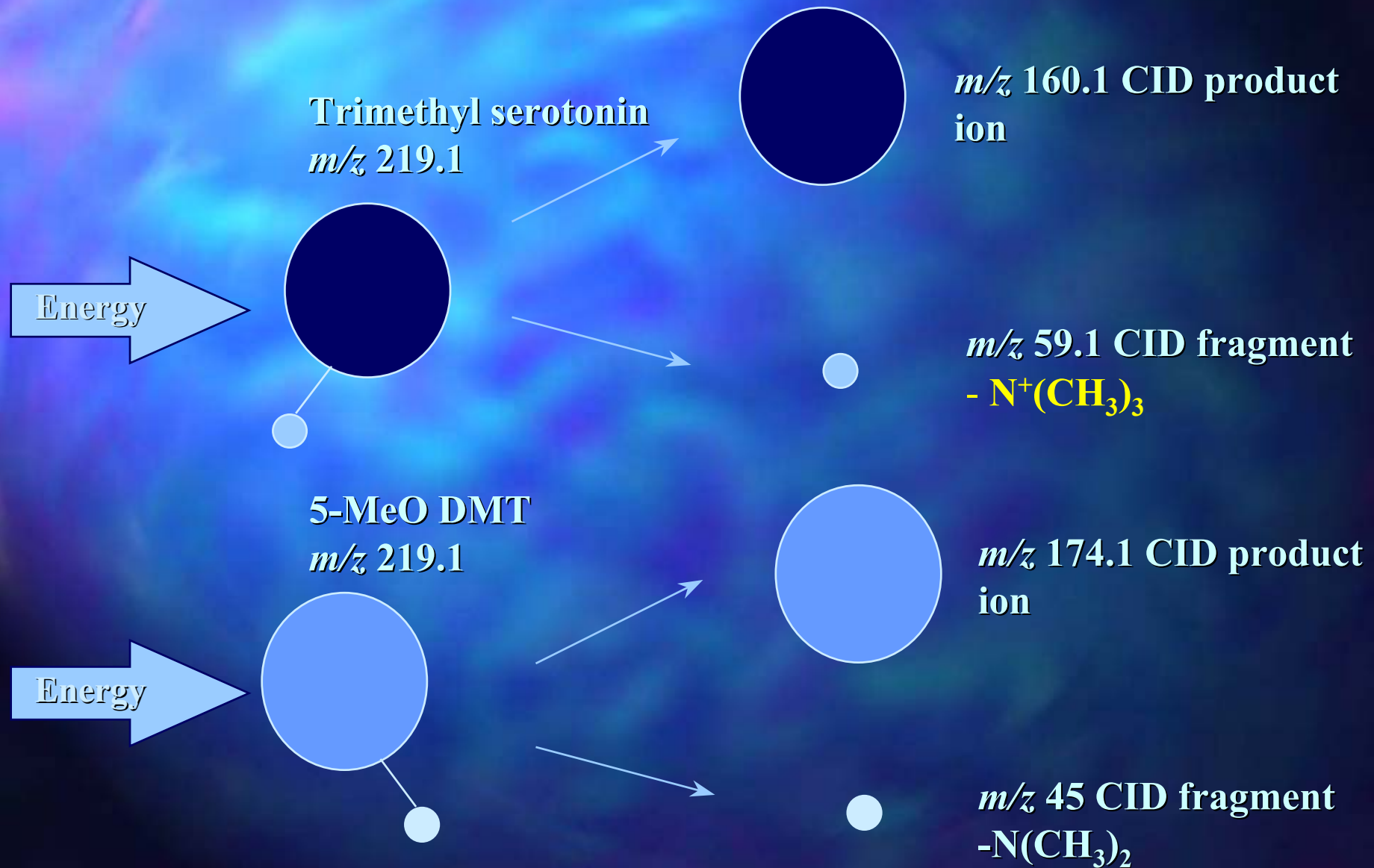
Bufotenine



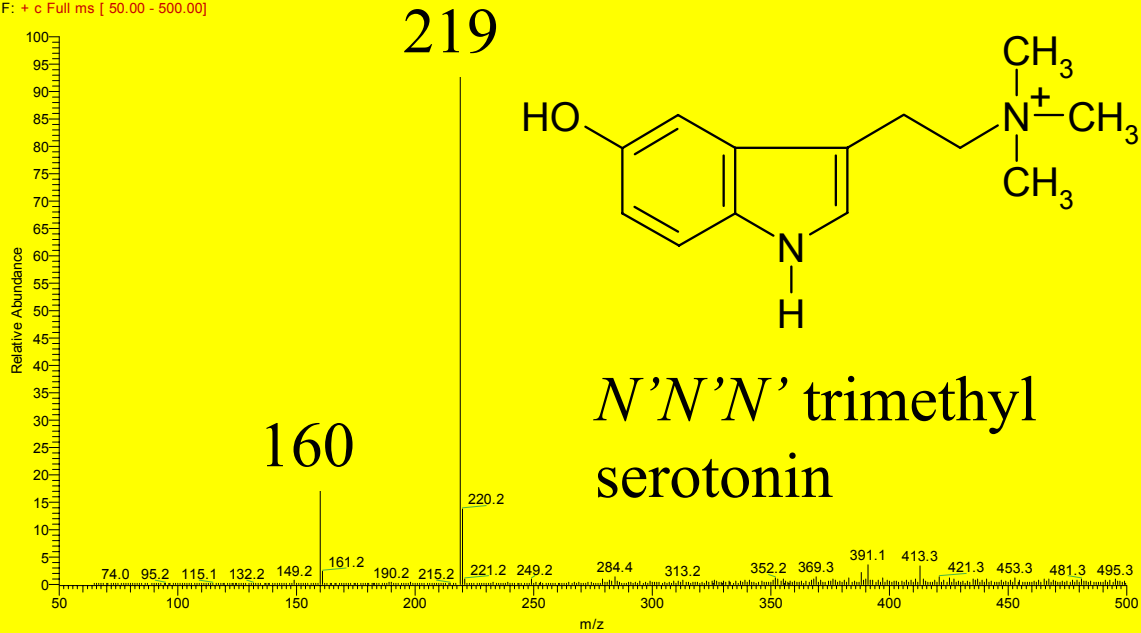
***N'**N'**N'* Trimethyl serotonin**



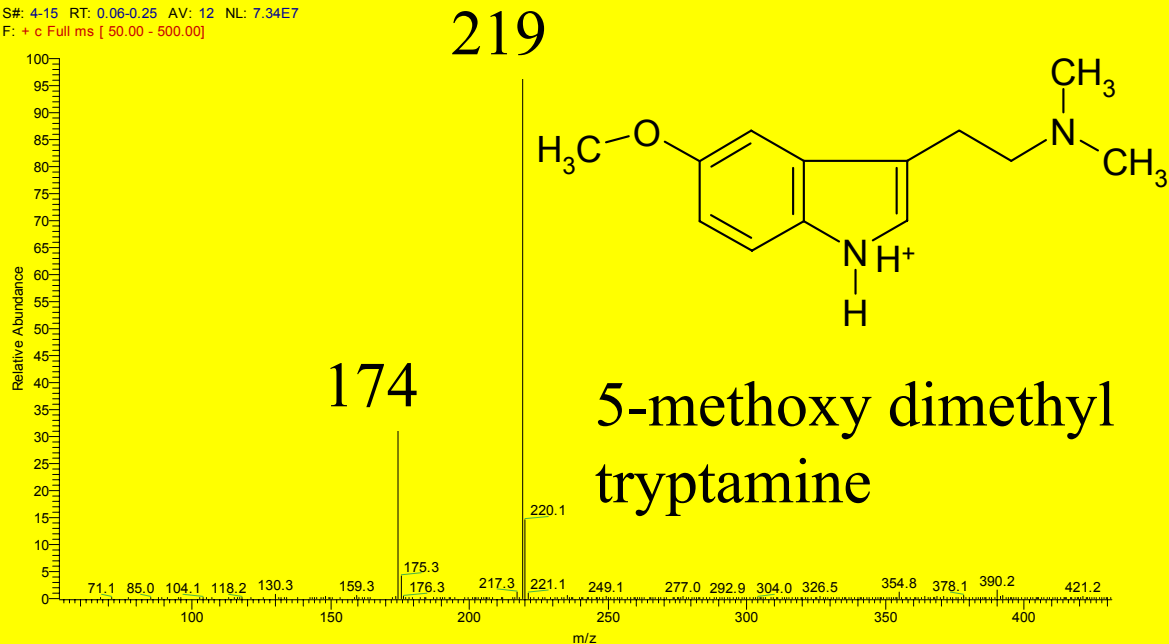
MS/MS of isobaric compounds



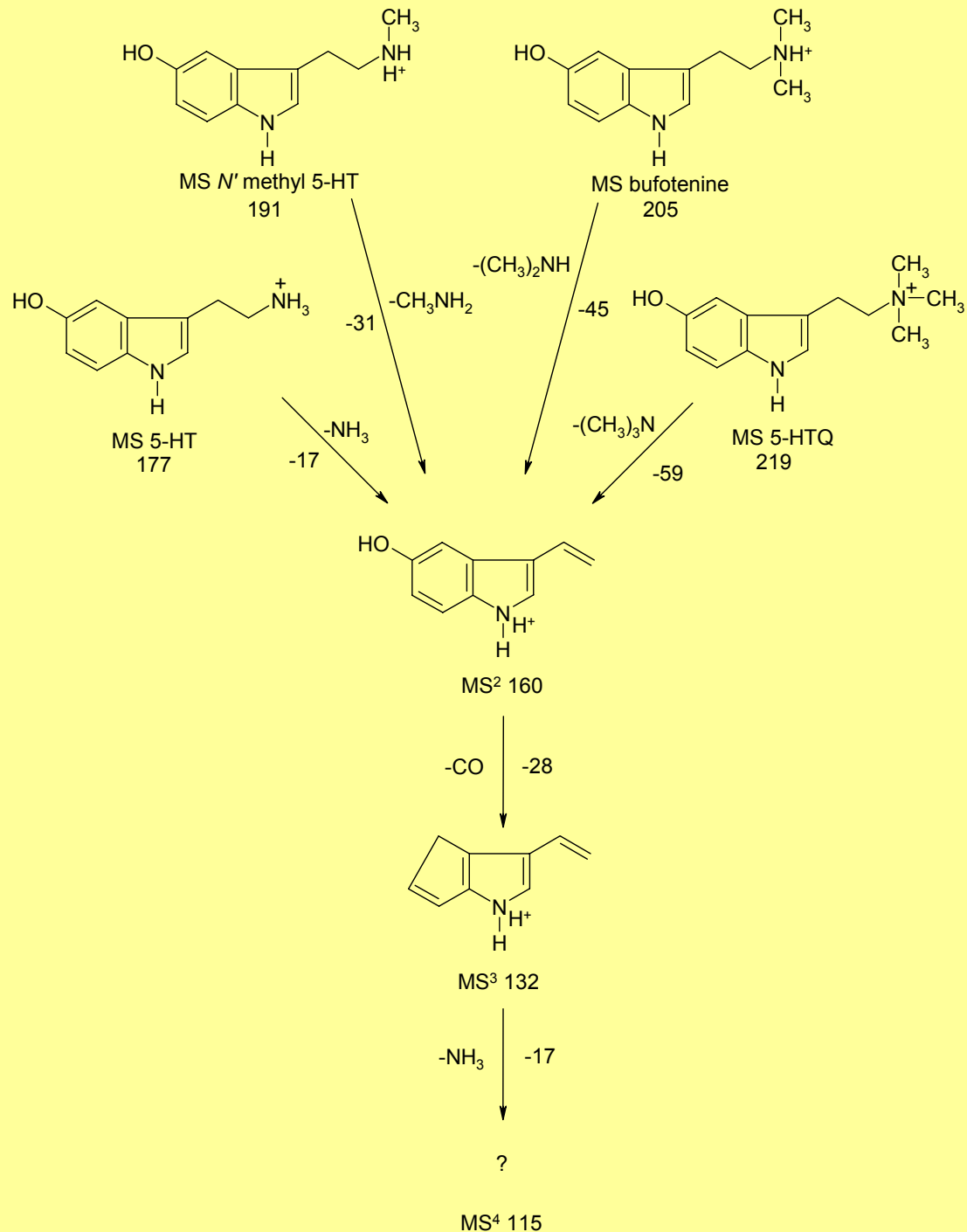
S#: 1-36 RT: 0.02-0.70 AV: 36 NL: 1.20E7
F: + c Full ms [50.00 - 500.00]



S#: 4-15 RT: 0.06-0.25 AV: 12 NL: 7.34E7
F: + c Full ms [50.00 - 500.00]

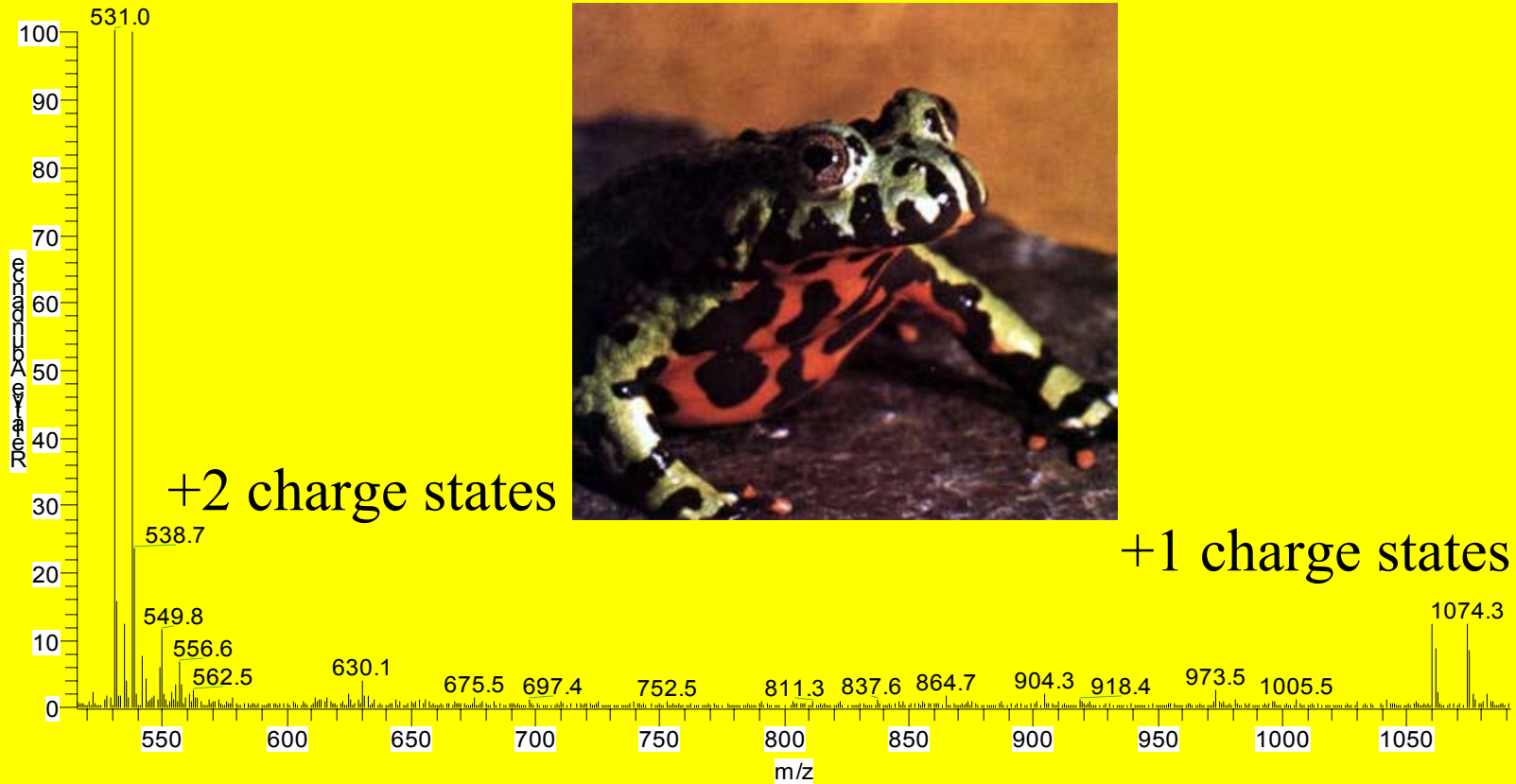


MS
analysis of
N,N,N'
trimethyl
serotonin
and
5-methoxy
dimethyl
tryptamine
DISTINGUISHED!



MS analysis of peptide mixtures

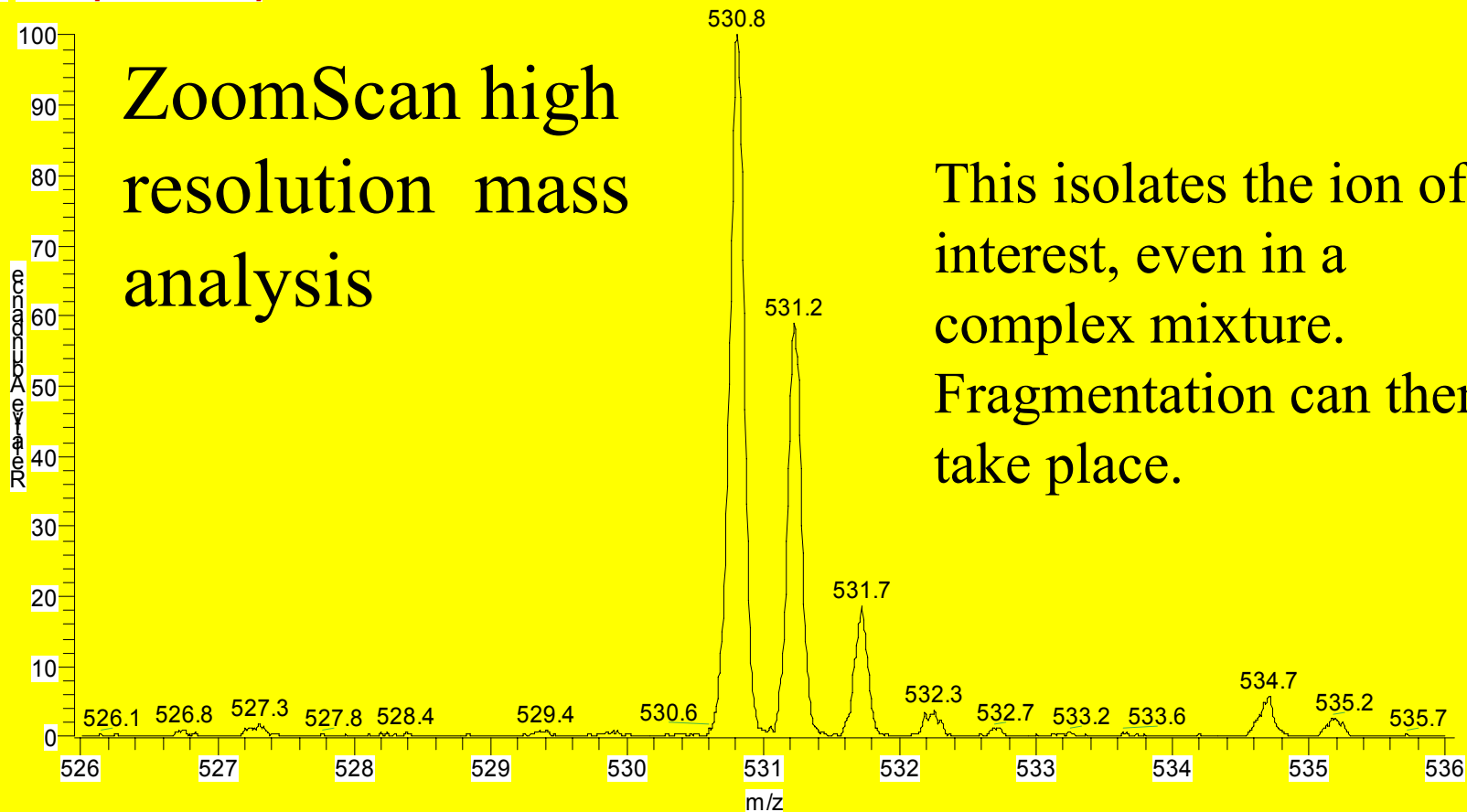
Bradykinin and threonine-6 bradykinin from *Bombina orientalis*



MS/MS Sequencing of Bradykinin

Mar02_eleanor_bo_fr98#69-92 RT: 1.31-1.82 AV: 14 NL: 5.42E3

F: + Z ms [526.00-536.00]



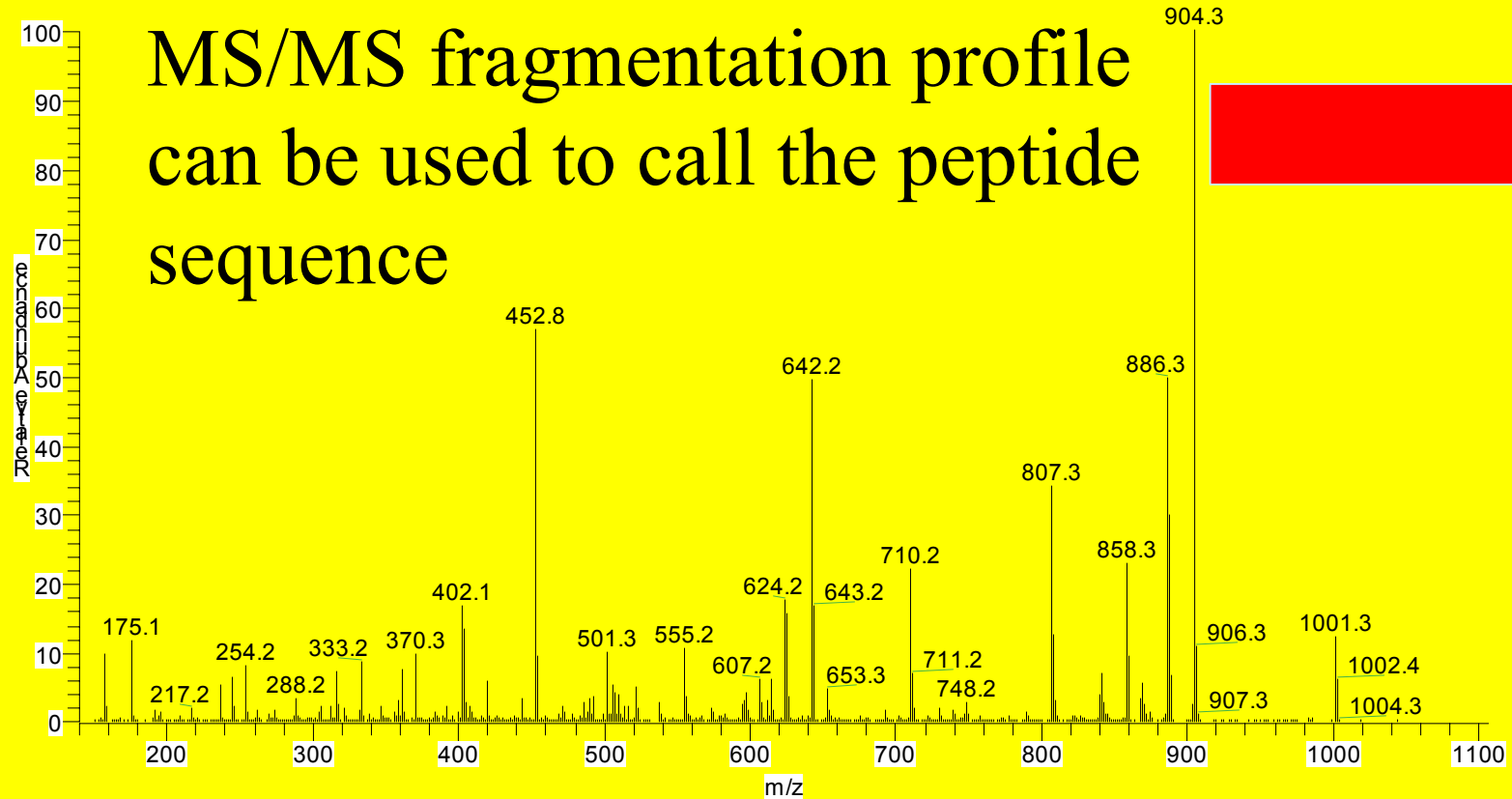
ZoomScan high resolution mass analysis

This isolates the ion of interest, even in a complex mixture. Fragmentation can then take place.

MS/MS Sequencing of Bradykinin

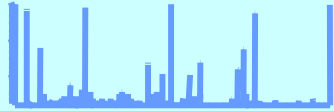
Mar02_eleanor_bo_fr97b#70-122 RT: 1.63-2.95 AV: 48 NL: 9.83E4
F: + c Full ms2 531.00@40.00 [145.00-1100.00]

MS/MS fragmentation profile
can be used to call the peptide
sequence



SEQUEST™

STEP 1.



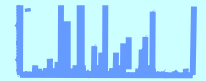
MS and MS/MS Spectrum



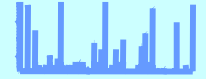
STEP 2.

Mass Search of Indexed Protein and DNA Databases based on the peptide parent molecular weight

SEQ 1 →



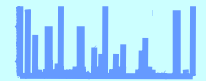
SEQ 2 →



SEQ 3 →



SEQ 4 →

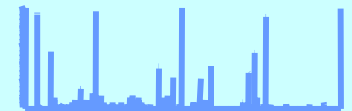
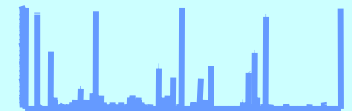


Predicted MS/MS Spectra



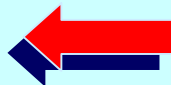
STEP 3.

Compares and correlates predicted and experimental MS/MS spectra



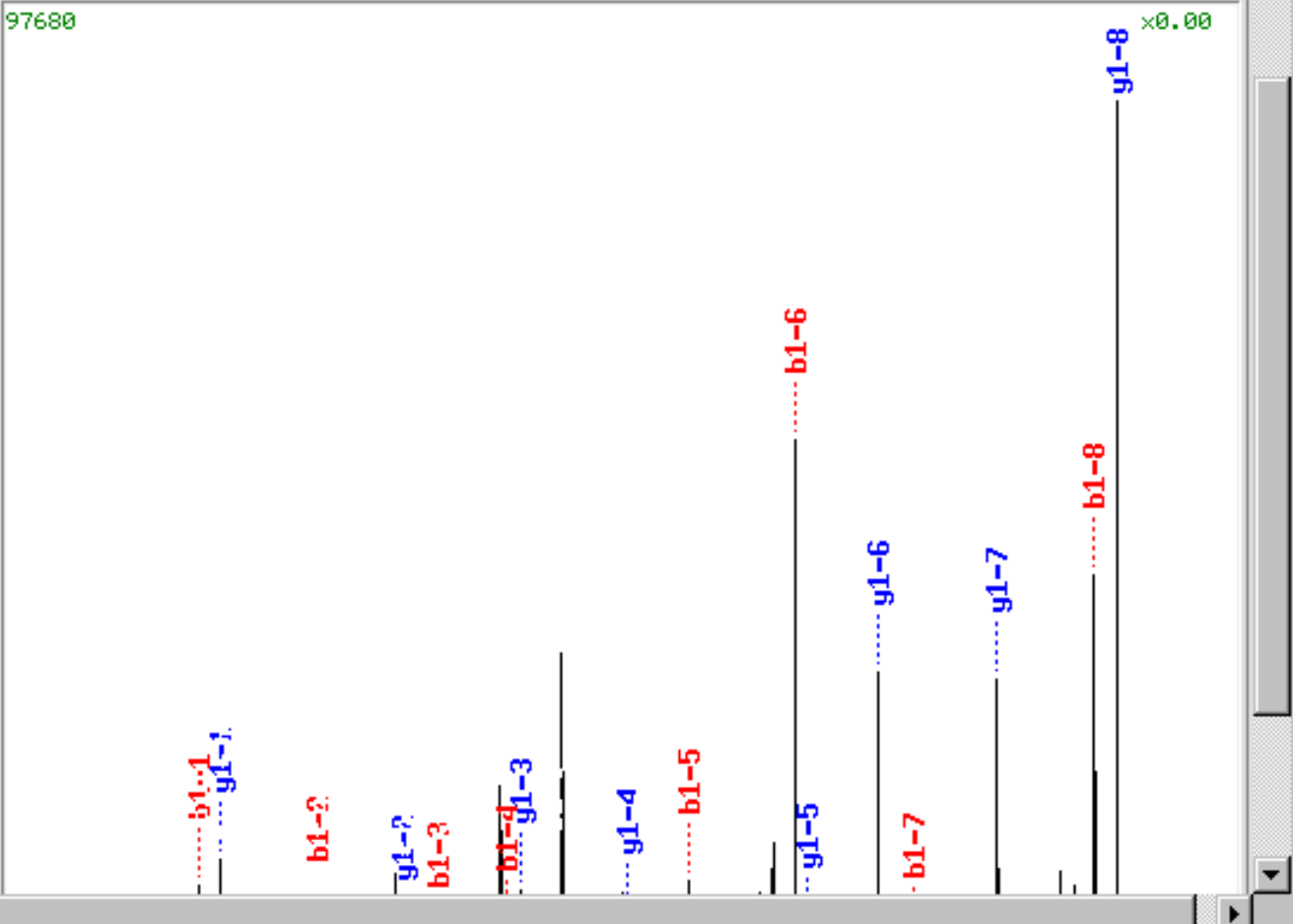
STEP 4.

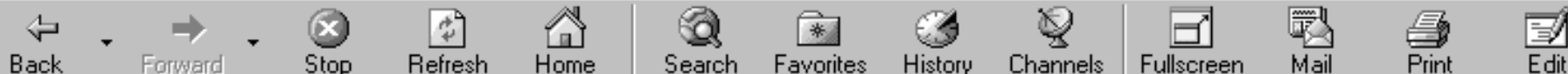
Rank hits, sequence peptides, create summary and Protein I.D.



Seq	#	b	y	(+1)
R	1	157.2	1061.2	9
P	2	254.3	905.0	8
P	3	351.4	807.9	7
G	4	408.5	710.8	6
F	5	555.6	653.7	5
S	6	642.7	506.6	4
P	7	739.8	419.5	3
F	8	887.0	322.4	2
R	9	1043.2	175.2	1

Seq	#	b	y	(+2)
R	1	79.1	531.1	9
P	2	127.7	453.0	8
P	3	176.2	404.5	7
G	4	204.7	355.9	6
F	5	278.3	327.4	5
S	6	321.9	253.8	4
P	7	370.4	210.2	3
F	8	444.0	161.7	2
R	9	518.1	113.2	1



Address 233/cgi-shl/web_retrieve.exe?Ref=gi|125510|sp|P01046|KNL1_BOVIN&Db=C:\Xcalibur\database\swissprot.fasta&Pep=RPPGFSPFR&MassType=0Links [Best of the Web](#) [Channel Guide](#) [Internet Explorer News](#) [Internet Start](#) [SACatalog](#)

```
database=C:\Xcalibur\database\swissprot.fasta, accession=gi|125510|sp|P01046|KNL1_BOVIN,  
peptide=RPPGFSPFR
```

Analyzing ...

```
>gi|125510|sp|P01046|KNL1_BOVIN KININOGEN, LMW I PRECURSOR (THIOL PROTEINASE INHIBITOR)  
(CONTAINS: BRADYKININ)
```

```
MKLITILELC SLLPSLTQE SSQEIDCNDQ DVFKAVDAAL TKYNSENKSG NOFVLYRITE VARMDNPDTF YSLKYPQIKEG  
DCPFQSNKTW QDCDYKDSRQ AATSECTATV AKRCNMKESV ALOTCLITRA ECPVYTAQTE CLGCVHPIST KSPDLEPVLR  
YAIQYFNMNT SHSHLFDLKE VKRAQRQVVS GWNVEVNYSI AQTNCSKEEF SFLTPDCKSL SSGDTGECTD KAHVDVKLRI  
SSFSQKCDLY PVKDFVQPPT RLCAGCPKPI PVDSPDLEEP LSHSIAKLNA EHDGAFYFKI DTVRKATVQW VAGLKYSIVF  
IARETTCSKG SNEELTKSCE INIHGQILHC DANVYVVPWE EKVYPTVNCQ PLGQTS LMKR PPGFSPFRSV QVMKTEGSTT  
THVKSCEYKG RPQEAGAEP A PQGEVSLPAE SPQLAR
```

```
>average mass = 48409, peptide at position 380-388
```

Search SWISS-PROT with [gi|125510|sp|P01046|KNL1_BOVIN](#) via [accession](#), [descr./ID](#), or [full text](#) field.

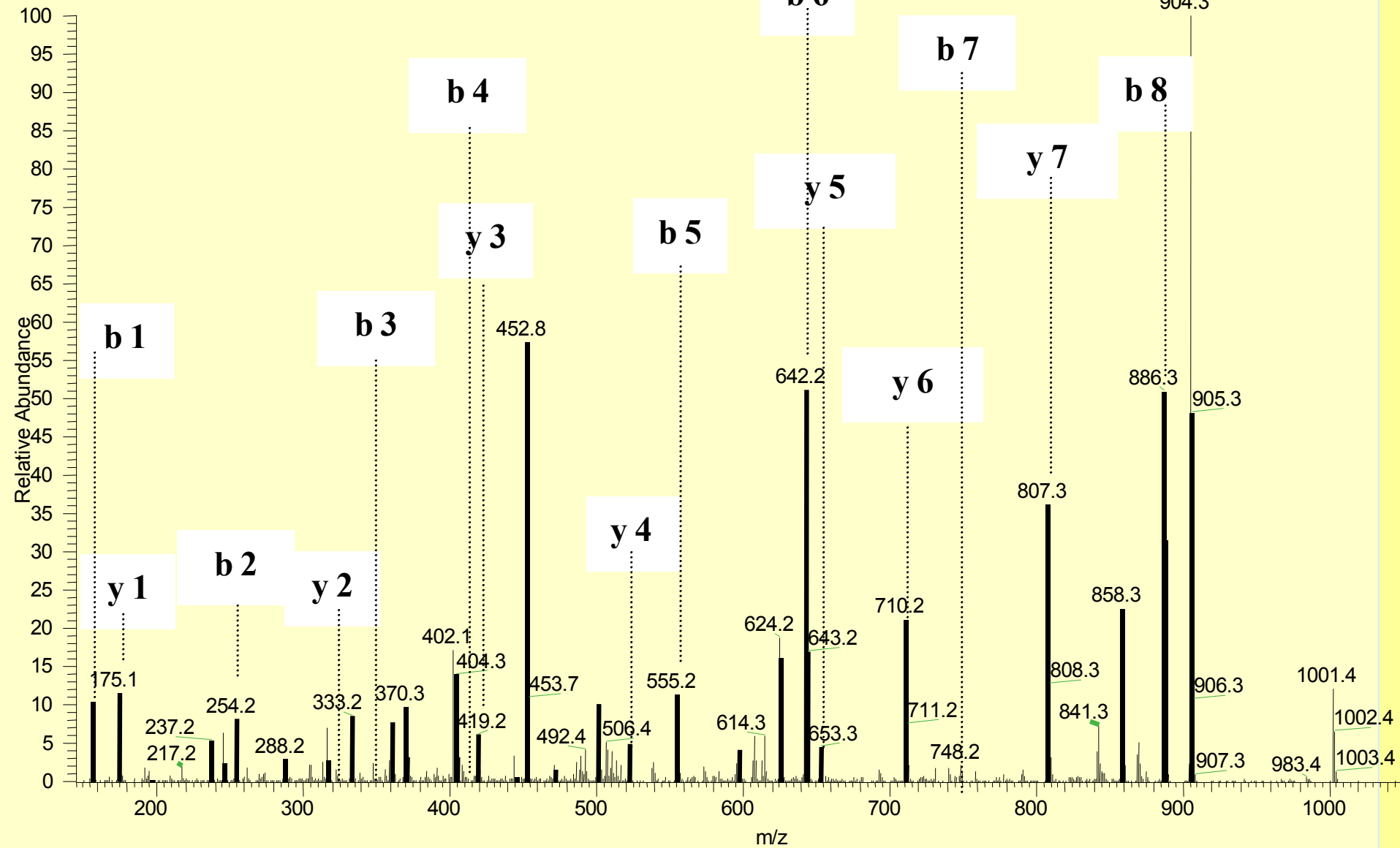
Perform NCBI Blast Search (nr protein database) on [RPPGFSPFR](#)

Done.

(a)

RPPGFSPFR

S#: 75-108 RT: 1.63-2.56 AV: 34 NL: 9.80E4
F: + c Full ms2 531.00 [145.00 - 1100.00]

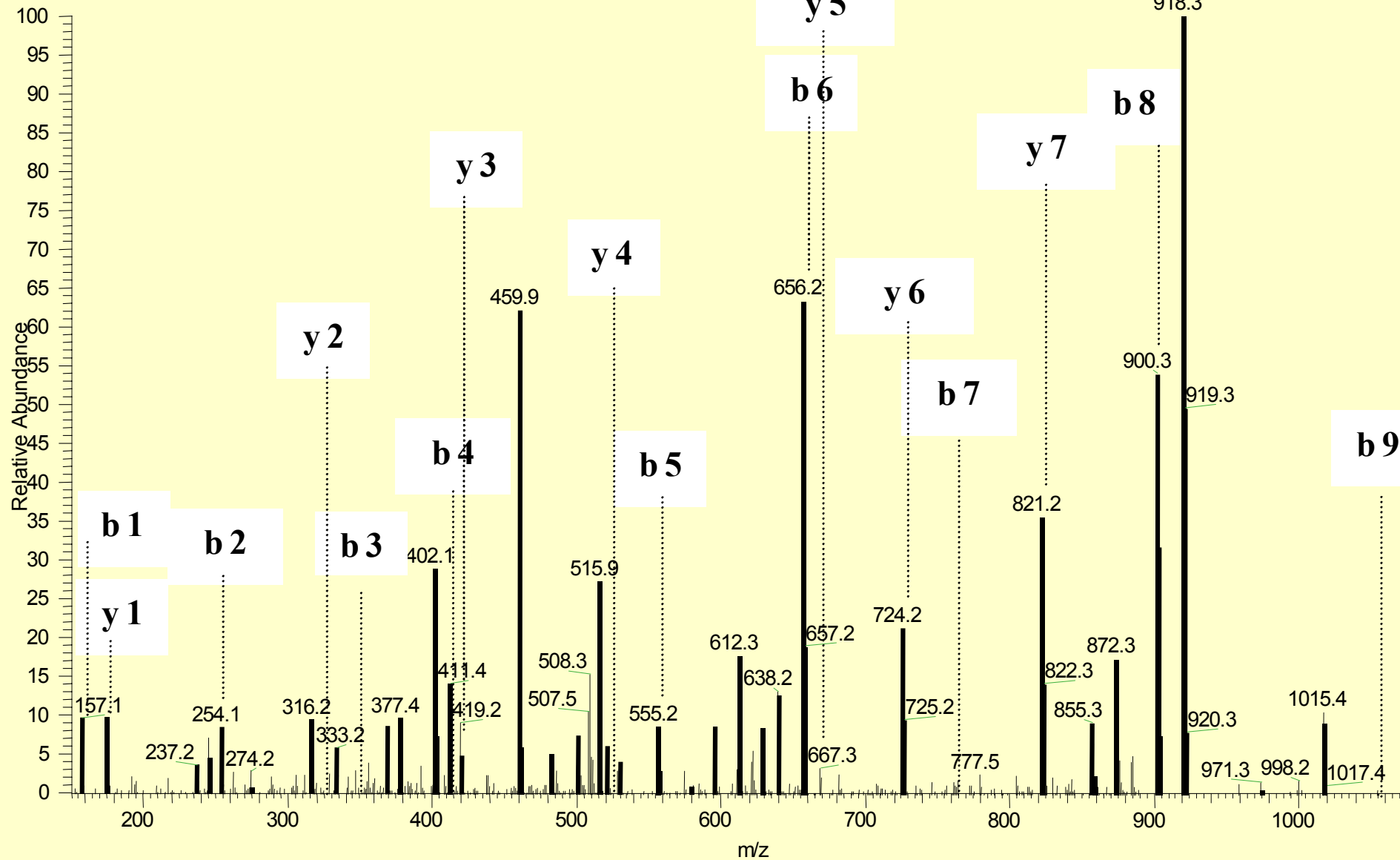


(b)

RPPGFTPFR

S#: 25-33 RT: 0.41-0.65 AV: 9 NL: 6.24E4

F: + c Full ms2 538.00 [150.00 - 1100.00]







cDNA CLONING

RT-PCR amplification of frog peptide mRNA



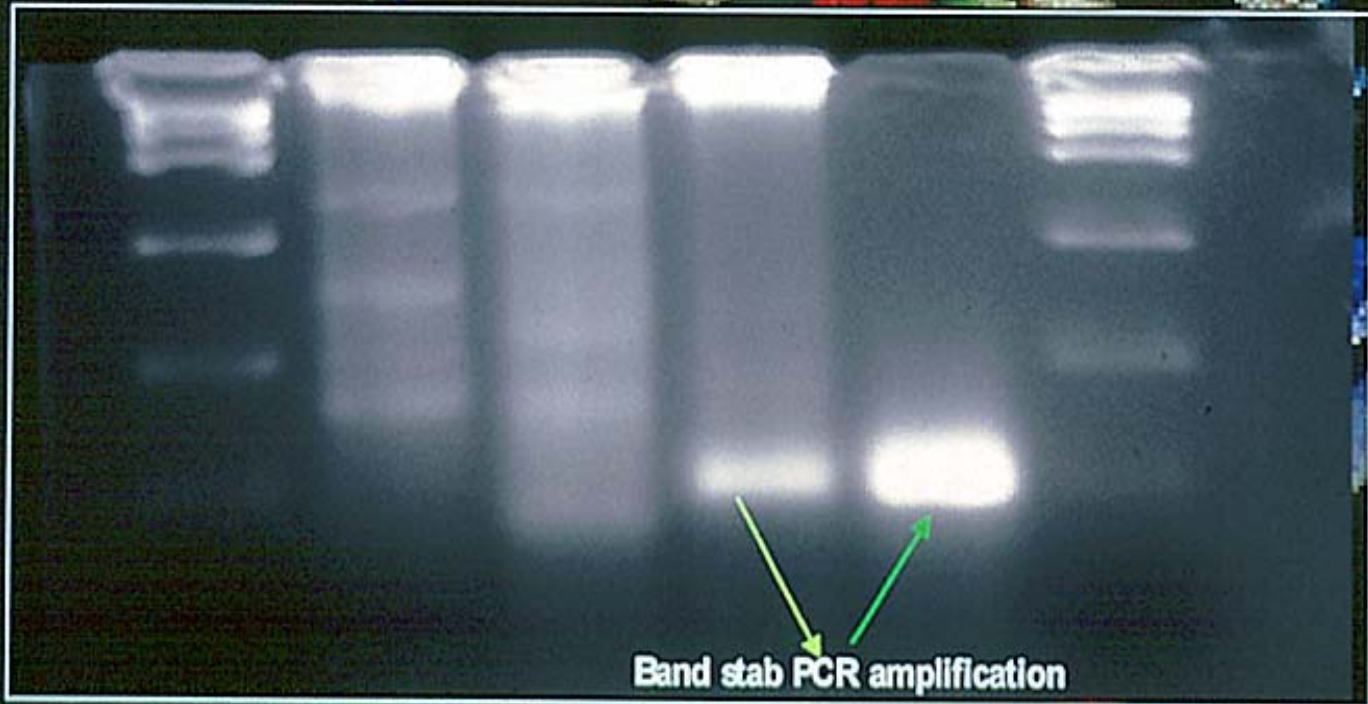
marker

Bradykinin

Mamba-like

RT-48
antimicrobial

marker



Band stab PCR amplification

Transcriptomic strategy

Extraction of peptide mixtures from frog sample followed by solvent extraction, centrifugation, lyophilization, etc



Fractionation of the mixtures by HPLC and verification of peptide mass by MALDI-TOF mass spectrometry



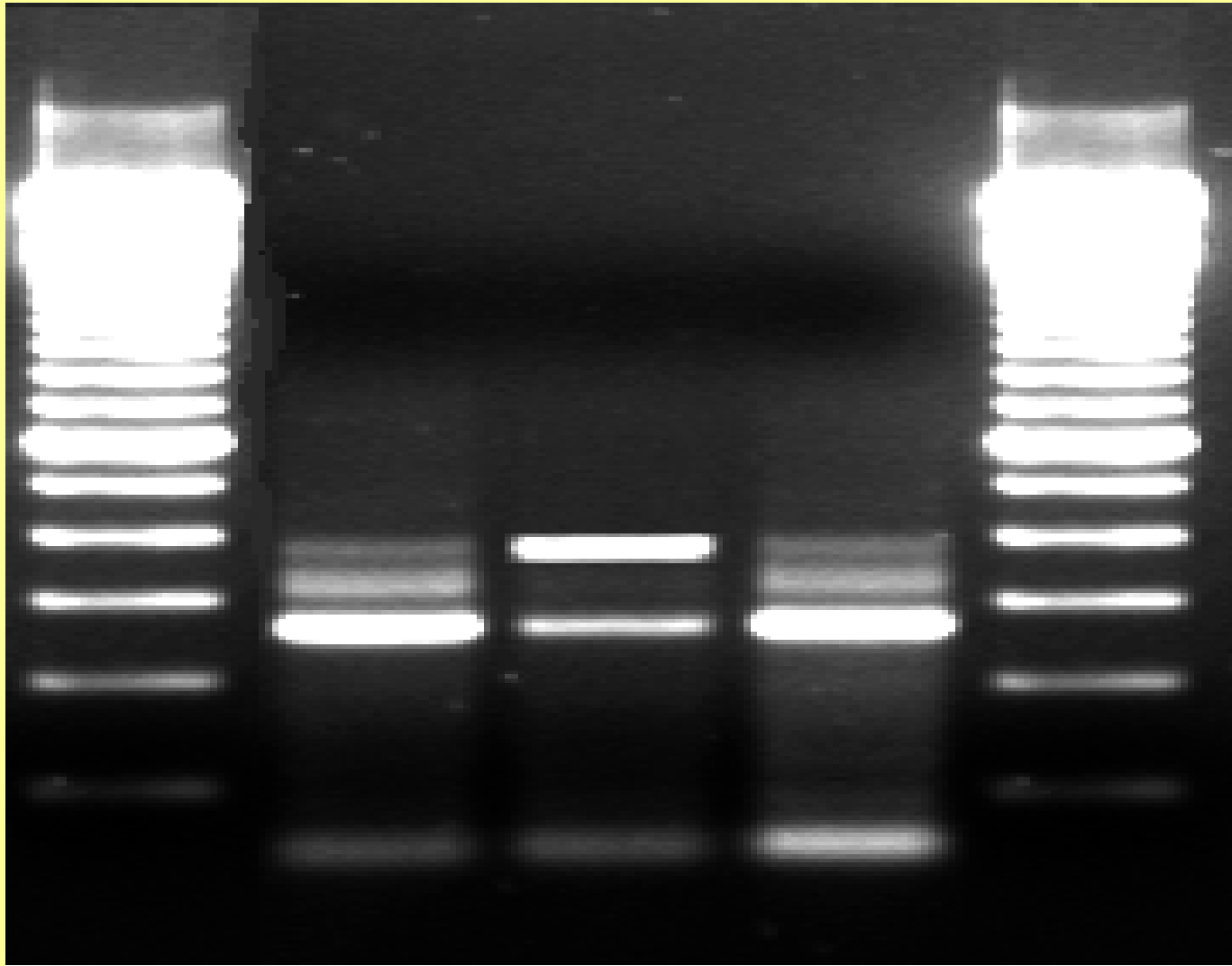
Verification of existing sequence by MS/MS (LCQ) and determination of novel peptide sequence by Edman degradation

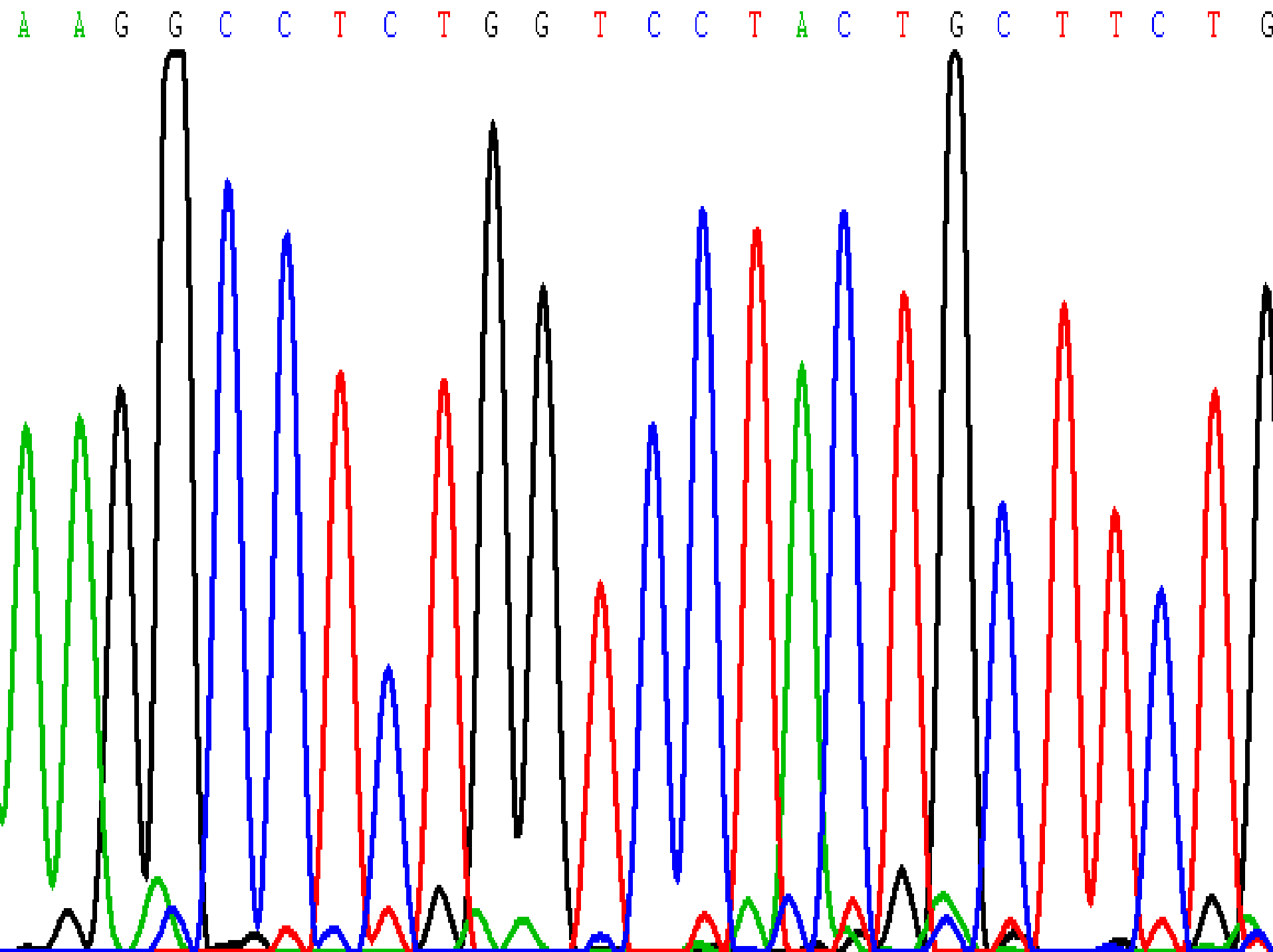


Screening of collected fraction for biological activity



Extraction of polyA RNA/total RNA for cDNA library





1 CCAATTAAGA TTTCCAGGTG GTATCCATTC TCAGAACCAG GTGAACCACC
GGTTAATTCT AAAGGTCCAC CATAGGTAAG AGTCTTGGTC CACTTGGTGG

1 M F T L K K S L L L L F F

51 AGAGCCCAA GATG TTCACC TTGAAGAAAT CCCTGTTACT CCTTTTCTTC
TCTCGGGTTT CTACAAGTGG AACTTCTTTA GGGACAATGA GGAAAAGAAG

14 L G T I N L S L C Q D E T N A E E .

101 CTTGGGACCA TCAACTTATC TCTTTGTCAG GATGAGACAA ATGCCGAAGA
GAACCCTGGT AGTTGAATAG AGAAACAGTC C TACTCTGTT TACGGCTTCT

31 . E R R D E E V A K M E E I K R G I .

151 AGAAAGAAGA GATGAAGAAG TTGCTAAAAT GGAAGAGATA AAACGCGGTA
TCTTTCTTCT C TACTTCTTC AACGATTTTA CCTTCTCTAT TTTGCGCCAT

48 .. L S G I L G A G K S L V C G L S

201 TATTAAGTGG CATCCTCGGT GCGGGGAAGA GCTTAGTATG TGGACTTAGC
ATAATTCACC GTAGGAGCCA CGCCCCTTCT CGAATCATAC ACCTGAATCG

64 G L C *

251 GGGCTGTGCT AAAGCTTGCA ATACCGAAAT CATCTGATGT GGAATATCAT
CCCGACACGA TTTCGAACGT TATGGCTTTA GTAGACTACA CCTTATAGTA

301 TTAGCTAAAT GCTAAATGTC TTATAAATAA TAAAAATGTC GCATACACTG
AATCGATTTA CGATTTACAG AATATTTATT ATTTTTACAG CGTATGTGAC

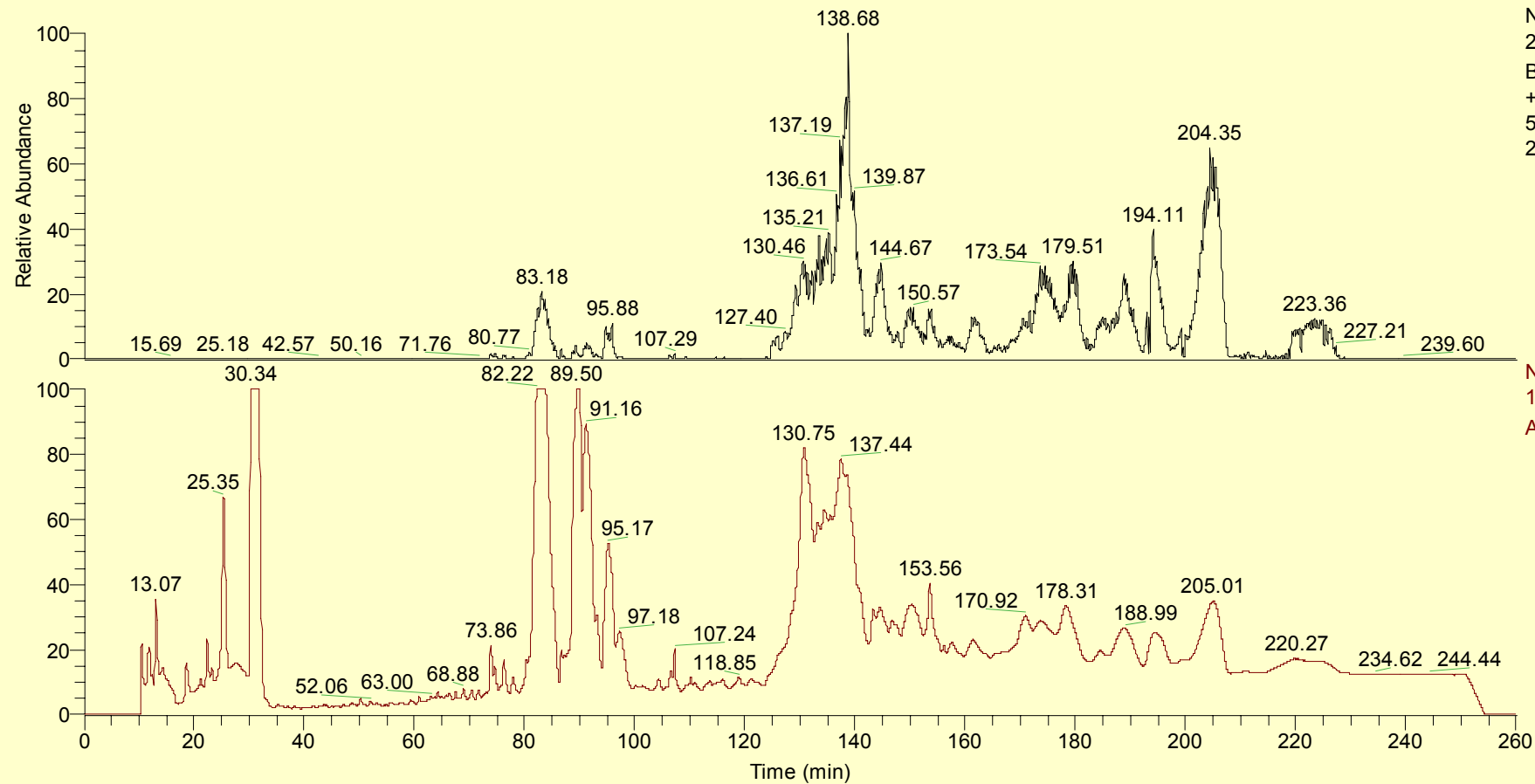
351 AAAAAAAAAA AAAAAAAAAA AAAAAA
TTTTTTTTTTTT TTTTTTTTTTTT TTTTTT

		1		50
Bm8a	(1)	MKCFAQIVVLLLVI	AFSHGAVITGV	CDRDAQCGSGTCCAASAFSRNIRFC
Bm8b	(1)	MKCFAQIVVLLLVI	AFSHGAVITGV	DRDAQCGSGTCCAASAFSRNIRFC
Bm8c	(1)	MKCFAQIVVLLLVI	AFSHGAVITGV	CDRDAQCGSGTCCAASAFSRNIRFC
Bm8d	(1)	MKCFAQIVVLLLVI	AFSHGAVITGV	CDRDAQCGSGTCCAASAFSRNIRFC
Bm8e	(1)	MKCFAQIVVLLLVI	AFSHGAVITGV	CDRDAQCGSGTCCAASAFSRNIRFC
Bm8f	(1)	MKCFAQIVVLLLVI	AFSHGAVITGV	CDRDAQCGSGTCCAASAFSRNIRFC

		51		96
Bm8a	(51)	VPLGNNGEECHPASHKVP	YNGKRLSSSLCPCNTGLTCSKS	GEKEQCS
Bm8b	(51)	VPLGNNGEECHPASHKVP	YNGKRLSSSLCPCNTGLTCSKS	GEKYQCS
Bm8c	(51)	VPLGNNGEECHPASHKVP	YNGKRLSSSLCPCNTGLTCSKS	GEKEQCS
Bm8d	(51)	VPLGNNGEECHPASHKVP	YNGKRLSSSLCPCNTGLTCSKS	GEKSQCS
Bm8e	(51)	VPLGNNGEECHPASHKVP	YNGKRLSSSLCPCNTGLTCSKS	GEKEQCS
Bm8f	(51)	VPLGNNGEECHPASHKVP	SDGKRLSSSLCPCNTGLTCSKS	GEKYQCS

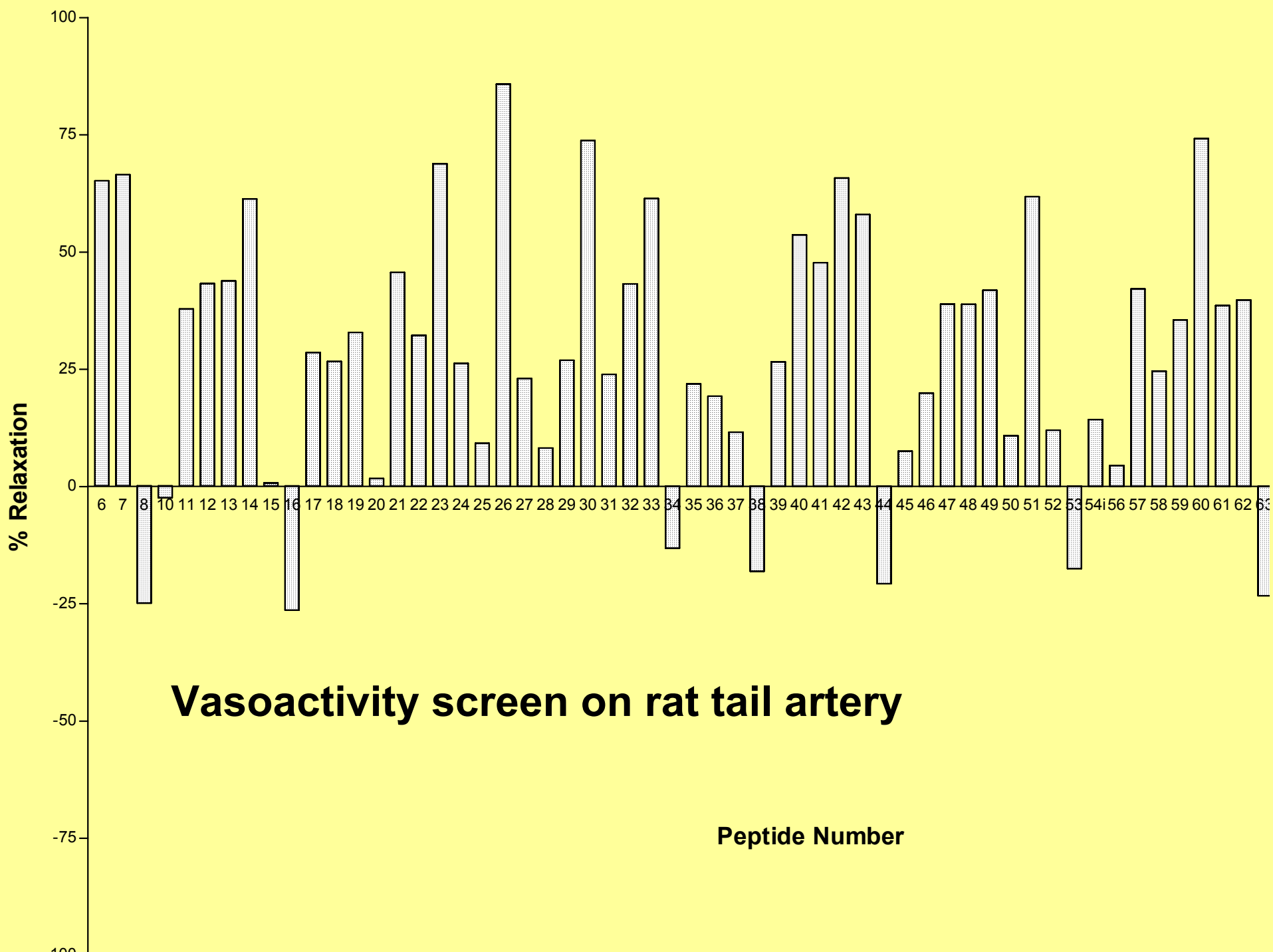


RT: 0.00 - 260.01 SM: 7B

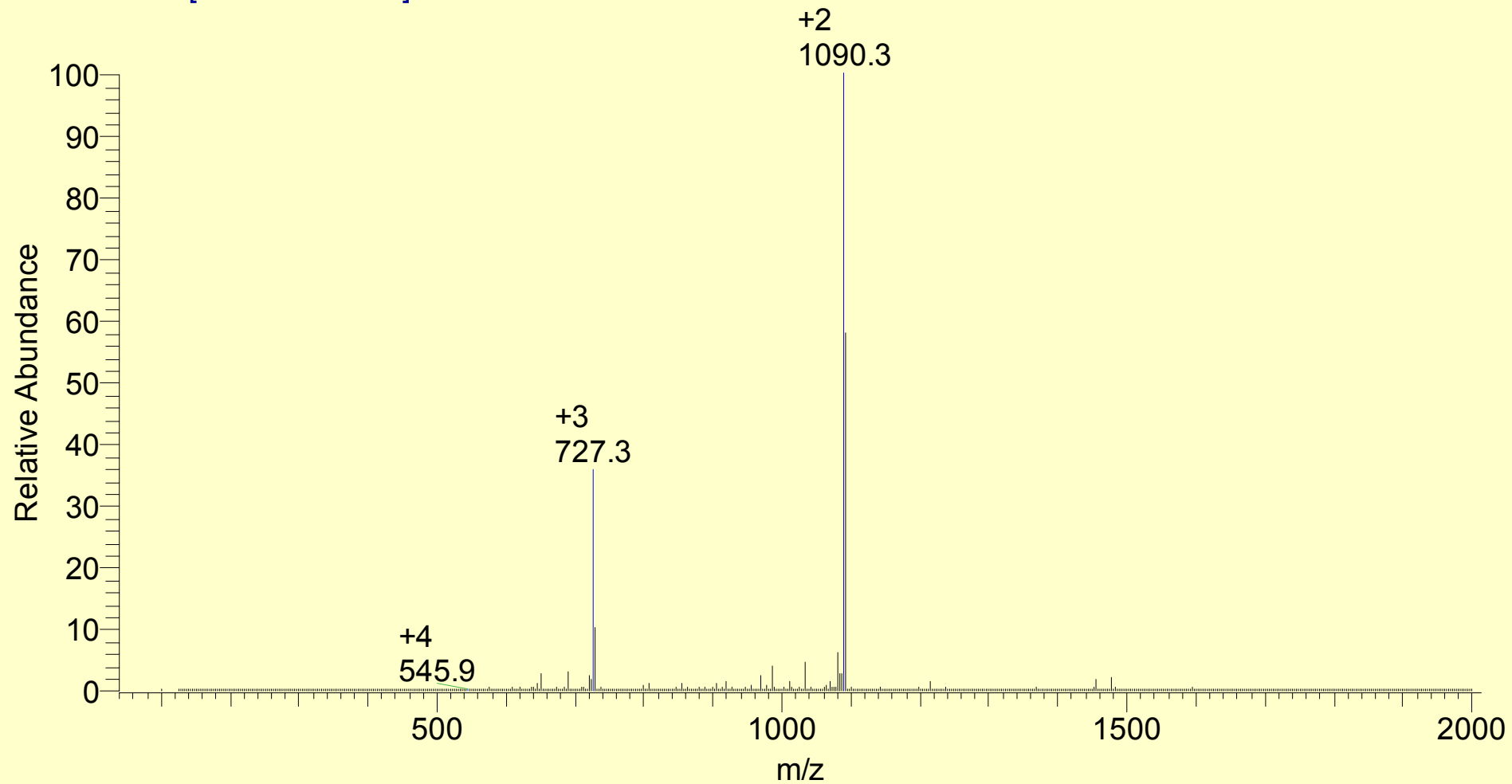


NL:
2.75E8
Base Peak F:
+ c Full ms [
50.00 -
2000.00]

NL:
1.00E6
Analog UV 1



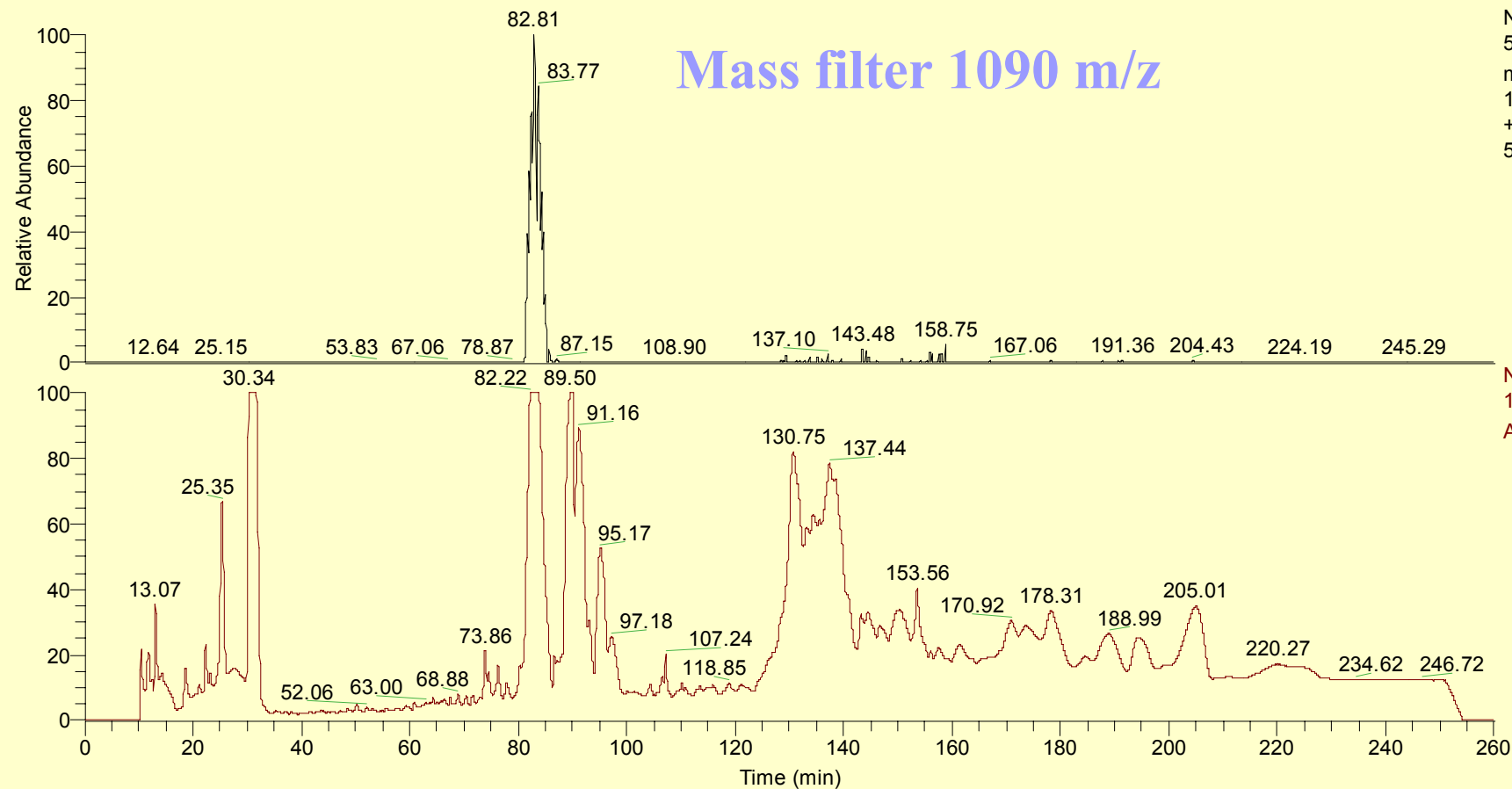
BMAX030101_01#2360-2636 RT: 78.29-85.34 AV: 277 Avg MW: 2179.0 +/- 0.4 NL: 1.73E7
T: + c Full ms [50.00-2000.00]



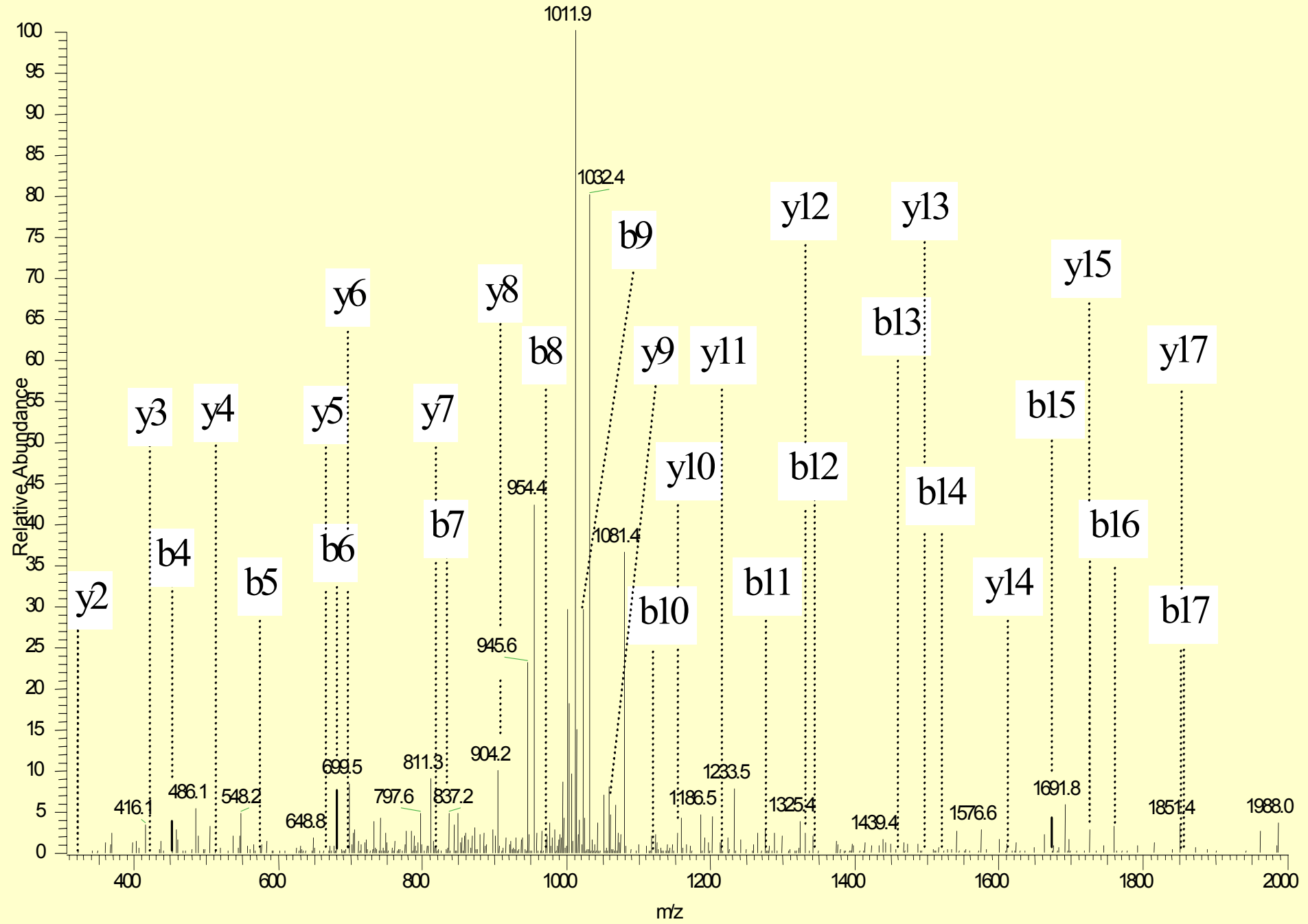
RT: 0.00 - 260.01 SM: 7B

Mass filter 1090 m/z

NL:
5.40E7
m/z=
1089.5-1090.5 F:
+ c Full ms [
50.00 - 2000.00]

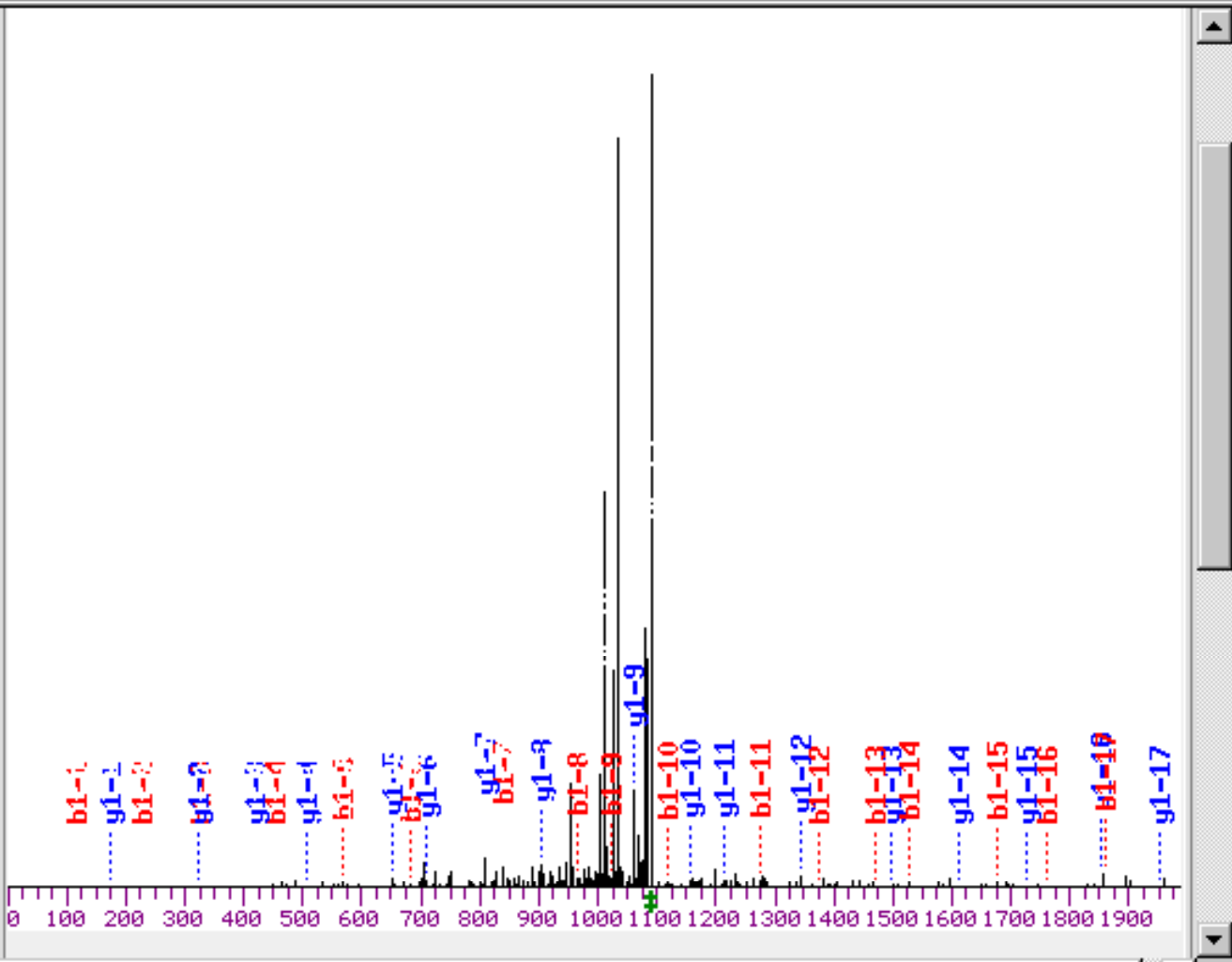


NL:
1.00E6
Analog UV 1



Seq #	b	y	(+1)
D 1	116.1	2179.5	19
L 2	229.2	2064.5	18
P 3	326.4	1951.3	17
K 4	454.5	1854.2	16
L 5	567.7	1726.0	15
N 6	681.8	1612.8	14
R 7	838.0	1498.7	13
K 8	966.2	1342.6	12
G 9	1023.2	1214.4	11
P 10	1120.3	1157.3	10
R 11	1276.5	1060.2	9
P 12	1373.6	904.0	8
P 13	1470.8	806.9	7
G 14	1527.8	709.8	6
F 15	1675.0	652.7	5
S 16	1762.1	505.6	4
P 17	1859.2	418.5	3
F 18	2006.4	321.4	2
R 19	2162.5	174.2	1

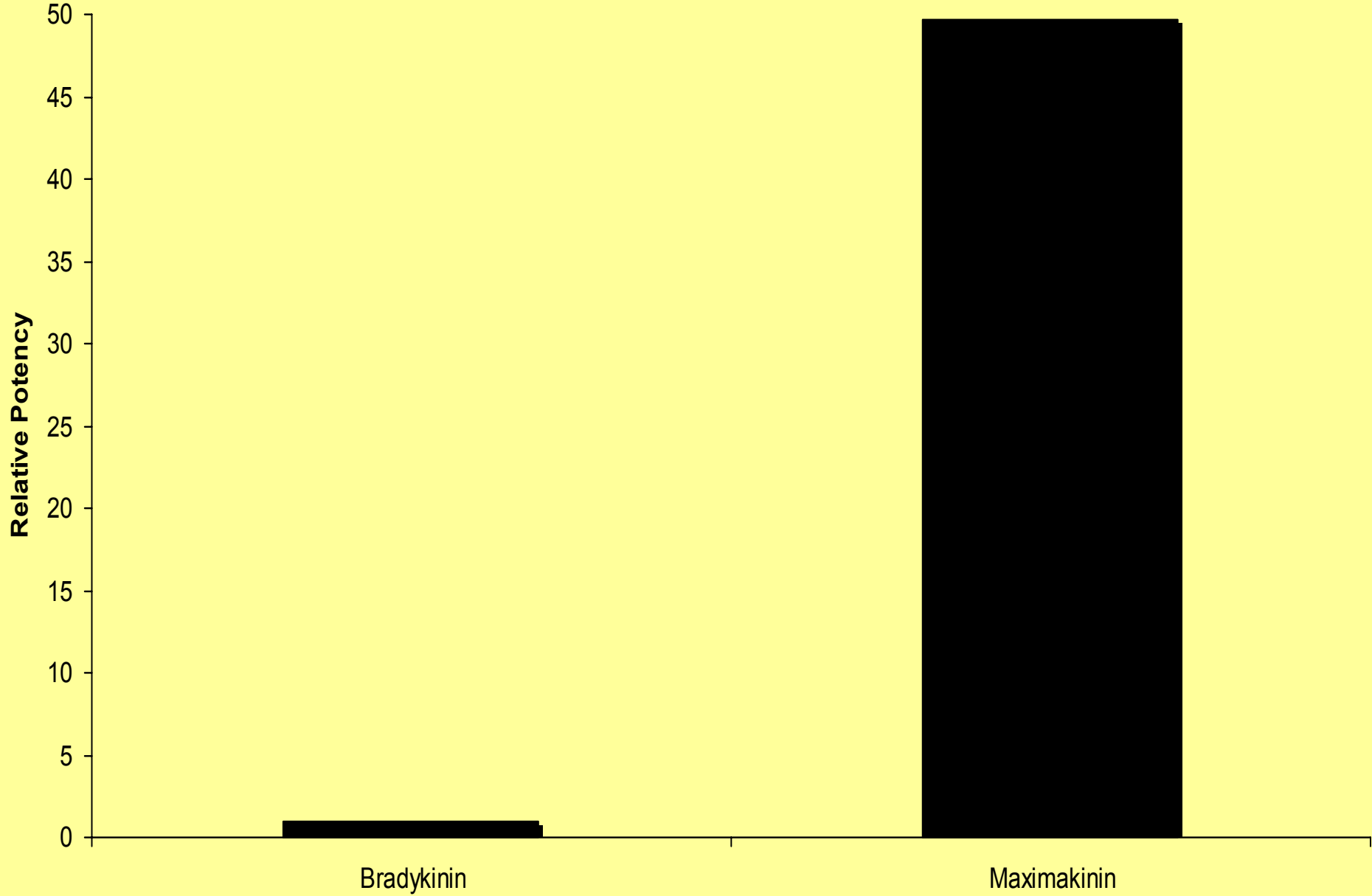
Seq #	b	y	(+2)
D 1	58.5	1090.3	19



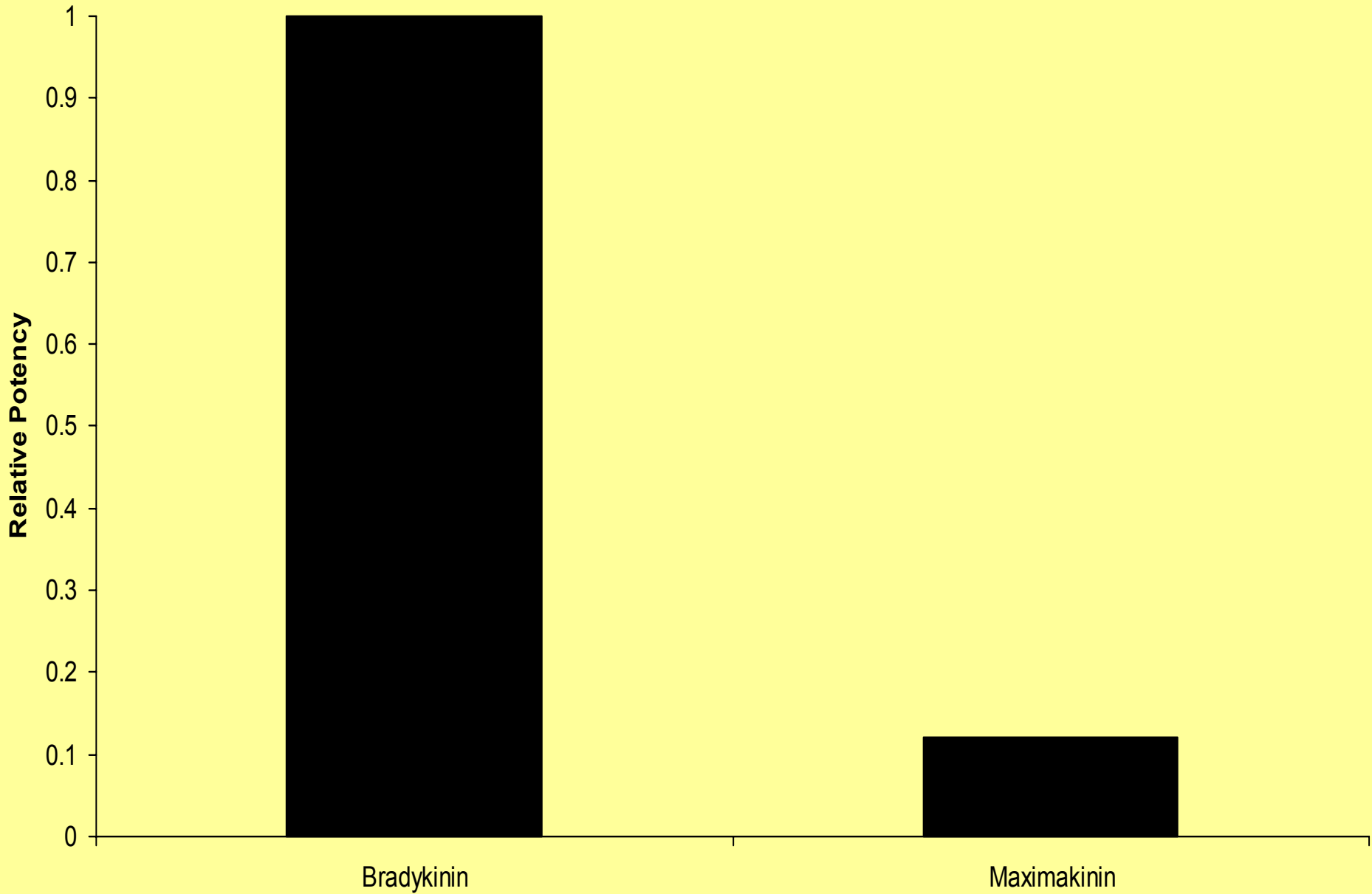
Cycle number	1	2	3	4	5	6	7	8	9	10
PTH-amino acid	D	L	P	K	I	N	R	K	G	P
Yield (pmol)	1194.3	787.9	560.0	416.9	585.9	514.6	542.0	299.1	374.5	279.7

Cycle number	11	12	13	14	15	16	17	18	19	
PTH-amino acid	R	P	P	G	F	S	P	F	R	
Yield (pmol)	328.7	295.4	361.4	164.0	137.1	48.7	98.0	76.3	14.1	

Artery



Small Intestine



M R L W F C L .
 1 TAGTTCTCAG TGTCACCTTCC AGCTCTGATC **ATGAGACTGT** **GGTTCTGTCT**
 . S F F I V L C L E H F P G T L A D .
 51 **AAGTTTTTTC** **ATCGTCCTGT** **GCCTGGAGCA** **TTTTCCAGGA** ACCCTGGCAG
 . . E R N N R D Y T I R T R L H G H
 101 ATGAAAGGAA TAATCGTGAC TACACCATCA GAACCCGCTT ACATGGCCAT
 H K P S R N N R Y A I K T S I H G .
 151 CATAAACCAA GCAGGAATAA CCGTTACGCC ATCAAACCA GCATACATGG
 . H H I P R N V P E S E E K T E Q L .
 201 CCATCATATA CCAAGGAATG TTCCAGAGAG TGAAGAAAAA ACTGAGCAGC
 . . L R D L P K I N R K G P R P P G
 251 TCCTGAGGGA TTTGCCTAAG ATCAACCGCA AAGGACCACG TCCACCGGGG
 F S P F R G K F H S Q S L R Q I P .
 301 TTCTCCCCTT TTCGAGGAAA ATTCCATAGC CAGTCCCTAC GACAAATTCC
 . G L G P L R G *
 351 TGGTTTAGGC CCTCTGCGTG GATAACGAAG CTCAGGGATA AGAATCTGCC
 401 CTATGTGTAT GCCATGTTCA CCATAGGCTA AAAAGTAGCG TCCCCTGCTA
 451 TAAATAAGCA TTGTTATGTC ACCTCTGTAA TACCAGCTCT GACTGACATG
 501 GTTTATTAAA CAGCAGATTT GTGCTCTCTA AAAAAAAAAA AAAAAAAAAA

Kinestatin

pGlu-Ile-Pro-**Gly**-Leu-Gly-**Pro**-Leu-**Arg**.NH₂

Bradykinin

Arg-Pro-Pro-**Gly**-Phe-Ser-**Pro**-Phe-**Arg**^[4, 9]

(Thr-6)-bradykinin

Arg-Pro-Pro-**Gly**-Phe-Thr-**Pro**-Phe-**Arg**^[5, 9]

(Leu-8)-bradykinin

Arg-Pro-Pro-**Gly**-Phe-Ser-**Pro**-Leu-**Arg**^[17]

(Val-1, Thr-6)-bradykinin

Val-Pro-Pro-**Gly**-Phe-Thr-**Pro**-Phe-**Arg**^[5]

(Ala-3, Thr-6)-bradykinin

Arg-Pro-Ala-**Gly**-Phe-Thr-**Pro**-Phe-**Arg**^[10]

(Val-1, Thr-3, Thr-6)-bradykinin

Val-Pro-Thr-**Gly**-Phe-Thr-**Pro**-Phe-**Arg**^[10]

References

The characterisation and determination of indole alkaloids in frog skin secretions by electrospray ionisation ion trap mass spectrometry

S. McClean, R.C. Robinson, C. Shaw and W.F. Smyth.

Rapid Communications in Mass Spectrometry 16 (2002) 346-354

Bradykinins and their precursor cDNAs from the skin of the Fire-Bellied Toad (*Bombina orientalis*)

T. Chen, D.F. Orr, A.J. Bjourson, S.McClean, M. O'Rourke, D.G. Hurst, P. Rao and C.Shaw

Peptides 23 (2002) 1547-1555

Novel bradykinins and their precursor cDNAs from the European Yellow-Bellied Toad (*Bombina variegata*) skin.

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GENOMICS

(Genes; Mutations)

PROTEOMICS

(Proteins: Identity; Expression; Activity)

MEDICINAL CHEMISTRY

(Orally Active Drugs: Natural Products; Synthetic; Combinatorial)

DRUG ACTIVITY/SPECIFICITY

(Protein/Drug Assays: Receptor Binding; Enzyme Inhibition)

BIOAVAILABILITY/METABOLISM

(In Vitro/In Vivo Preclinical)

CLINICAL PHARMACOKINETICS/METABOLISM

(Human Pharmacokinetics/Metabolism)

METHODS DEVELOPMENT FOR PRODUCTION

(Formulation, Stability)

PRODUCTION QC

(Purity)

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*'The toad, ugly and venomous, wears yet
a precious jewel in his crown'*

Troilus & Cressida

Shakespeare