How do HYNIC-conjugated peptides bind technetium? Insights from LC-MS and stability studies.

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Dalton Discussion 10

Supplementary Information

Tables A – H: LC-MS data for ^{99}Tc peptide conjugates, including HPLC profile and ES-MS assignments
Table I – HPLC data on ^{99m}Tc and ^{99}Tc labeled peptides
Figure: High resolution detail of molecular ions to demonstrate confidence in number of protons displaced during Tc-binding

Throughout, calculated m/z values are based on the following exact molecular mass data calculated for uncharged molecules and atoms using ChemDraw analytical tool

HYNIC-nanogastrin conjugate = 1279.5 (M^+ 1280.5, M 1278.5)
HYBA-nanogastrin conjugate = 1278.5 (M^+ 1279.5, M 1277.5)
Tc = 98.9
Nicotinic acid = 123.03
Tricine = 179.08
N,N'-Ethylenediamine diacetic acid (EDDA) = 176.08
Trifluoroacetic acid (TFA) = 113.99
Acetonitrile (ACN) = 41.03

In each table, P refers to the unlabelled peptide conjugate (HYNIC-nanogastrin or HYBA-nanogastrin)

TABLE A
Unlabelled peptides

peptide	Method A, B, C	% of base peak	m/z calc	m/z obs	Assignment	Inferred solution species from which in originates
HYNIC-nanogastrin	C	100	640.8	640.9	$[P+2H]^{2+}$	P
	C	45	1280.5	1280.5	$[P+H]^+$	
HYBA-nanogastrin	C	100	639.3	639.3	$[(P-2H)+2H]^{2+}$	P–2H
	C	30	1277.5	1277.3	$[(P-2H)+H]^+$	
	C	30	640.5	640.2	$[P+2H]^{2+}$	P
	C	15	1279.5	1279.4	$[P+H]^+$	
HYNIC-[gly^8]nanogastrin	B	100	1208.2	1208.4	$[P+H]^+$	P
	B	20	604.1	604.8	$[P+2H]^{2+}$	

TABLE B
P = Nanogastrin-hynic, coligand = tricine

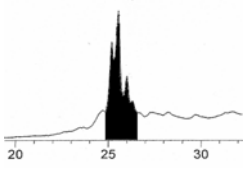
HPLP peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	Assignment	Inferred solution species from which in originates
	B	100	777.2	777.5	$[(P+Tc+Tri-5H)+2H]^{2+}$	(P+Tc+Tri–5H)
	B	15	1553.5	1553.2	$[(P+Tc+Tri-5H)+H]^+$	
	A	90	1551.5	1551.4	$[(P+Tc+Tri-5H)-H]^-$	
	A	100	1665.5	1664.7	$[(P+Tc+Tri-5H+TFA)-H]^-$	
	A	80	1179.5	1778.5	$[(P+Tc+Tri-5H)+2TFA-H]^-$	
	C	100	1553.5	1553.3	$[(P+Tc+Tri-5H)+H]^+$	
	C	40	777.2	777.3	$[(P+Tc+Tri-5H)+2H]^{2+}$	

TABLE C
P = nanogastrin-hynic, colignd = tricine + nicotinic acid

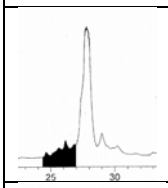
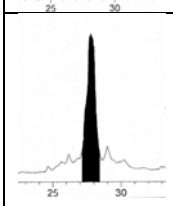
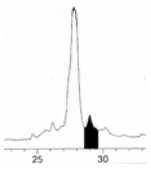
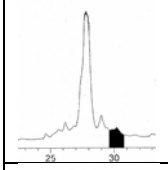
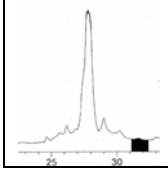
HPLC peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	assignment	Inferred solution species from which in originates
	B	100	846.8	846.9	$[(P+O+Tc+Tri+Nic-5H)+2H]^{2+}$	(P+O+Tc+Nic−5H)
	B	2	1692.5	1692.2	$[(P+O+Tc+Tri+ Nic-5H)+H]^{+}$	
	A	100	1690.5	1690.3	$[(P+O+Tc+Tri+ Nic-5H)-H]^{-}$	
	A	30	1804.5	1803.0	$[(P+O+Tc+Tri+Nic+TFA-5H)-H]^{-}$	
	A	25	1551.5	1551.0	$[(P+Tc+Tri-5H)-H]^{-}$	(P+Tc+Tri−5H)
	B	100	839.2	838.9	$[(P+Tc+Tri+Nic-5H)+2H]^{2+}$	(P+Tc+Tri+Nic−5H)
	B	30	1676.5	1677.3	$[(P+Tc+Tri+Nic-5H)+H]^{+}$	
	A	100	1674.5	1674.3	$[(P+Tc+Tri+Nic-5H)-H]^{-}$	
	A	15	1788.5	1787.0	$[(P+Tc+Tri+Nic+TFA-H)]^{-}$	
	C	100	839.2	838.9	$[(P+Tc+Tri+Nic-5H)+2H]^{2+}$	
	C	10	1676.5	1676.1	$[(P+Tc+Tri+Nic5H)+H]^{+}$	
	B	25		1118.0	?	
	B	15	899.8	900.2	$[(P+Tc+Tri+2Nic-5H)+2H]^{2+}$	(P+Tc+Tri+2Nic−5H)
	C	30	899.8	900.6	$[(P+Tc+Tri+2Nic-5H)+2H]^{2+}$	
	C	2	1799.5	1799.9	$[(P+Tc+Tri+2Nic-5H)+2H]^{2+}$	
	B	100		1115.5	?	
	B	50	829.8	829.9	$[(P+Tc+Tri+Nic-H_2O-5H)+2H]^{2+}$	($P-H_2O$+Tc+Tri+Nic−5H)
	A	80	1656.5	1656.3	$[(P+Tc+Tri+Nic-H_2O-5H)-H]^{-}$	
	A	40	1674.5	1674.2	$[(P+Tc+Tri+Nic-5H)-H]^{-}$	(P+Tc+Tri+Nic−5H)
	A	35	1770.5	1768.9	$[(P+Tc+Tri+Nic+TFA-5H)-H]^{-}$	
	B	45	839.2	838.8	$[(P+Tc+Tri+Nic-5H)+2H]^{2+}$	
	A	100		1340.5	?	
	B	100	866.8	867.0	$[(P+Tc+2Tri-5H)+H]^{+}$	(P+Tc+2Tri−5H)
	A	100	1730.6	1731.4	$[(P+Tc+2Tri-5H)-H]^{-}$	
	B	45	839.2	838.9	$[(P+Tc+Tri+Nic-5H)+2H]^{2+}$	(P+Tc+Tri+Nic−5H)
	B	25		1043.2	?	
	B	20		930.1	?	
	A	20		1843.3	?	
	B	100		1128.2	?	
	B	40		838.8	?	

TABLE D
P =NG-HYNIC, coligand = Tricine+EDDA

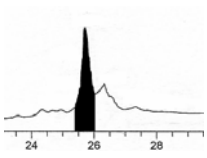
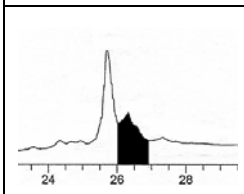
HPLC peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	assignment	Inferred solution species from which in originates
	B	100	863.8	864.0	$[(P+Tc+2EDDA-5H)+2H]^{2+}$	P+Tc+2EDDA−5H
	B	12	1726.6	1726.2	$[(P+Tc+2EDDA-5H)+H]^{+}$	
	A	70	1724.6	1724.3	$[(P+Tc+2EDDA-5H)-H]^{-}$	
	A	35	1838.6	1838.5	$[(P+Tc+2EDDA+TFA-5H)-H]^{-}$	
	C	100	863.8	863.9	$[(P+Tc+2EDDA-5H)+2H]^{2+}$	
	C	7	1726.6	1726.1	$[(P+Tc+2EDDA-5H)+H]^{+}$	
	B	3	1550.5	1550.2	$[(P+Tc+EDDA-5H)+H]^{+}$	P+Tc+EDDA−5H
	C	3	1550.5	1551.3	$[(P+Tc+EDDA-5H)+H]^{+}$	
	B	20		1151.4		?
	A	100		1376.8		?
	A	65		1263.6		?
	B	100		668.9		?
	B	60		863.9		?
	B	45		1017.3		?
	B	35		1336.4		?
	A	100		1334.5		?
	A	80		1447.6		?

TABLE E
P = NG-HYBA, coligand = tricine

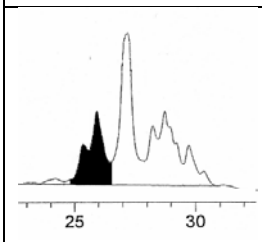
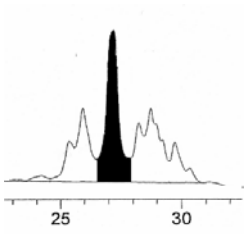
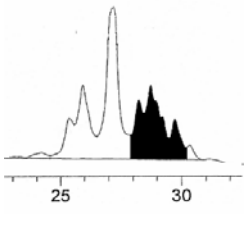
HPLC peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	assignment	Inferred solution species from which in originates
	A	100	1550.5	1550.3	$[(P+Tc+Tri-5H)-H]^-$	[P+Tc+Tri−5H]
	A	95	1664.5	1663.7	$[(P+Tc+Tri+TFA-5H)-H]^-$	
	B	100	797.3	797.3	$[(P+Tc+Tri+ACN-5H)+2H]^{2-}$	
	B	15	776.8	776.9	$[(P+Tc+Tri-5H)+2H]^{2-}$	
	B	5	1552.5	1552.2	$[(P+Tc+Tri-5H)+H]^-$	
	B	3	1593.5	1593.4	$[(P+Tc+Tri+ACN-5H)+H]^-$	
	A	35		1704.5	?	
	A	30		1818.5	?	
	A	100	1550.5	1550.4	$[(P+Tc+Tri-5H)-H]^-$	[P+Tc+Tri−5H]
	A	25	1664.5	1663.2	$[(P+Tc+Tri+TFA-5H)H]^-$	
	B	40	776.8	777.0	$[(P+Tc+Tri-5H)+2H]^{2-}$	
	B	20		1552.3	$[(P+Tc+Tri-5H)+H]^+$	
	A	25	1729.6	1729.2	$[(P+Tc+2Tri-5H)-H]^-$	[P+Tc+2Tri−5H]
	B	100	866.3	866.4	$[(P+Tc+2Tri-5H)+2H]^{2+}$	
	B	20	1731.6	1732.1	$[(P+Tc+2Tri-5H)+H]^+$	
	A	25		1859.2	?	
	A	20		1745.8	?	
	A	50		1704.6	?	
	A	30		1436.5	?	
	B	30		1719.8	?	
	B	25		817.8	?	
	A	100		1375.9	?	
	A	65		1418.7	?	
	A	15		1460.5	?	
	A	100		1333.4	?	
	A	85		1446.6	?	
	A	40		1460.9	?	
	A	35		1347.5	?	
	A	100		1387.7	?	
	A	55		1274.6	?	
	A	100		1444.6	?	
	A	85		1331.5	?	
	A	100		1444.8	?	
	A	95		1331.5	?	
	B	100		1335.4	?	
	B	70		1349.4	?	
	B	50		668.4	?	
	B	40		632.9	?	

TABLE F
P = NG-HYBA , coligand = Tricine + Nicotinic acid

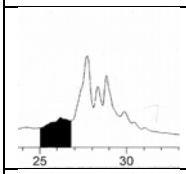
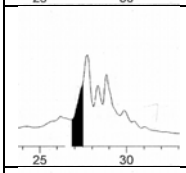
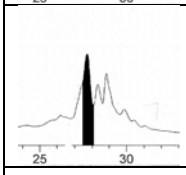
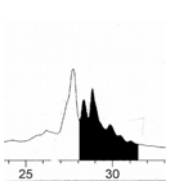
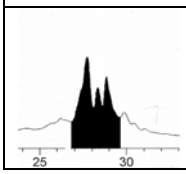
HPLC peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	assignment	Inferred solution species from which in originates
	A	100	1275.5	1275.5	$[(P-2H)-H]^-$	[P−2H]
	A	60	1389.5	1388.6	$[(P+TFA-2H)-H]^-$	
	A	65		1811.8	?	
	A	100		1256.9	?	
	A	70		1370.3	?	
	A	70		1437.5	?	
	A	70		1549.9	?	
	A	50		1462.7	?	
	A	100	1796.5	1795.9	$[(P+Tc+Tri+2Nic-5H)-5H]^-$	[P+Tc+Tri+2Nic−5H]
	A	20	1910.5	1909.1	$[(P+Tc+Tri+2Nic+TFA-5H)-5H]^-$	
	A	45	1673.5	1673.2	$[(P+Tc+Tri+Nic-5H)-5H]^-$	[P+Tc+Tri+Nic−5H]
	A	25	1550.5	1550.6	$[(P+Tc+Tri)-5H]^-$	[P+Tc+Tri−5H]
	A	100		1375.8	?	
	A	80		1262.6	?	
	A	55		1418.7	?	
	A	45		1305.6	?	
	A	100		1333.4	?	
	A	95		1456.7	?	
	A	80		1343.5	?	
	A			1446.4	?	
	A	100		1403.6	?	
	A			1440.7	?	
	B	100	899.8	899.7	$[(P+Tc+Tri+2Nic-5H)+2H]^{2+}$	[P+Tc+Tri+2Nic−5H]
	B	3	1798.6	1799.1	$[(P+Tc+Tri+2Nic-5H)+H]^+$	
	C	100	899.8	899.6	$[(P+Tc+Tri+2Nic-5H)+2H]^{2+}$	
	B	40	838.3	838.8	$[(P+Tc+Tri+Nic-5H)+2H]^{2+}$	[P+Tc+Tri+Nic−5H]
	B	3	1675.5	1677.3	$[(P+Tc+Tri+Nic-5H)+H]^+$	
	C	70	838.3	838.5	$[(P+Tc+Tri+Nic-5H)+2H]^{2+}$	

TABLE G
P = NG-HYBA, coligand = tricine + EDDA

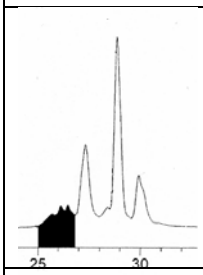
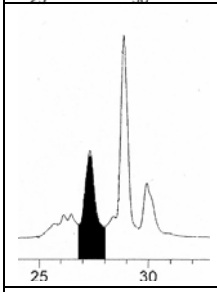
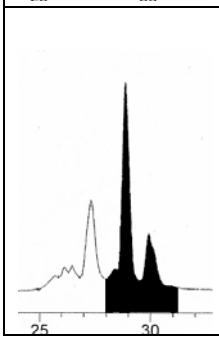
HPLC peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	Assignment	Inferred solution species from which in originates
	B	100	775.2	775.4	$[(P+Tc+EDDA-5H)+2H]^{2+}$	[P+Tc+EDDA−5H]
	B	10	1549.5	1549.3	$[(P+Tc+EDDA-5H)+H]^{+}$	
	A	100	1661.5	1660.7	$[(P+Tc+EDDA+TFA-5H)-H]^{-}$	
	A	35	1547.5	1547.4	$[(P+Tc+EDDA-5H)-H]^{-}$	
	C	100	775.2	775.2	$[(P+Tc+EDDA-5H)+2H]^{2+}$	
	C	20	1549.5	1549.4	$[(P+Tc+EDDA-5H)+H]^{+}$	
	A	20	1389.5	1388.6	$[(P-2H+TFA)-H]^{-}$	[P−2H]
	B	100	863.3	863.5	$[(P+Tc+2EDDA-5H)+2H]^{2+}$	[P+Tc+2EDDA−5H]
	B	50	1725.6	1725.3	$[(P+Tc+2EDDA-5H)+H]^{+}$	
	A	100	1723.6	1723.3	$[(P+Tc+2EDDA-5H)-H]^{-}$	
	A	25	1837.6	1835.9	$[(P+Tc+2EDDA+TFA-5H)-H]^{-}$	
	C	100	863.3	863.3	$[(P+Tc+2EDDA-5H)+2H]^{2+}$	
	C	10	1725.6	1725.1	$[(P+Tc+2EDDA-5H)+H]^{+}$	
	B	60	1549.5	1549.3	$[(P+Tc+EDDA-5H)+H]^{+}$	[P+Tc+EDDA−5H]
	B	20		676.4	?	
	B	20		1351.4	?	
	B	20		1281.3	?	
	A	70		1462.6	?	
	A	55		1349.4	?	
	B	100		1336.0	?	
	B	20		668.4	?	
	B	5		1781.0	?	
	A	100		1333.5	?	
	A	90		1446.6	?	
	A	3		1583.5	?	
	B	100		1335.5	?	
	B	40		668.4	?	
	B	20		1249.4	?	
	A	100		1333.5	?	
	A	60		1446.6	?	
	A	35		1416.8	?	
	A	25		1303.6	?	

TABLE H
P = HYNIC-[gly^{8}]nanogastrin, coligand = tricine

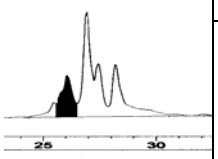
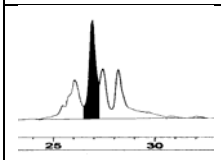
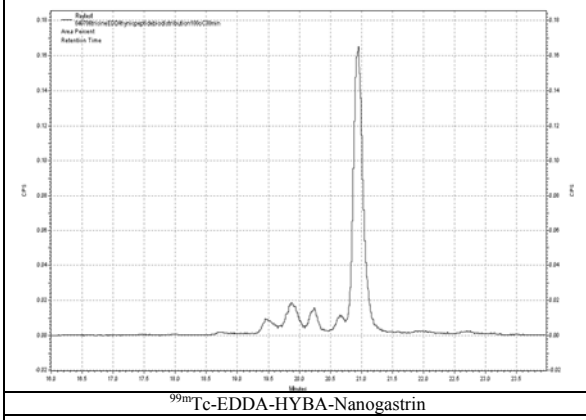
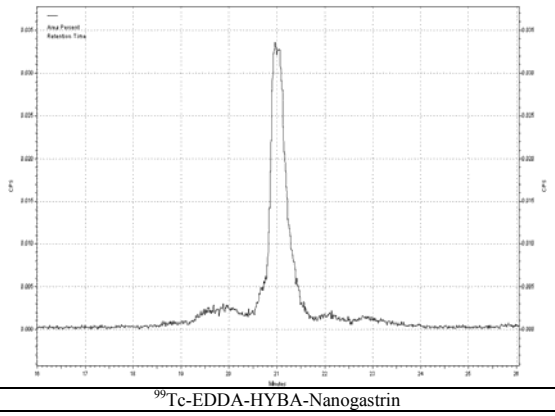
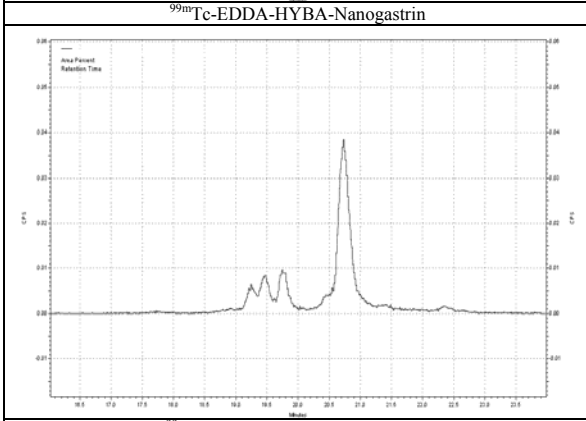
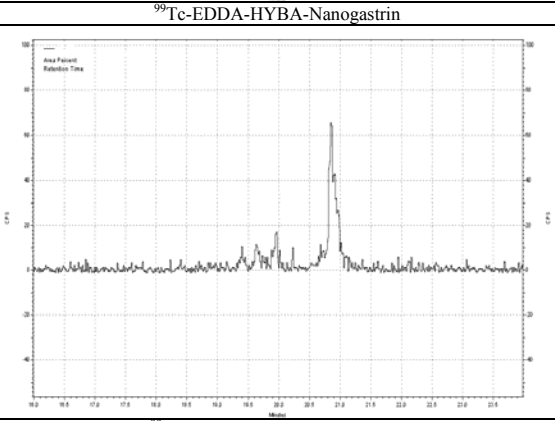
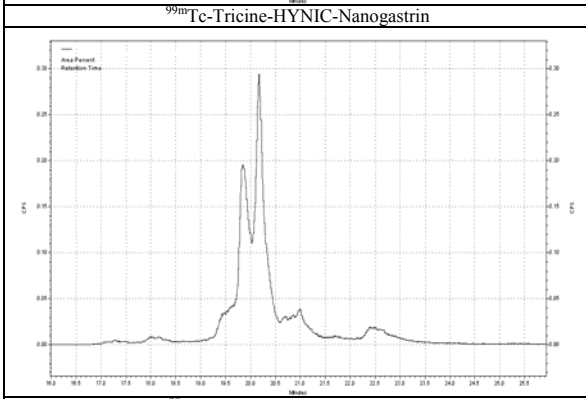
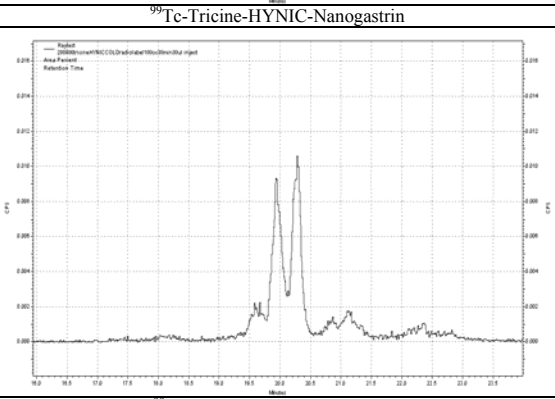
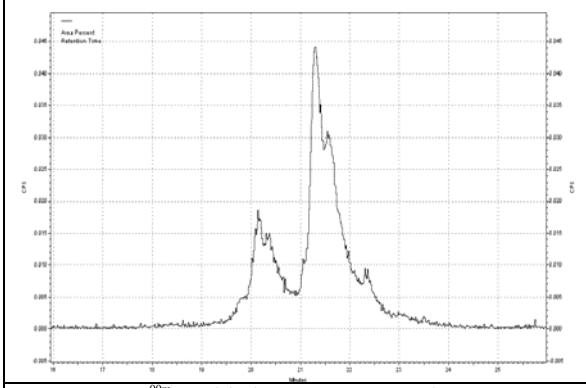
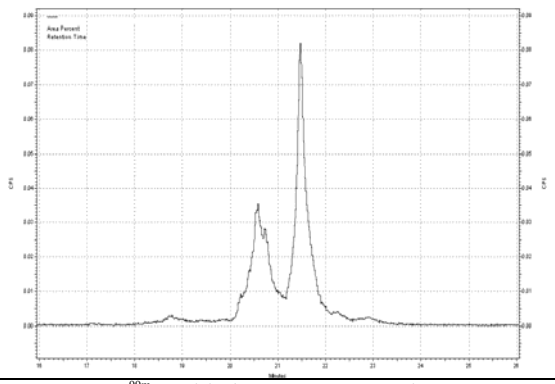
HPLC peak selected	Method A,B,C	% of base peak	m/z calc	m/z obs	Assignment	Inferred solution species from which in originates
	C	100	741.1	741.5	$[(P+Tc+Tri-5H)+2H]^{2+}$	[P+Tc+Tri−5H]
	C	40	1481.2	1481.3	$[(P+Tc+Tri-5H)+H]^{+}$	
	C	100	741.1	741.9	$[(P+Tc+Tri-5H)+2H]^{2+}$	
	C	70	1481.2	1482.4	$[(P+Tc+Tri-5H)+H]^{+}$	
	C	100	830.6	830.7	$[(P+Tc+2Tri-5H)+2H]^{2+}$	[P+Tc+2Tri−5H]
	C	15	1660.2	1660.9	$[(P+Tc+2Tri-5H)+H]^{+}$	

Table I. RP-HPLC analysis – Radiometric traces (Method 2) of ^{99m}Tc and ^{99}Tc labeled peptides

^{99m}Tc-EDDA-HYNIC-Nanogastrin	^{99}Tc-EDDA-HYNIC-Nanogastrin
	
^{99m}Tc-EDDA-HYBA-Nanogastrin	^{99}Tc-EDDA-HYBA-Nanogastrin
	
^{99m}Tc-Tricine-HYNIC-Nanogastrin	^{99}Tc-Tricine-HYNIC-Nanogastrin
	
^{99m}Tc-Tricine-HYBA-Nanogastrin	^{99}Tc-Tricine-HYBA-Nanogastrin
	
^{99m}Tc-Tricine/NA-HYNIC-Nanogastrin	^{99m}Tc-Tricine/NA-HYNIC-Nanogastrin

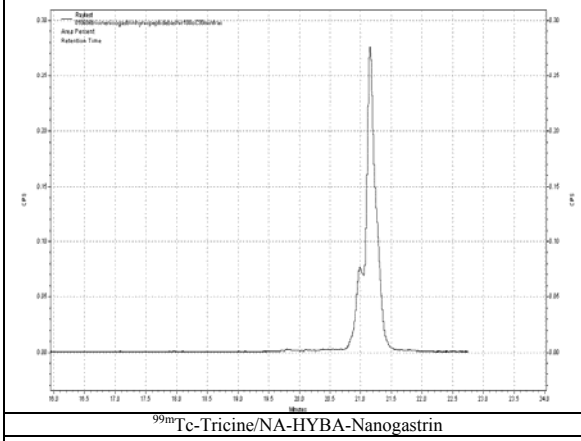
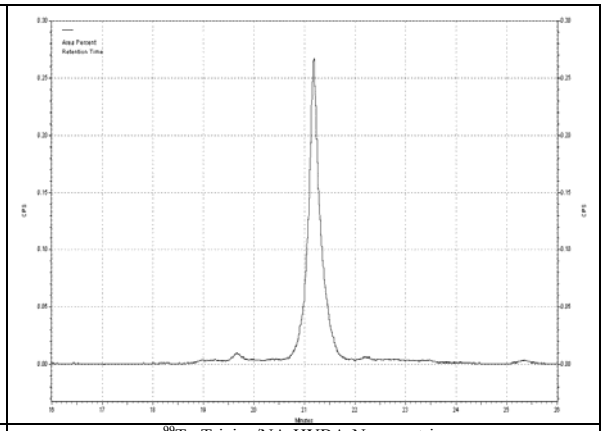
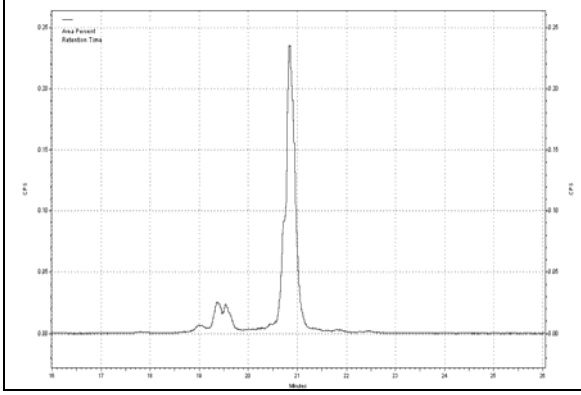
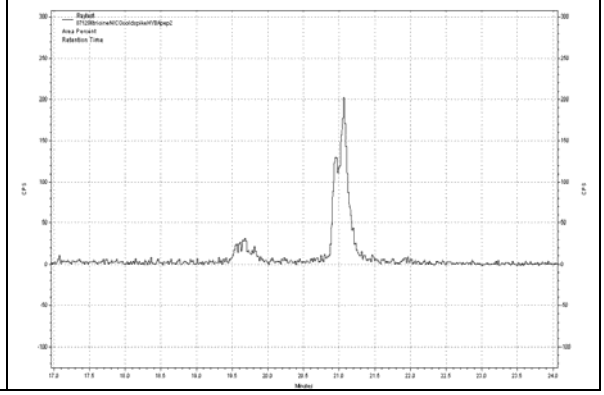
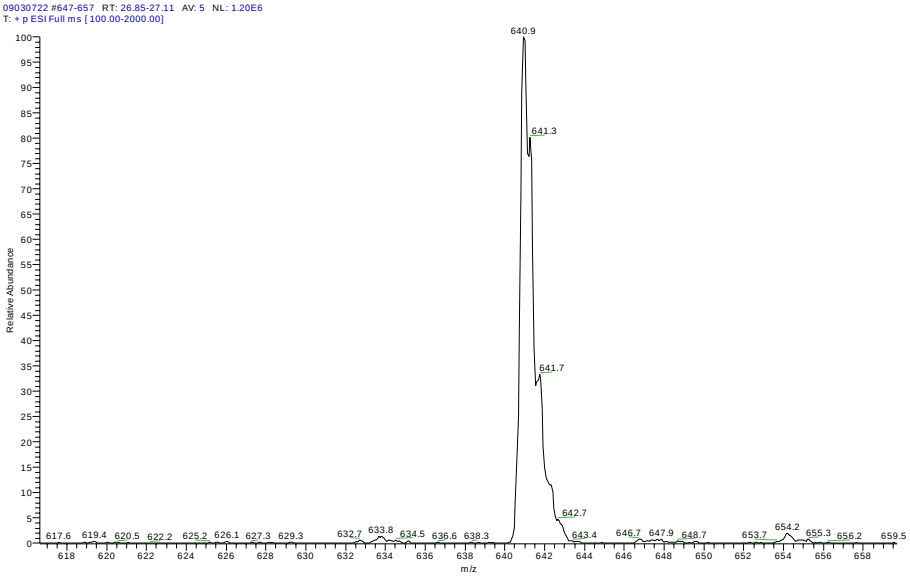
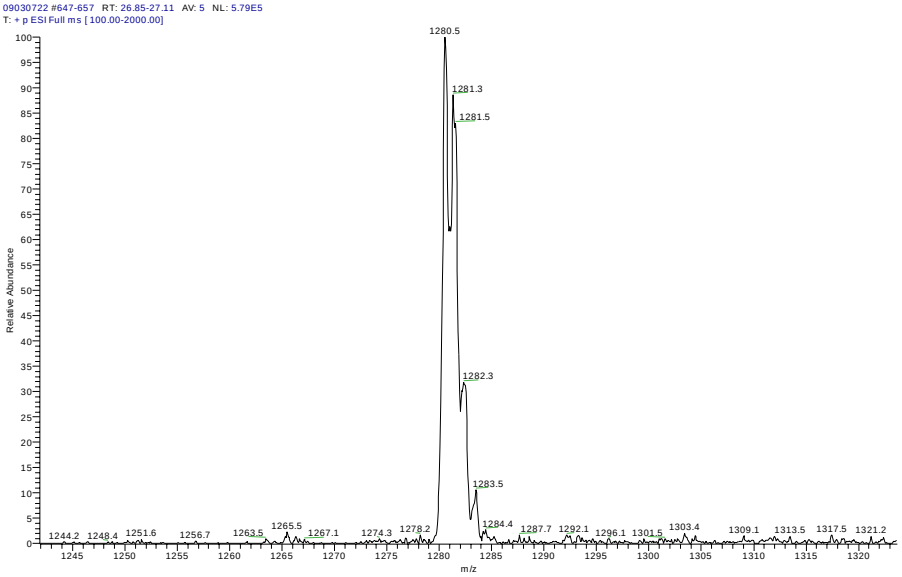
	
^{99m}Tc-Tricine/NA-HYBA-Nanogastrin	^{99}Tc-Tricine/NA-HYBA-Nanogastrin
	

Figure
High resolution detail of molecular ions to demonstrate confidence in number of protons displaced during Tc-binding

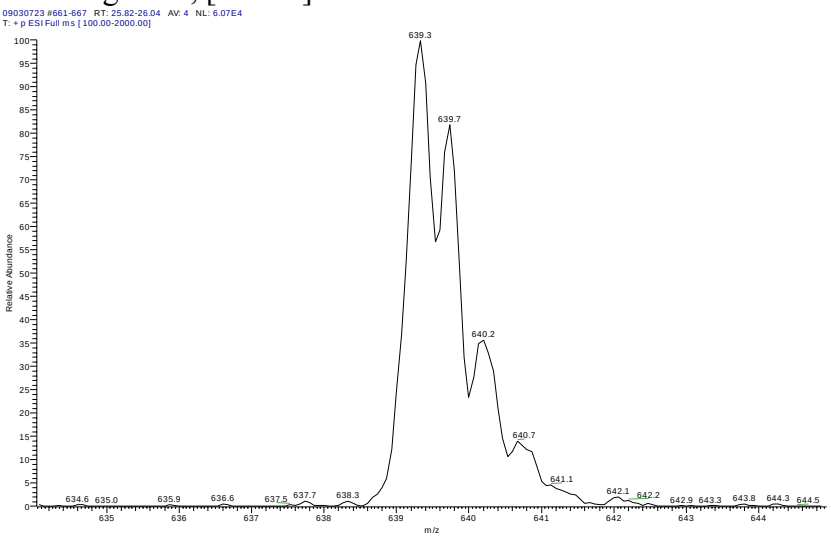
HYNIC-gastrin, $[M+2H]^{2+}$



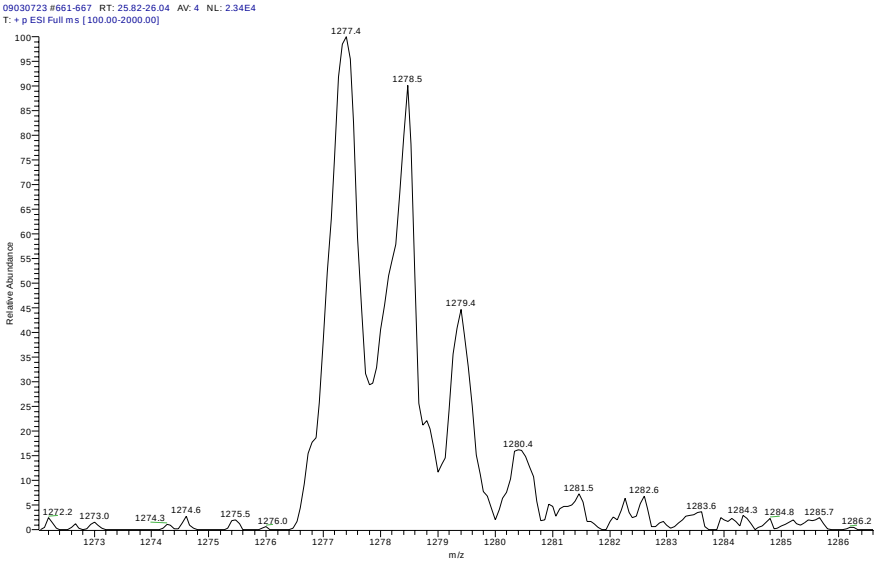
HYNIC-gastrin, $[M+H]^{+}$



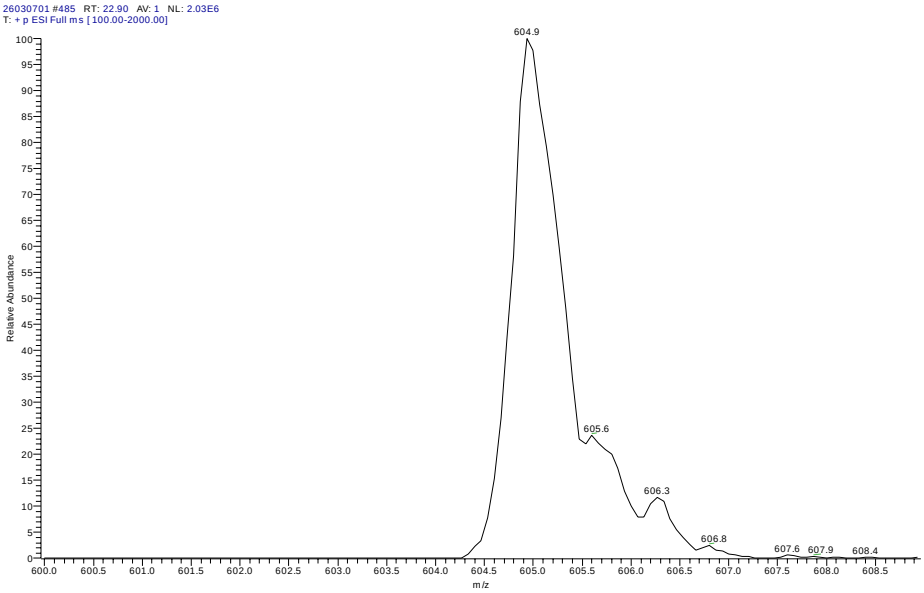
HYBA-gastrin, $[M+2H]^{2+}$



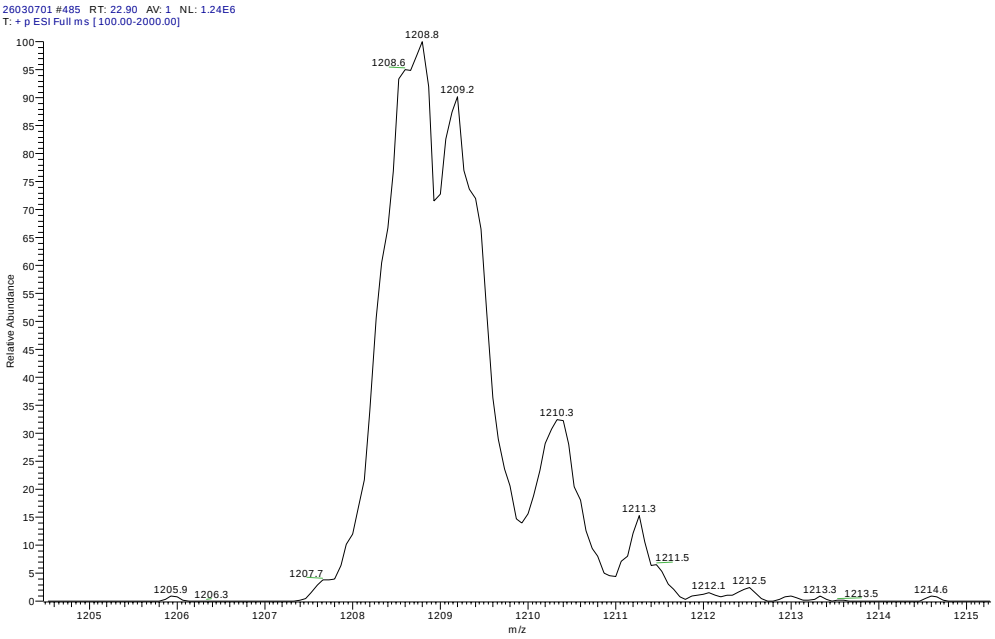
HYBA-gastrin, $[M+H]^{+}$



HYNIC-[Gly^8-gastrin], $[M+2H]^{2+}$



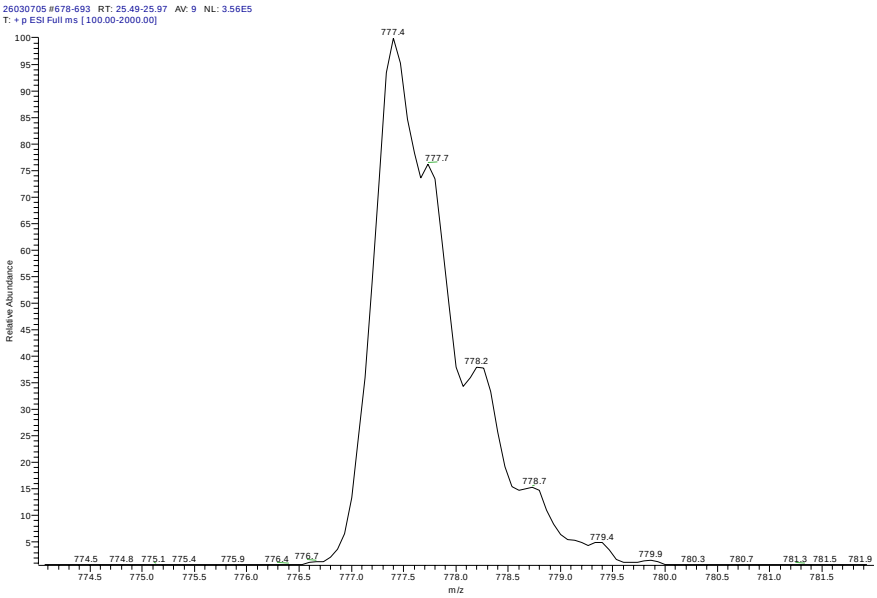
HYNIC-[Gly^8-gastrin], $[M+H]^{+}$



HYNIC-nanogastrin

$[(\text{Peptide} + \text{Tc} + \text{tricine} - 5\text{H}) + 2\text{H}]^{2+}$

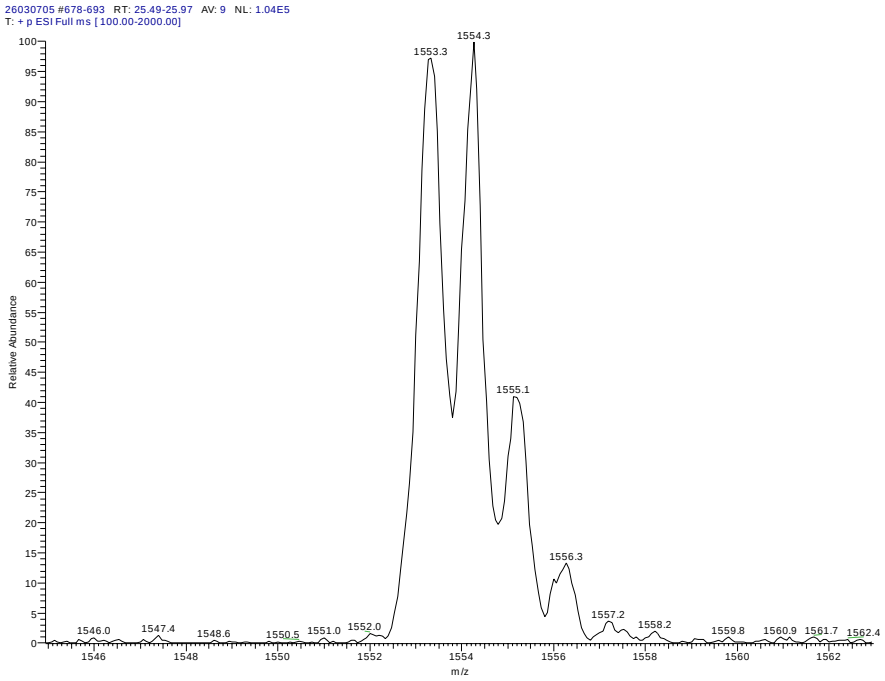
m/z = 777.4



HYNIC-nanogastrin

$[(\text{Peptide} + \text{Tc} + \text{tricine} - 5\text{H}) + \text{H}]^{+}$

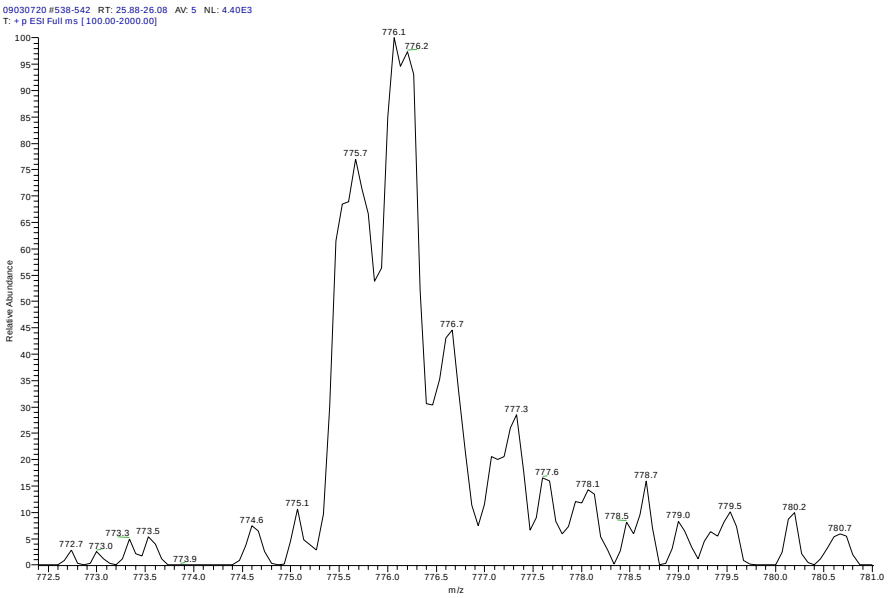
m/z = 1553.3



HYNIC-nanogastrin

$[(Peptide + Tc + EDDA – 5H) + 2H]^{2+}$

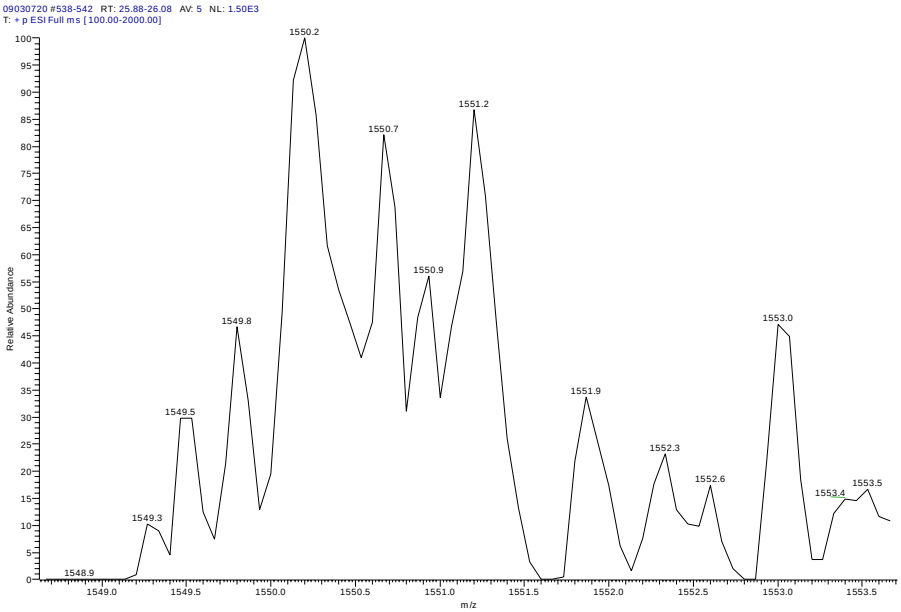
m/z = 775.7



HYNIC-nanogastrin

$[(Peptide + Tc + EDDA – 5H) + H]^{+}$

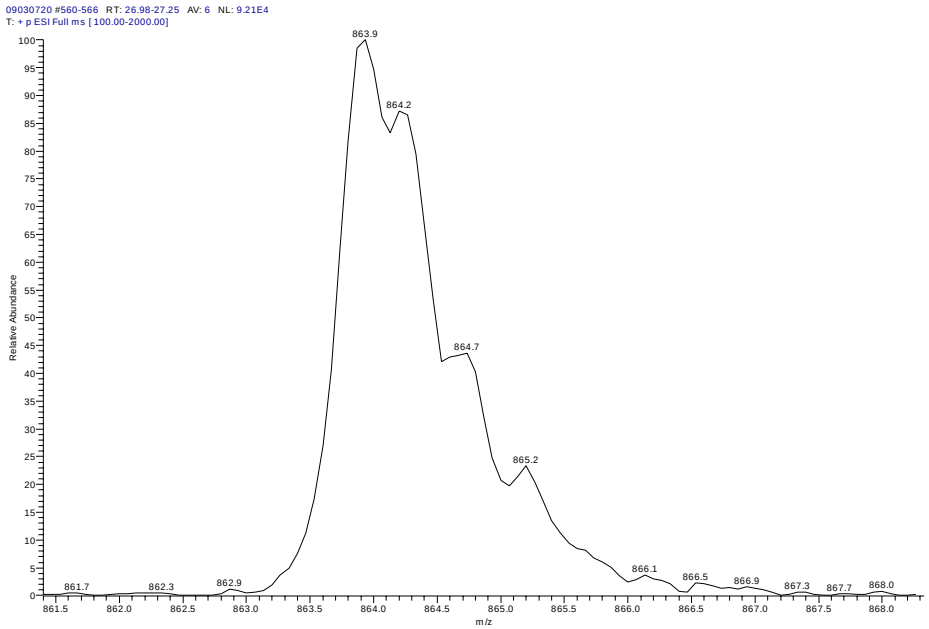
m/z = 1550.2



HYNIC-nanogastrin

$[(Peptide + Tc + 2EDDA - 5H) + 2H]^{2+}$

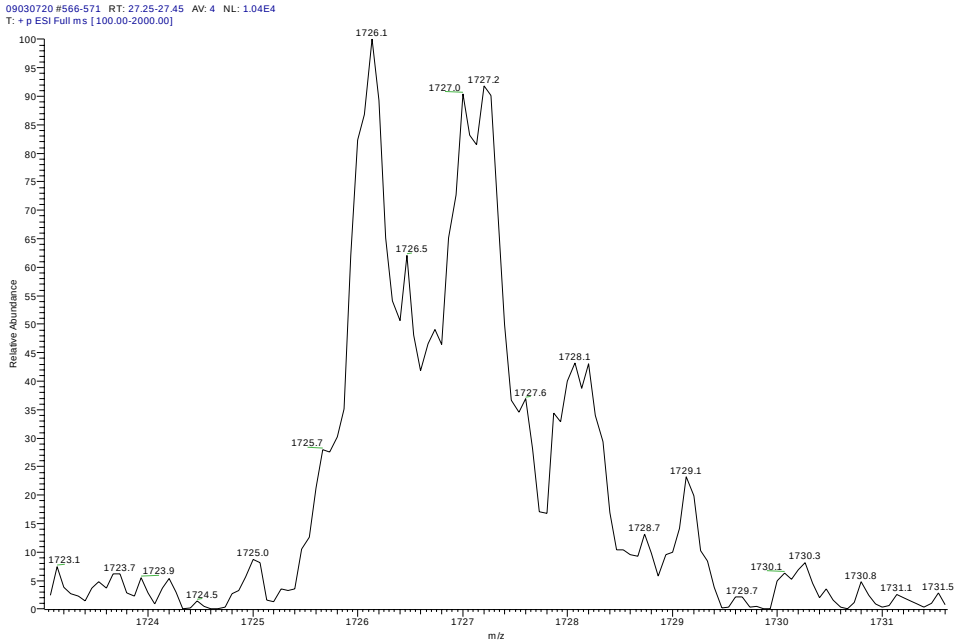
m/z = 863.9



HYNIC-nanogastrin

$[(Peptide + Tc + 2EDDA - 5H) + H]^{+}$

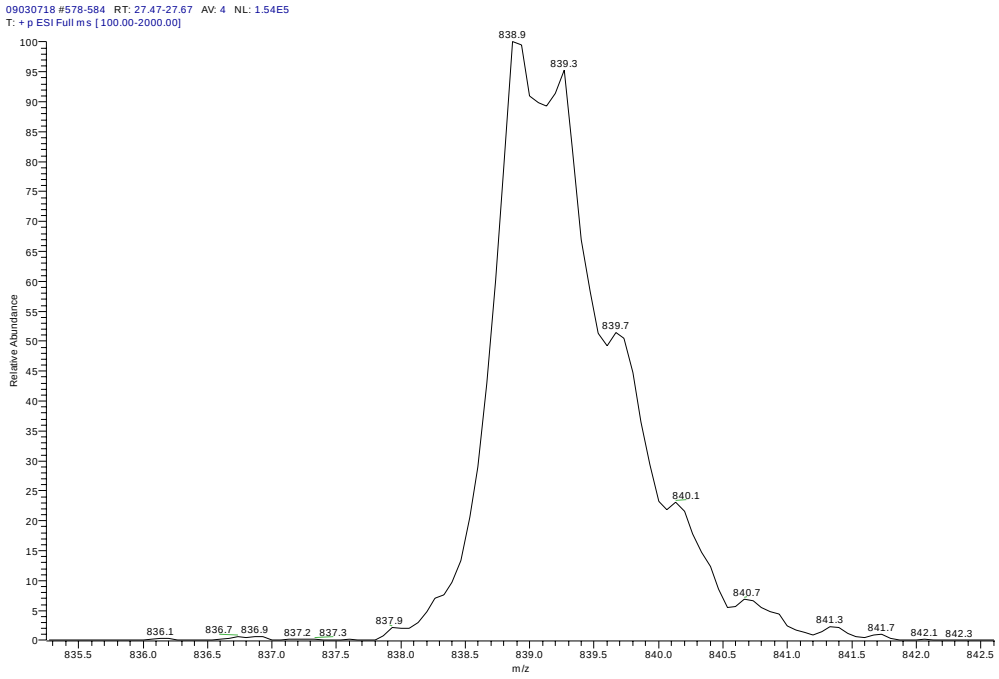
m/z = 1726.1



HYNIC-nanogastrin

$[(Peptide + Tc + tricine + nic - 5H) + 2H]^{2+}$

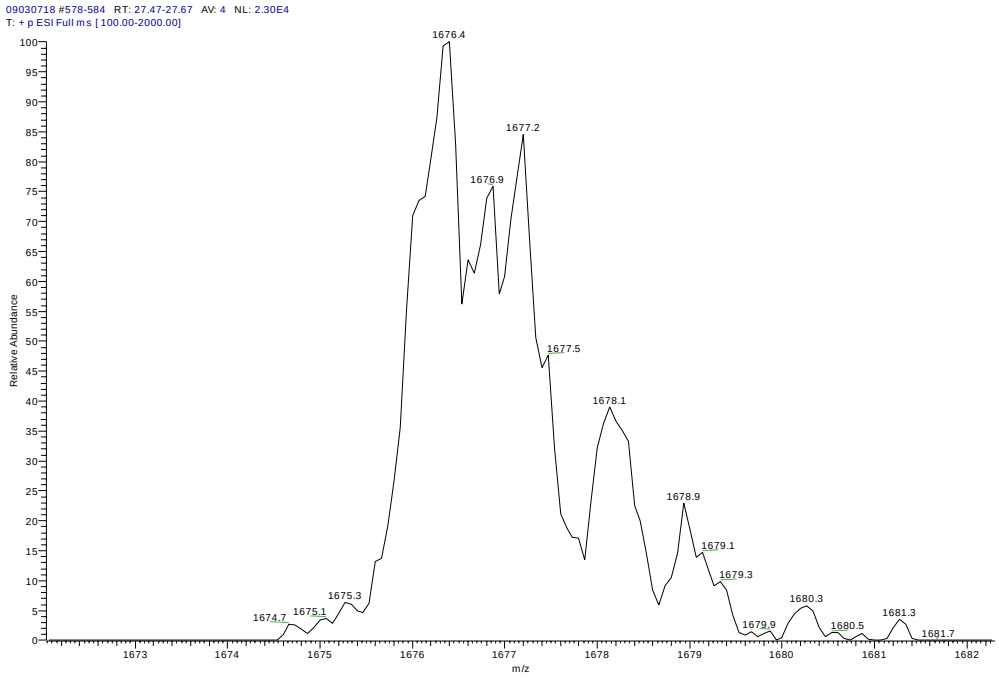
m/z = 838.9



HYNIC-nanogastrin

$[(Peptide + Tc + tricine + nic - 5H) + 2H]^{2+}$

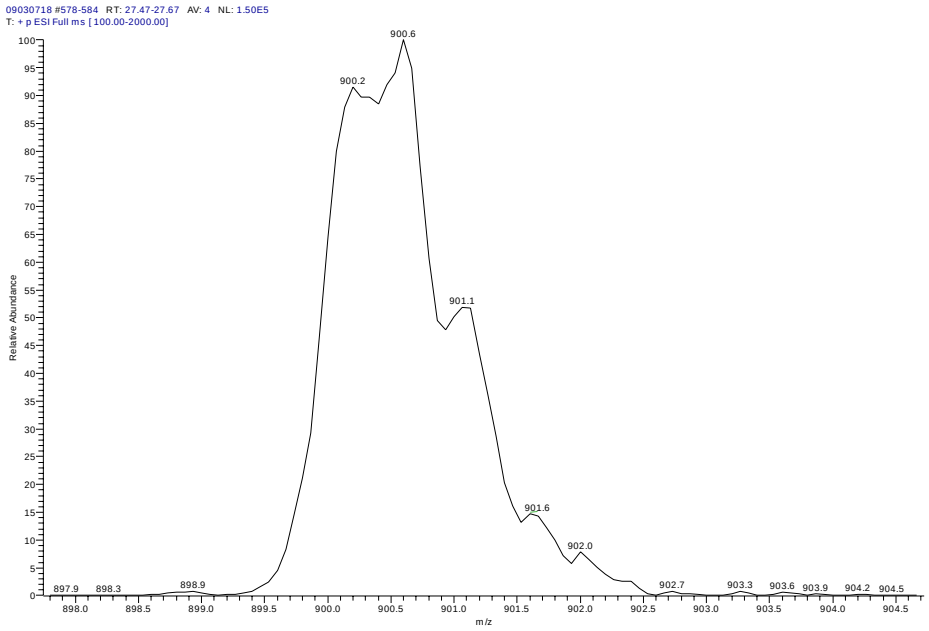
m/z = 1676.4



HYNIC-nanogastrin

$[(Peptide + Tc + tricine + 2nic - 5H) + 2H]^{2+}$

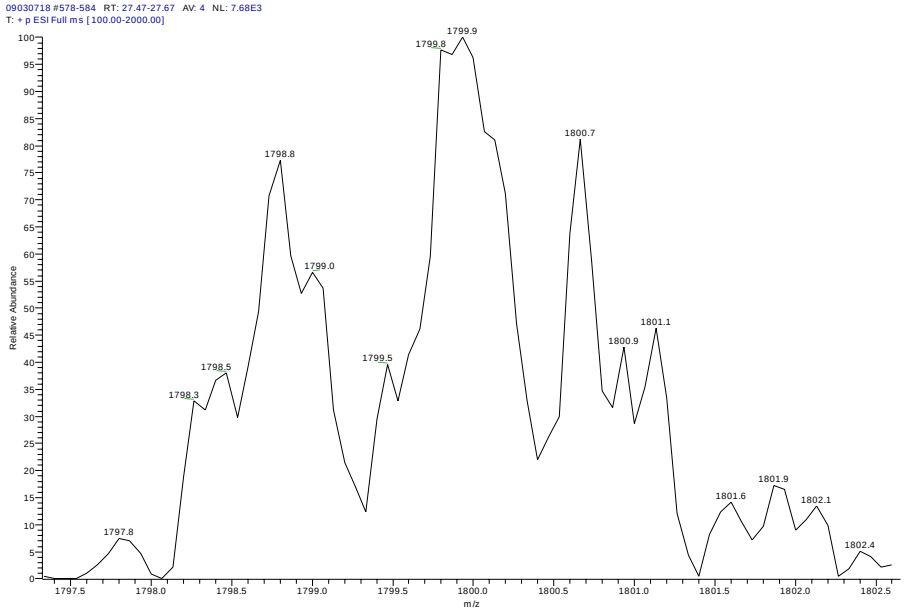
m/z = 900.2



HYNIC-nanogastrin

$[(Peptide + Tc + tricine + 2nic - 5H) + H]^{+}$

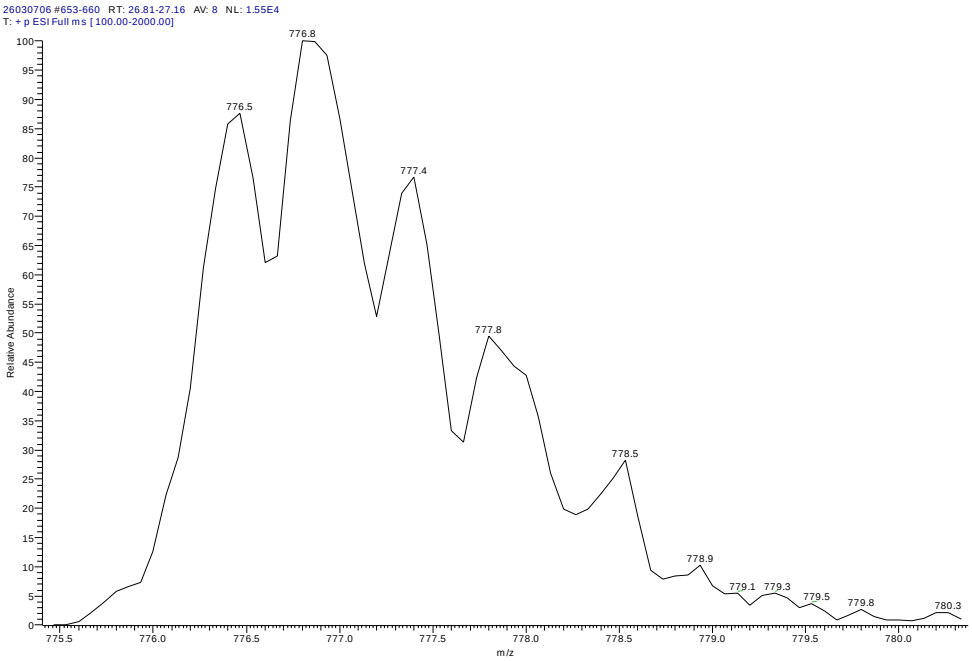
m/z = 1798.8



HYBA-nanogastrin

$[(\text{Peptide} + \text{Tc} + \text{tricine} - 5\text{H}) + 2\text{H}]^{2+}$

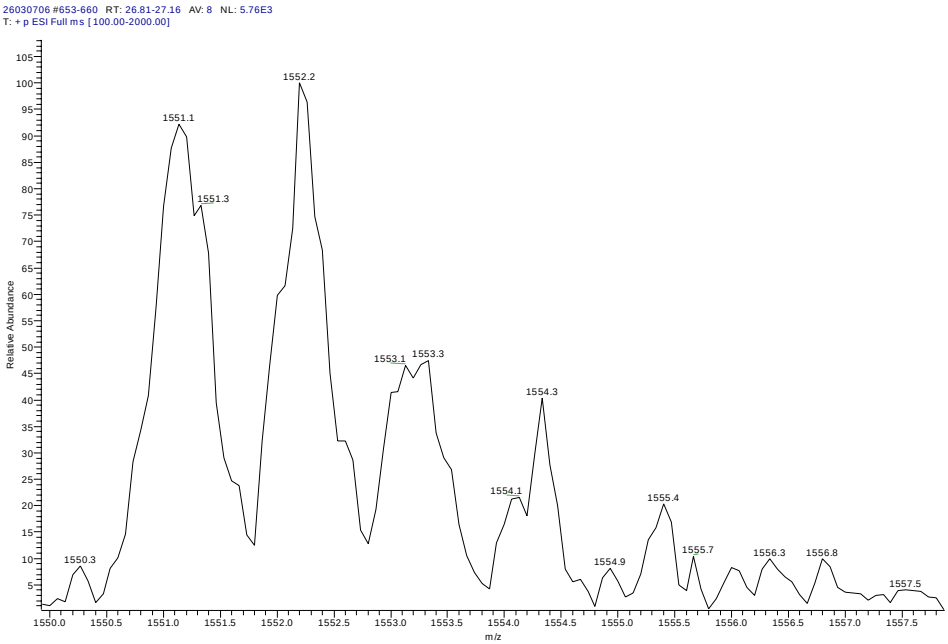
m/z = 776.8



HYBA-nanogastrin

$[(\text{Peptide} + \text{Tc} + \text{tricine} - 5\text{H}) + \text{H}]^{+}$

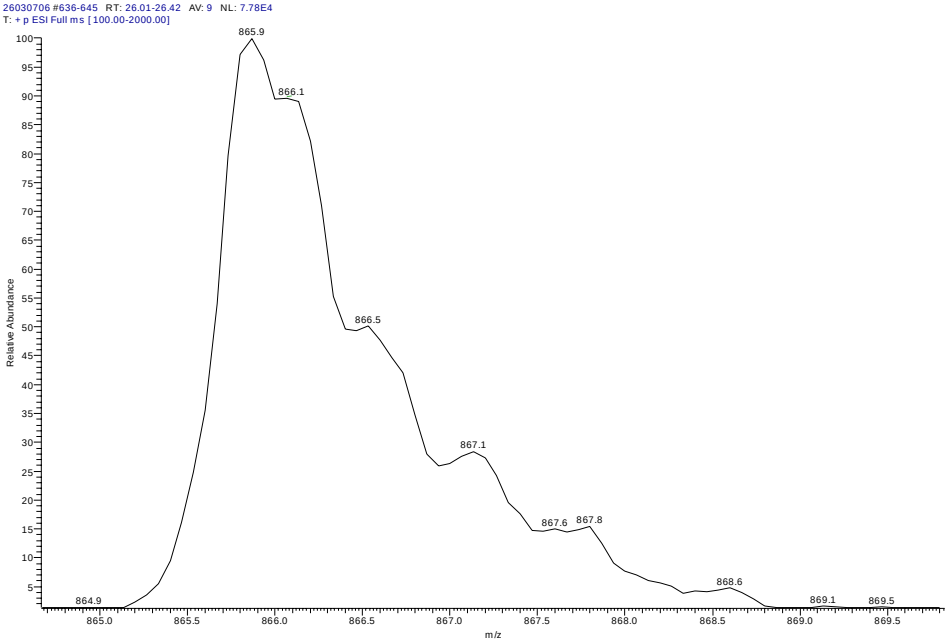
m/z = 1552.2



HYBA-nanogastrin

$[(\text{Peptide} + \text{Tc} + 2\text{tricine} - 5\text{H}) + 2\text{H}]^{2+}$

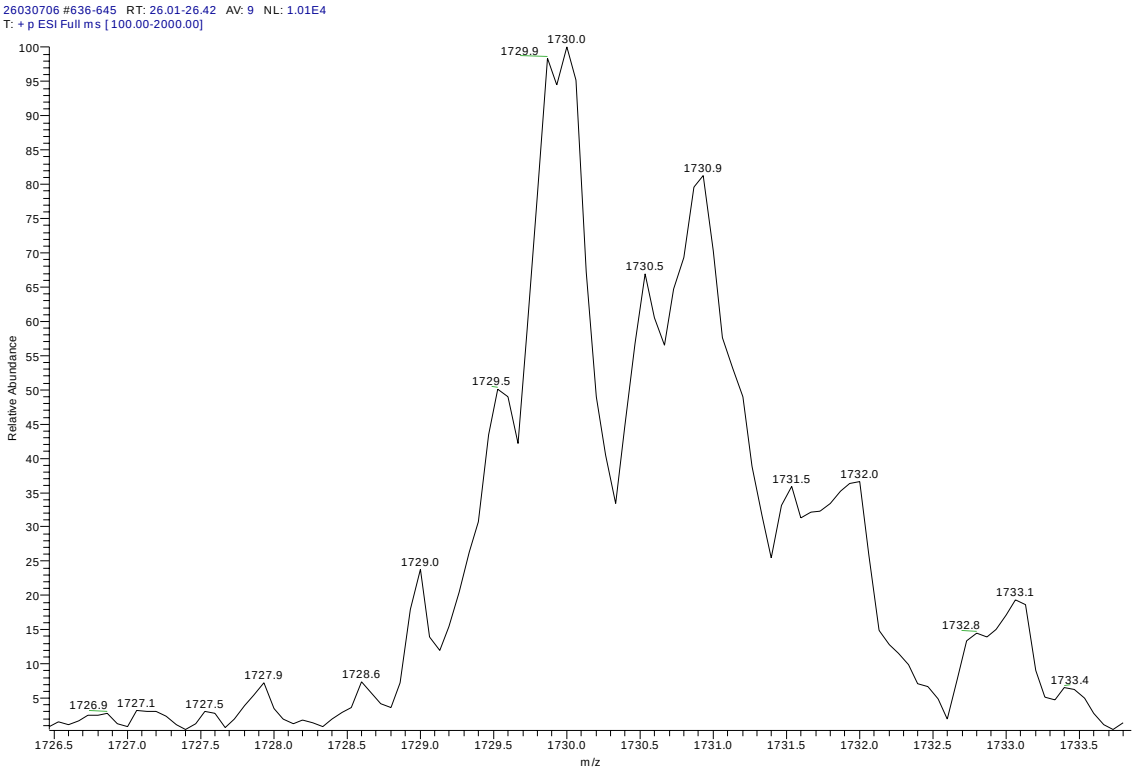
m/z = 865.9



HYBA-nanogastrin

$[(\text{Peptide} + \text{Tc} + 2\text{tricine} - 5\text{H}) + \text{H}]^{+}$

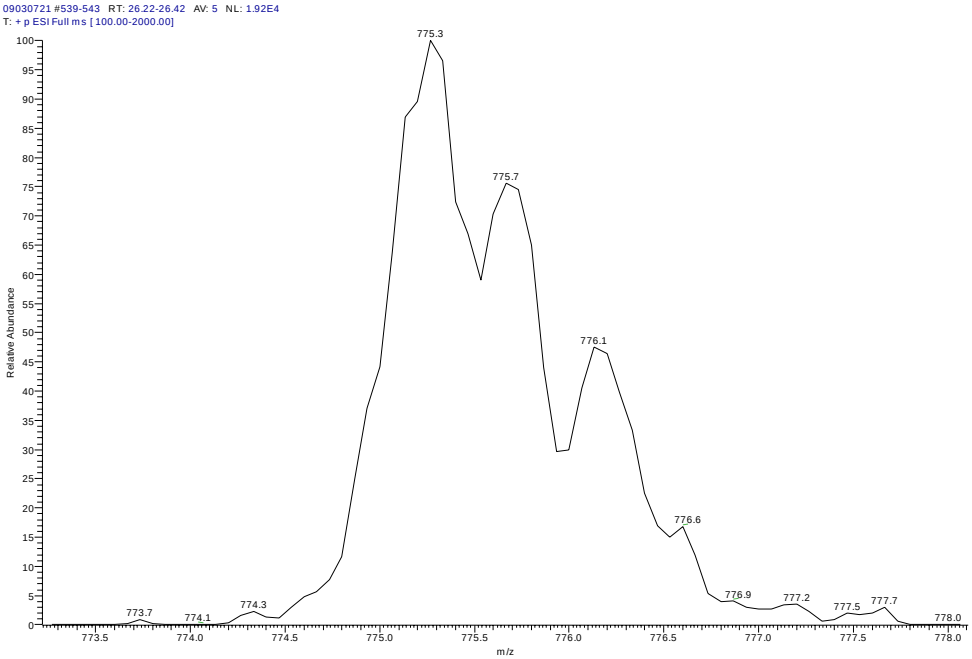
m/z = 1729.9



HYBA-nanogastrin

$[(Peptide + Tc + EDDA - 5H) + 2H]^{2+}$

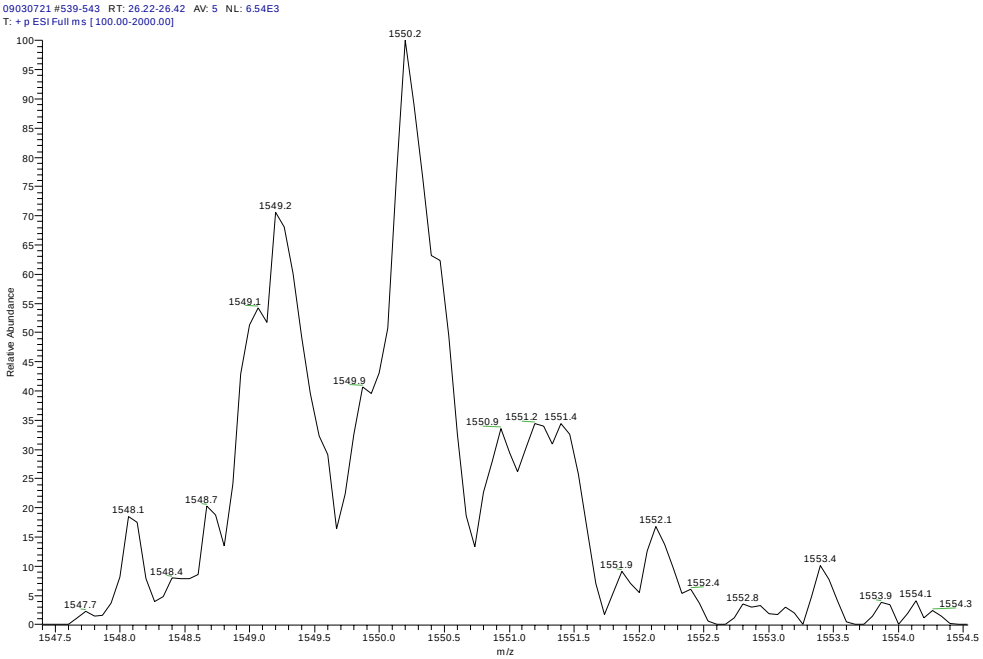
m/z = 775.3



HYBA-nanogastrin

$[(Peptide + Tc + EDDA - 5H) + H]^{+}$

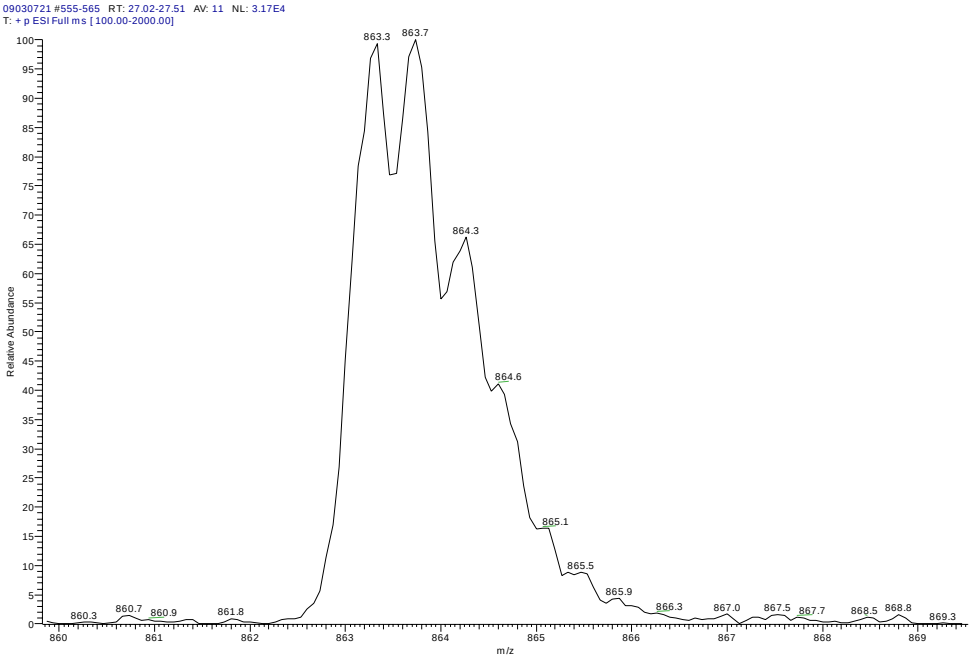
m/z = 1549.1



HYBA-nanogastrin

$[(Peptide + Tc + 2EDDA - 5H) + 2H]^{2+}$

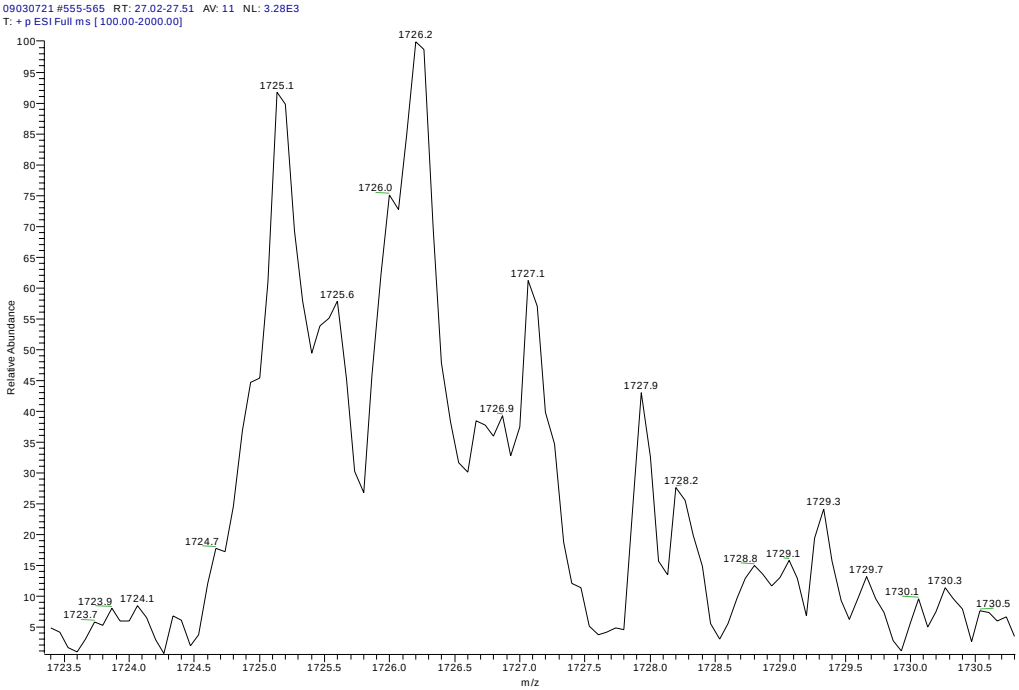
m/z = 863.3



HYBA-nanogastrin

$[(Peptide + Tc + 2EDDA - 5H) + H]^{+}$

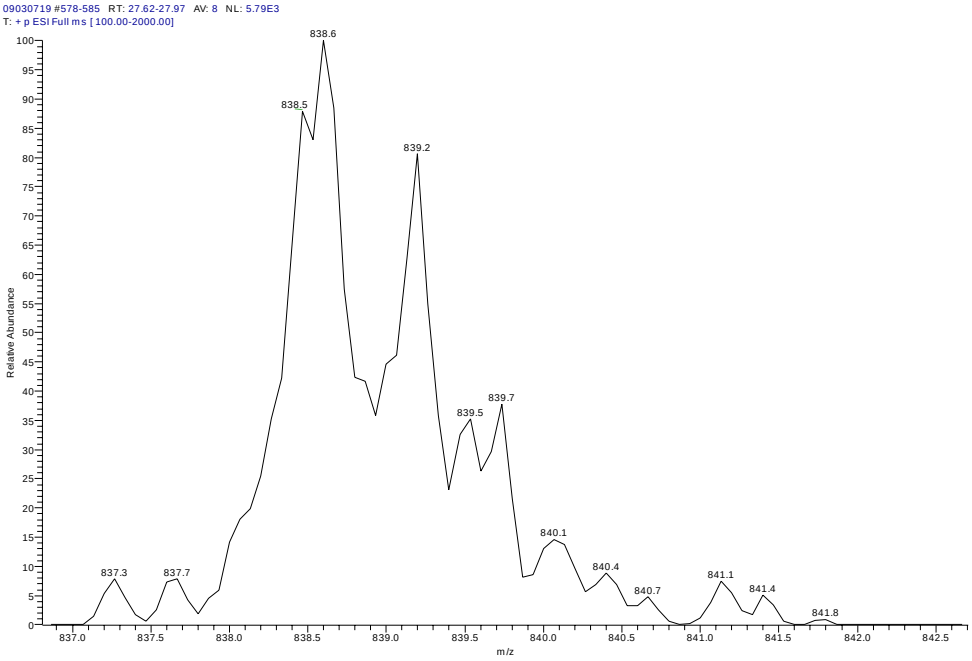
m/z = 1725.1



HYBA-nanogastrin

$[(Peptide + Tc + tricine + nic – 5H) + 2H]^{2+}$

m/z = 838.5

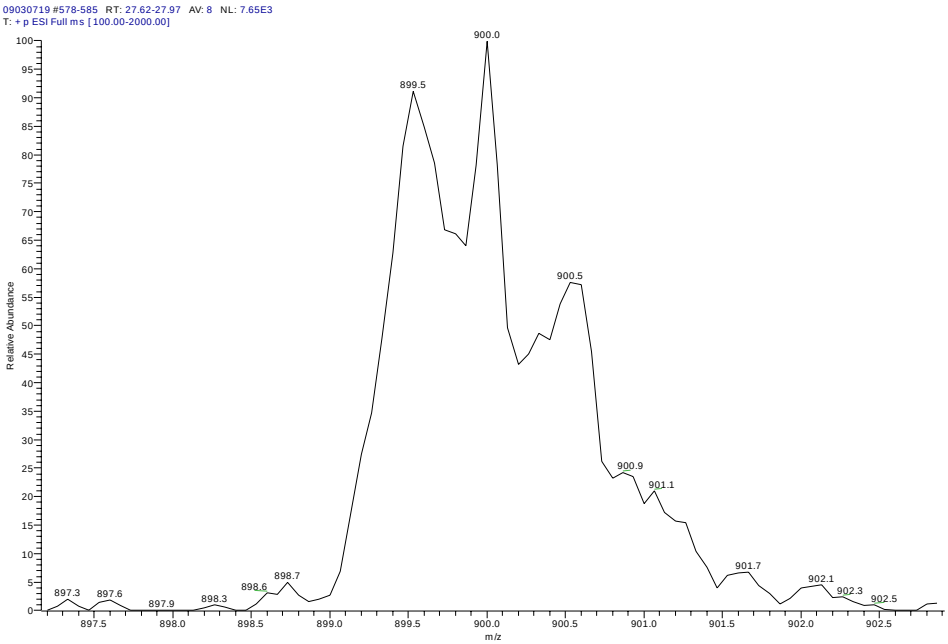


(did not detect $[(Peptide + Tc + tricine + nic – 5H) + H]^{+}$ due to weak sample)

HYBA-nanogastrin

$[(Peptide + Tc + tricine + nic – 5H) + 2H]^{2+}$

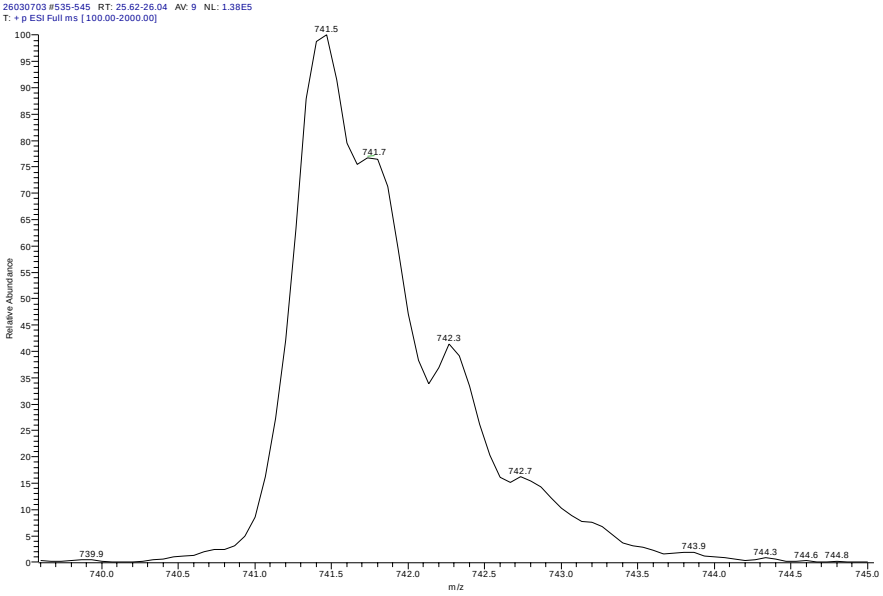
m/z = 899.5



HYNIC-[Gly^{8}]-nanogastrin

$[(\text{Peptide} + \text{Tc} + \text{tricine} - 5\text{H}) + 2\text{H}]^{2+}$

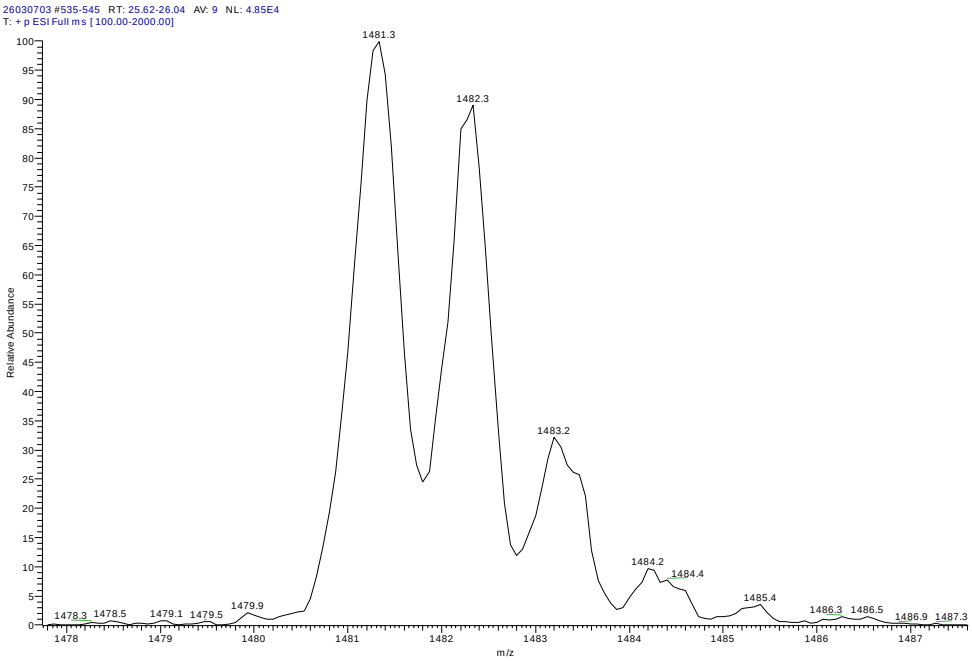
m/z = 741.5



HYNIC-[Gly^{8}]-nanogastrin

$[(\text{Peptide} + \text{Tc} + \text{tricine} - 5\text{H}) + \text{H}]^{+}$

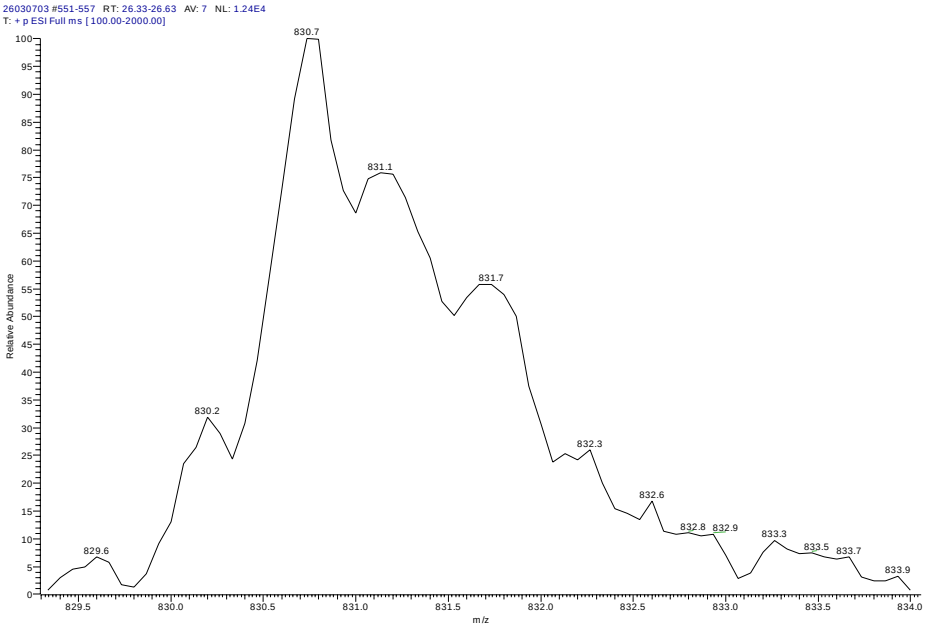
m/z = 1481.3



HYNIC-[Gly^{8}]-nanogastrin

$[(\text{Peptide} + \text{Tc} + 2\text{tricine} - 5\text{H}) + 2\text{H}]^{2+}$

m/z = 830.7



HYNIC-[Gly^{8}]-nanogastrin

$[(\text{Peptide} + \text{Tc} + 2\text{tricine} - 5\text{H}) + \text{H}]^{+}$

m/z = 1660.1