Supplementary Figure and Table Legends

Supplementary Figure 1: Skyline Extracted Ion Chromatograms for each of the CiRT_SW reference peptides selected by manual curation. Extracted chromatograms for peak groups representing each of the 14 peptides hand-selected for the CiRT_SW peptide set are shown as they were detected in SWATH files from human, yeast, and mouse cell lysates. Additional peptides not selected for inclusion in the CiRT_SW list are also demonstrated at the end of the figure set for comparison.

Supplementary Figure 2: Comparison of the accuracy in peptide prediction between automated CiRT peptide selection algorithm to the manually curated CiRT_SW peptides. (A) The difference between observed and predicted retention time (ΔRT) of each of the confidently identified (FDR < 1%) peak groups defined by an RT-normalized assay library and extracted from a human, yeast, or mouse SWATH-MS run are compared between conditions where the SWATH-MS normalization were performed with manually curated, 14 CiRT peptides (CiRT_SW) or by automated selection of peptides from the larger 113 CiRT list (CiRT_ALL) using the –best_peptides adaptation to the RTNormalizer algorithm. Data are presented as box and whisker plots, with the middle quartiles surrounding the median for the entire assay library represented by the box, whiskers showing the 95% data range and the upper and lower 2.5%

of all values plotted as individual data points. **(B)** Correlation between the intensity of a given peptide as determined by the CiRT_SW and CiRT_ALL normalization set. Each dot represents the summed intensity of all transitions extracted for a given peptide peak group from the same raw file, with the only difference being the method of RT normalization. **(C)** Distribution of matching and mis-matching peptide peak group intensities for the human (left) and yeast (middle) and mouse (right) derived samples. Pie charts depict overall distribution peptides with matching or mismatching intensity values between CiRT_SW and CiRT_ALL aligned datasets. Horizontal bars show distribution of peptides among different categories explaining mismatched intensity values.

Supplementary Table 1: Descriptions of the 113 peptides contained in the CiRT list.

Supplementary Table 2: A breakdown of the CiRT peptides that were detected and used for RT alignment within each of the 12 data files generated from the fractionated peptide set (described in methods).

Supplementary Table 3. A breakdown of the CiRT peptides that were detected and use for RT alignment within each of the 11 OFFGEL data files generated from the human lysate fractionated peptide set (described in methods).

Supplementary Table 4: An outline of the conceptual steps and specific softwares used in the peptide assay library generation workflow and the openSWATH workflow for quantitative analysis of DIA data. For each step, the substitution needed, if any, for the workflows using CiRT rather than SiRT normalization peptides is provided.

Additional Supplementary Files

CiRT_SW.TraML – a document containing the transitions needed as input to the openSWATH workflow for the OpenSwathRTNormalization function.

CiRT_ALL.TraML – a document containing the transitions needed as input to the openSWATH workflow for the OpenSwathRTNormalization function, recommended usage with the option " –best_peptides true "

CiRT.txt– the list of peptides and their iRTs formatted to copy and paste after the –k option in the spectrast2spectrast_iRT.py script.

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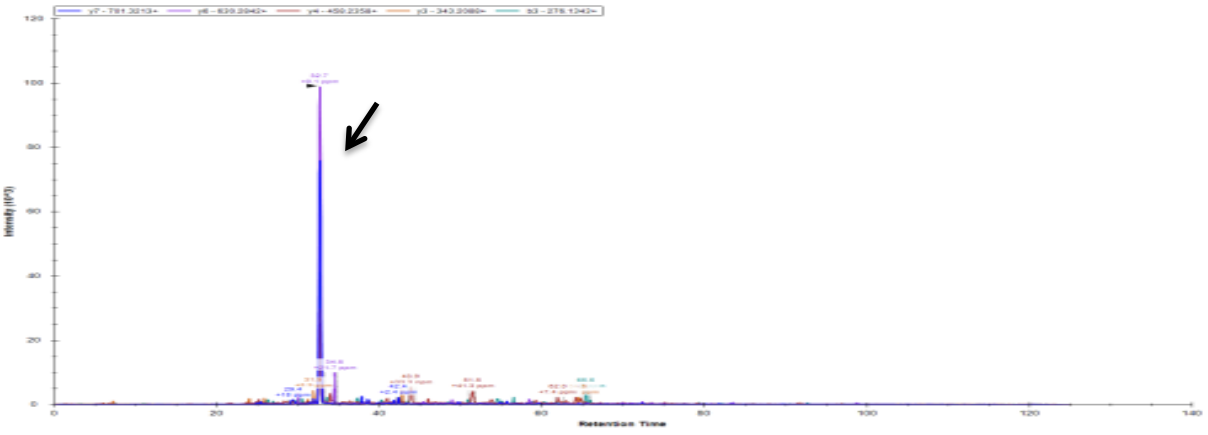

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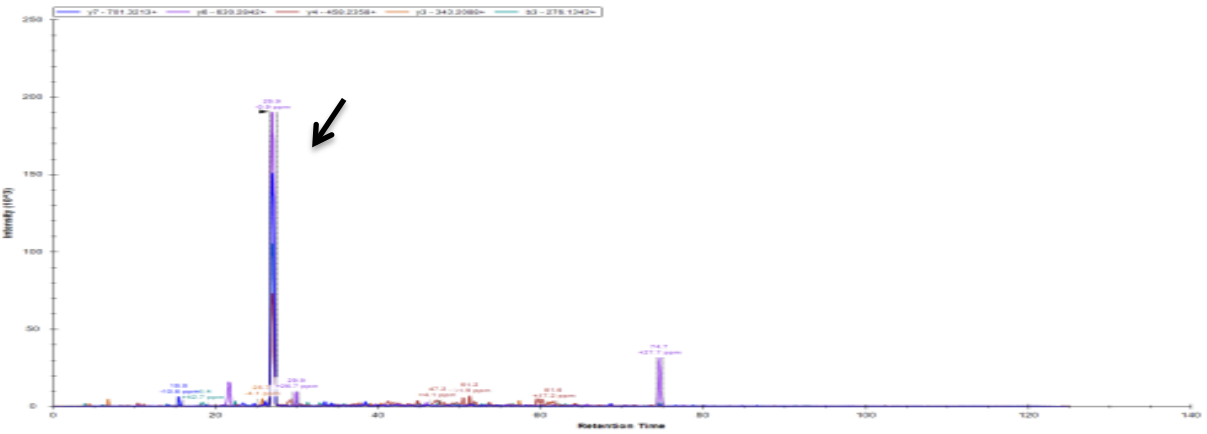

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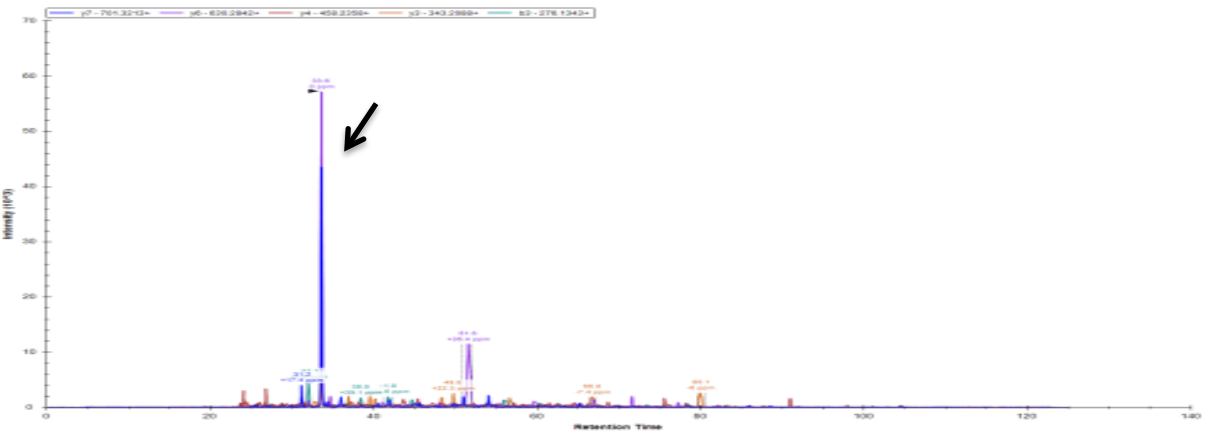

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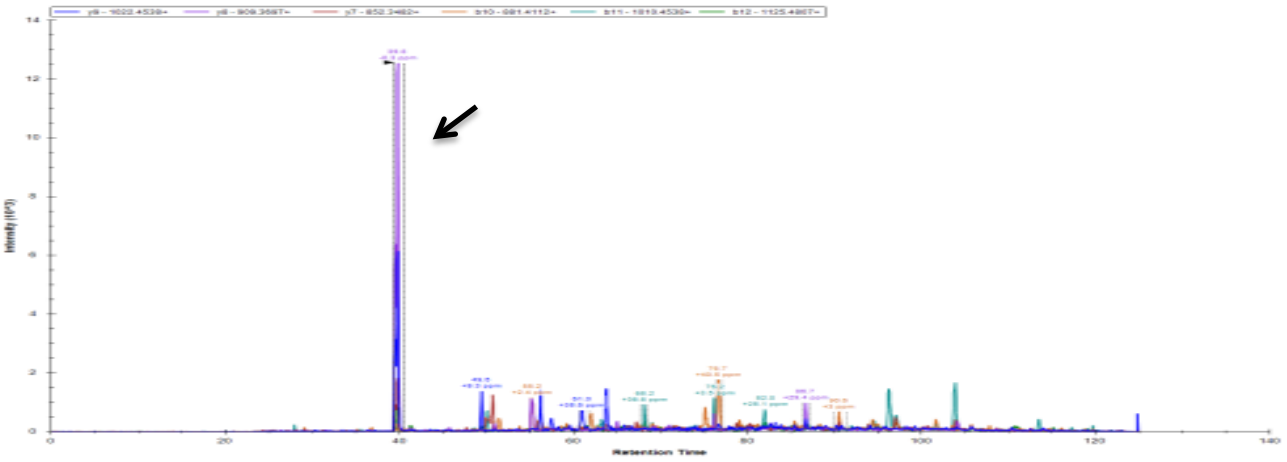

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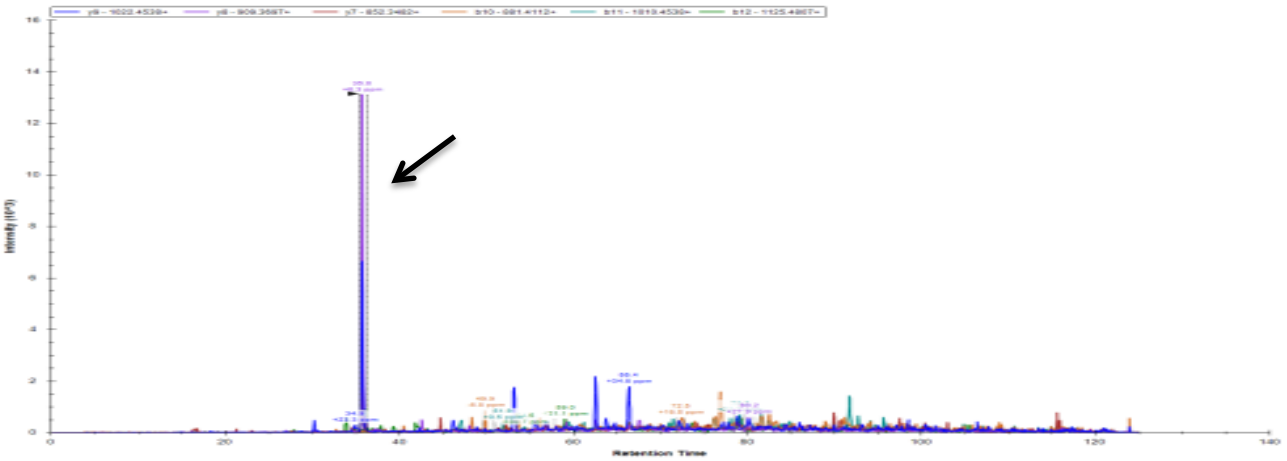

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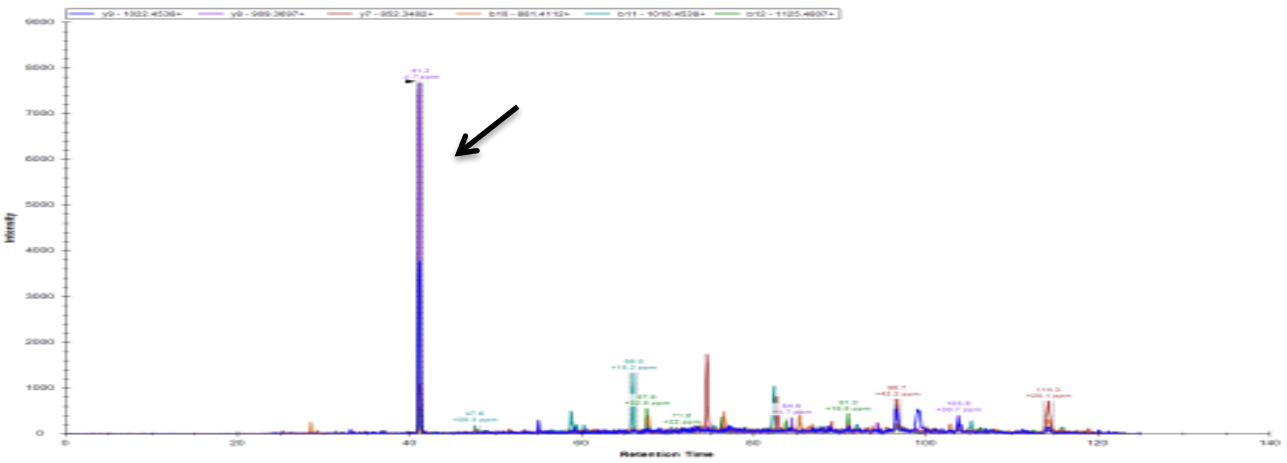

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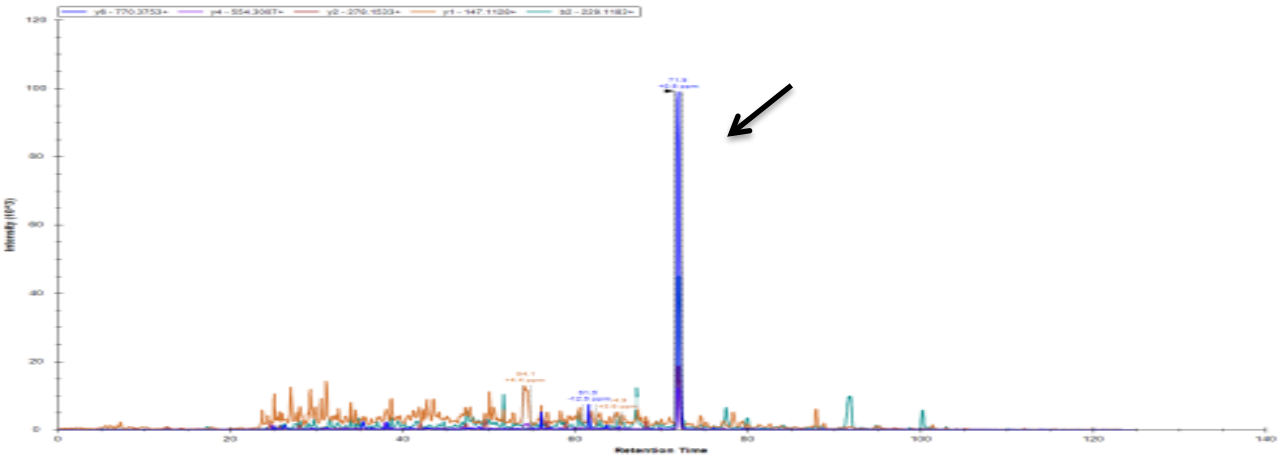

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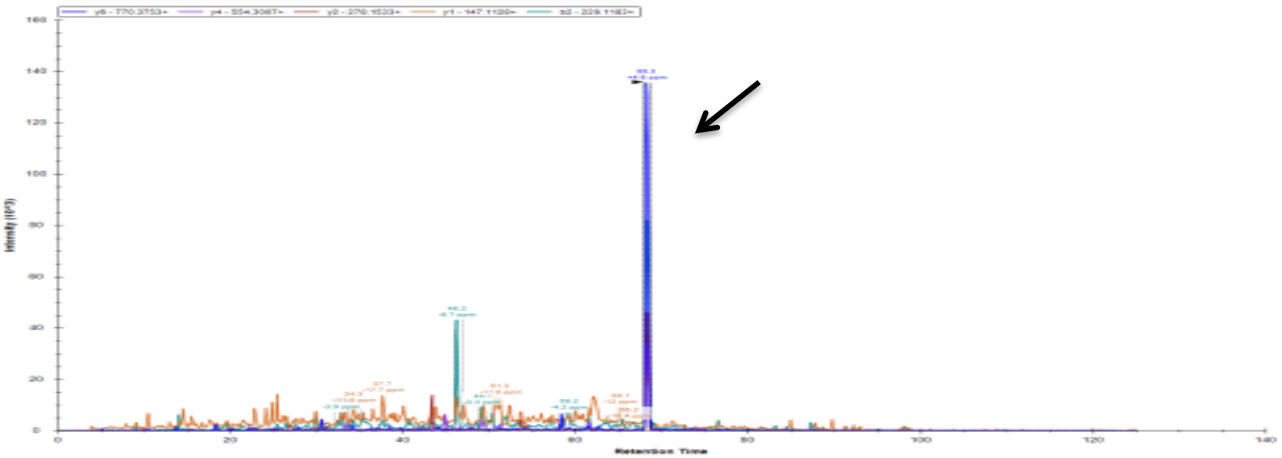

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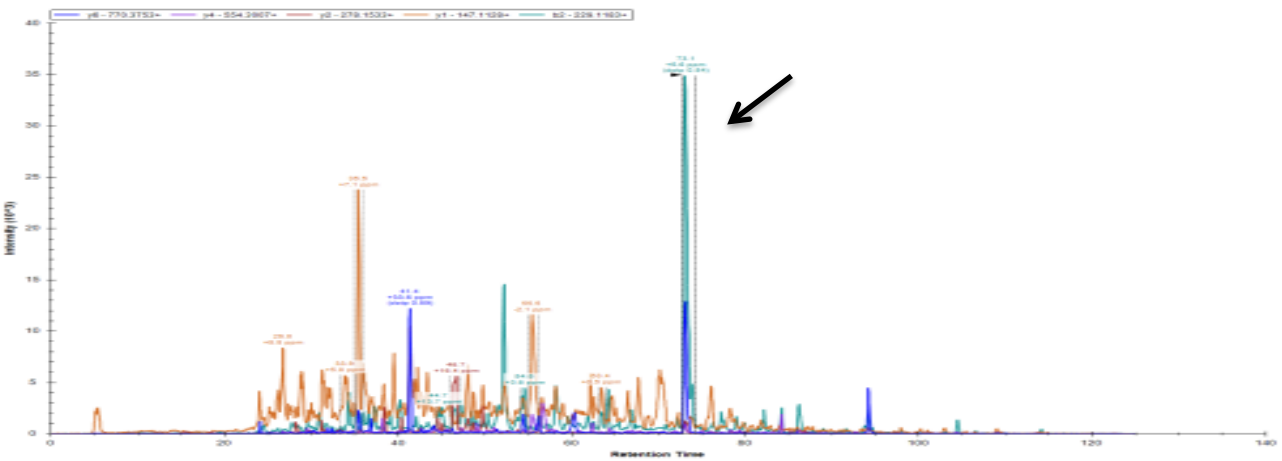

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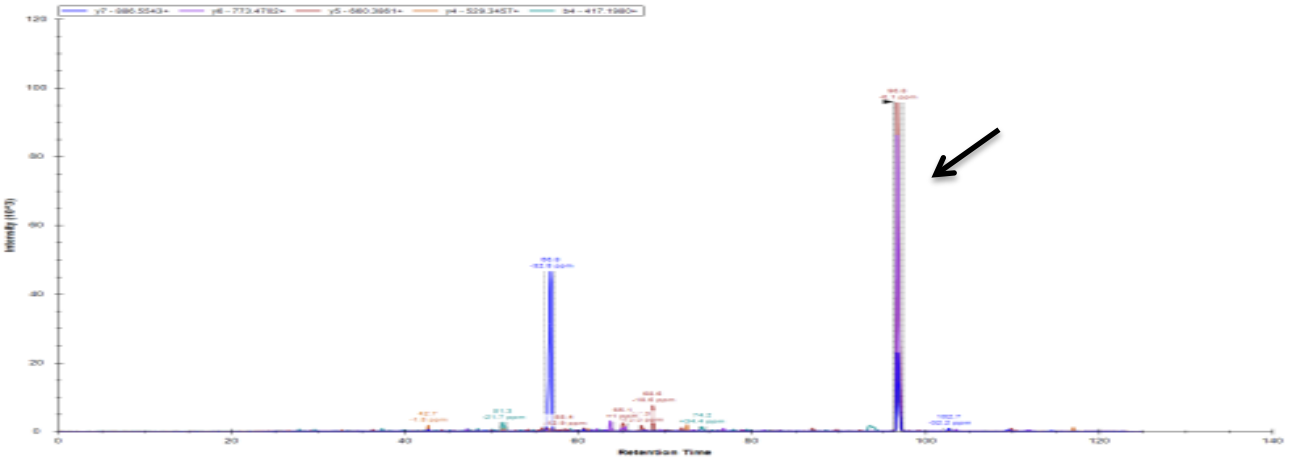

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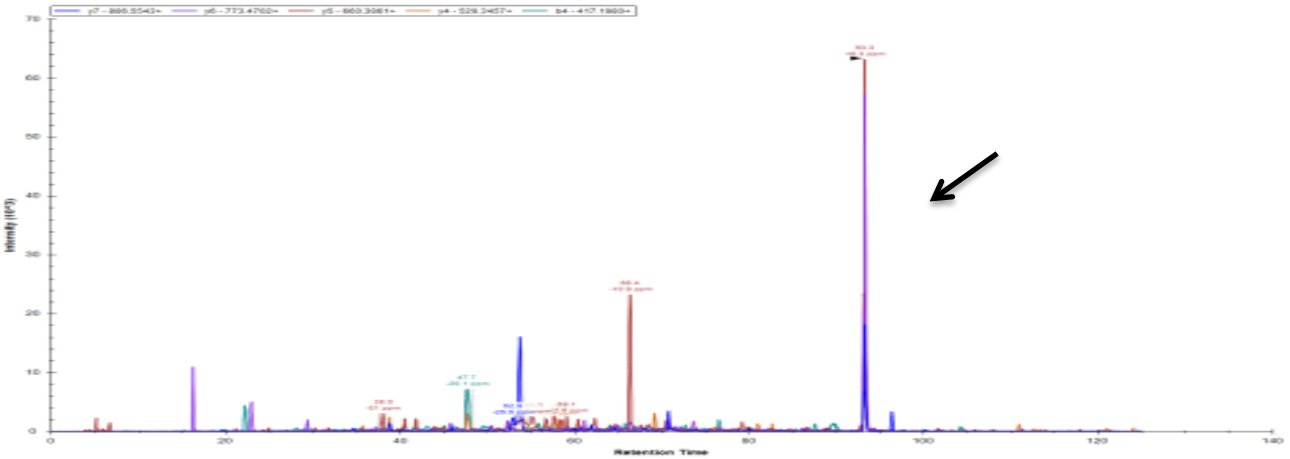

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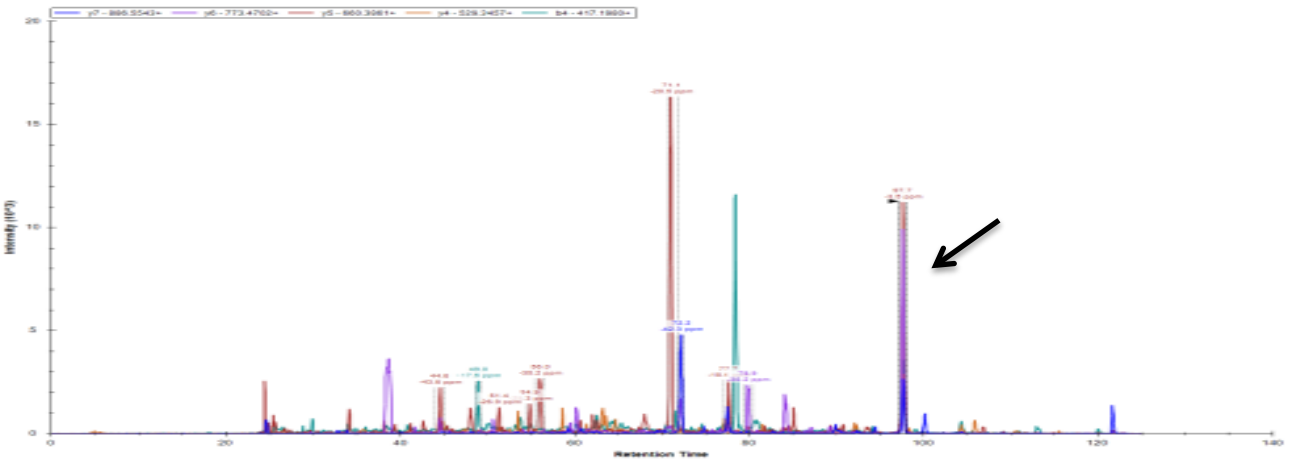

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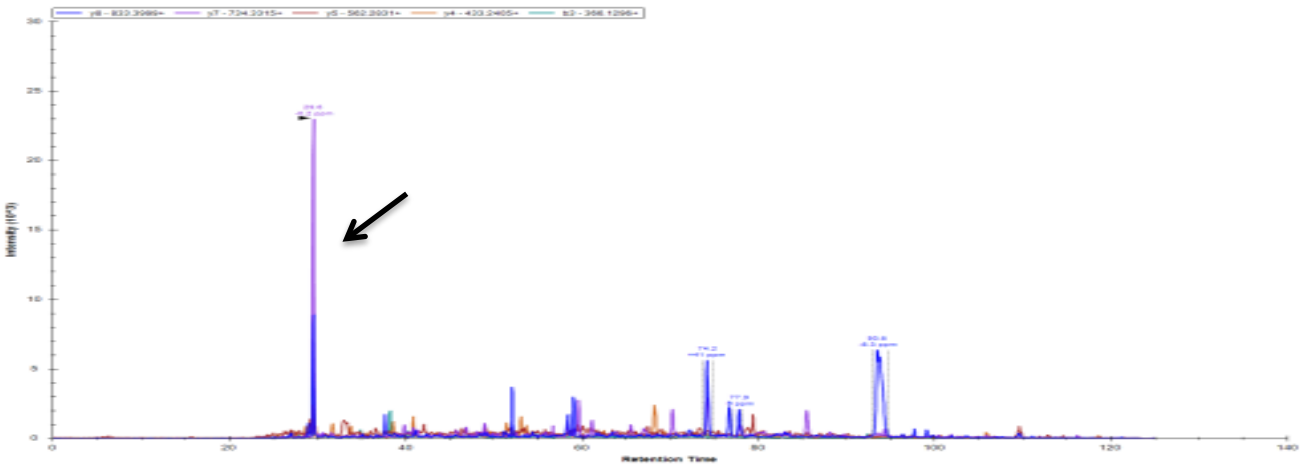

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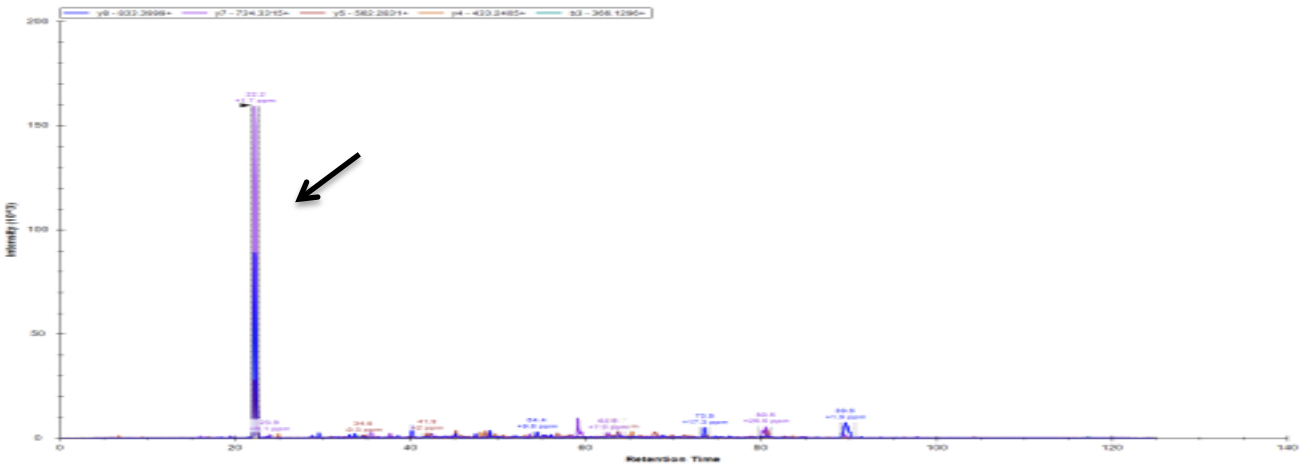

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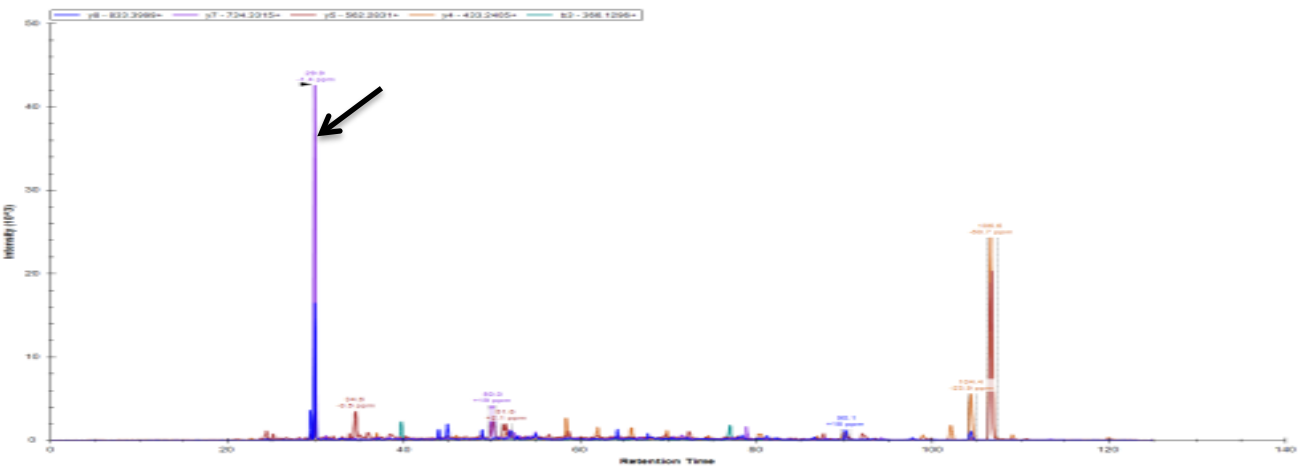

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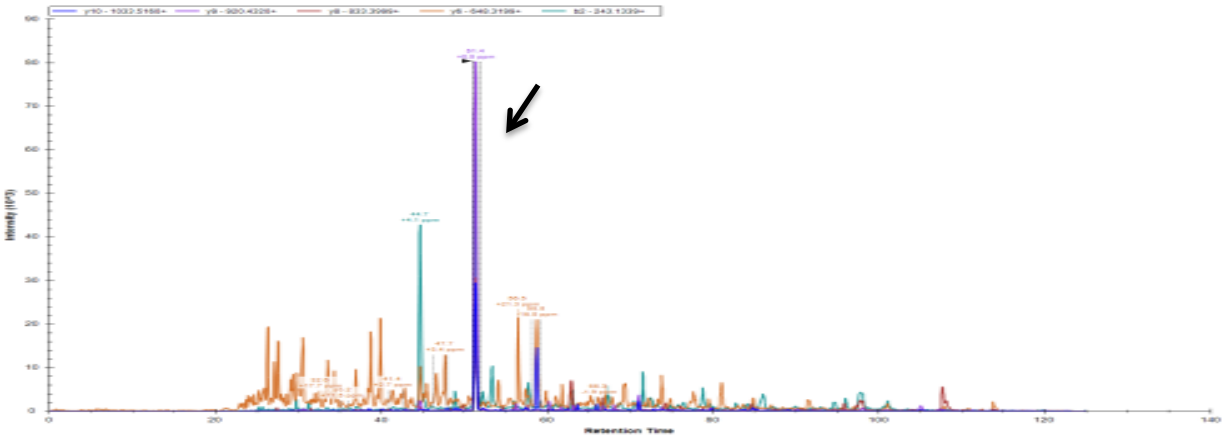

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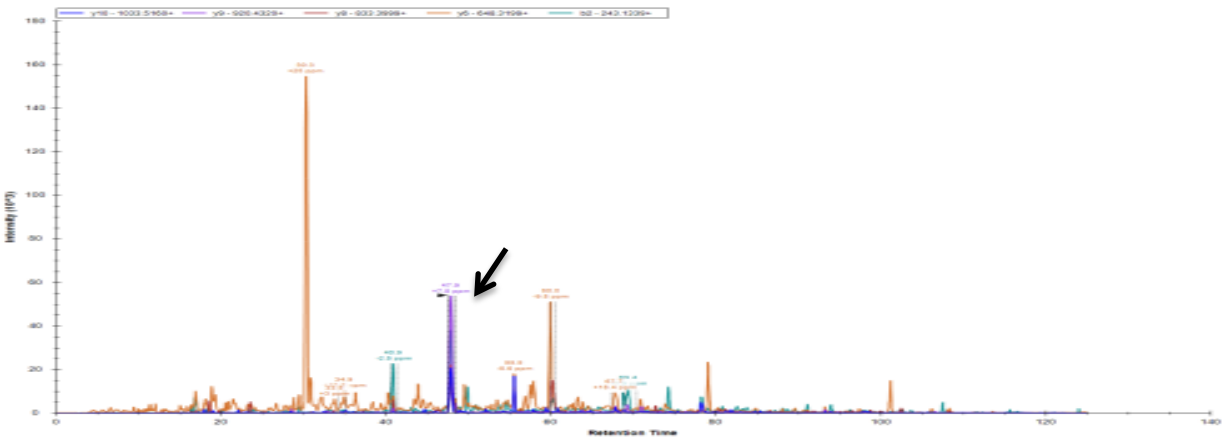

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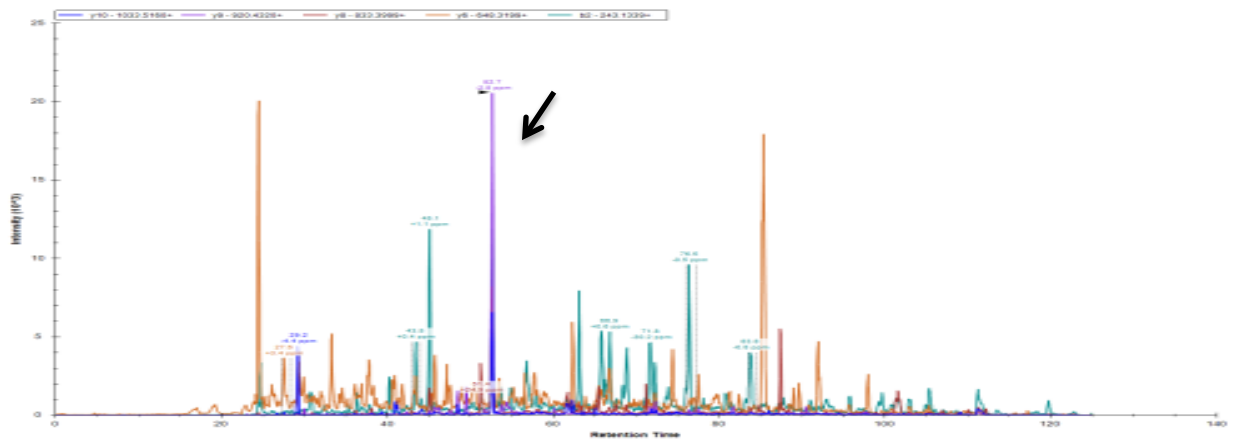

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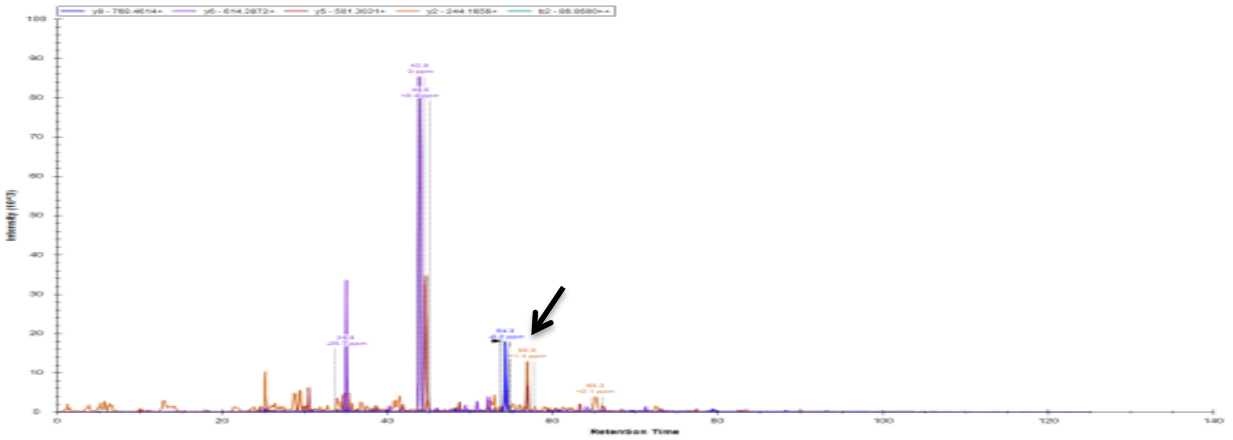

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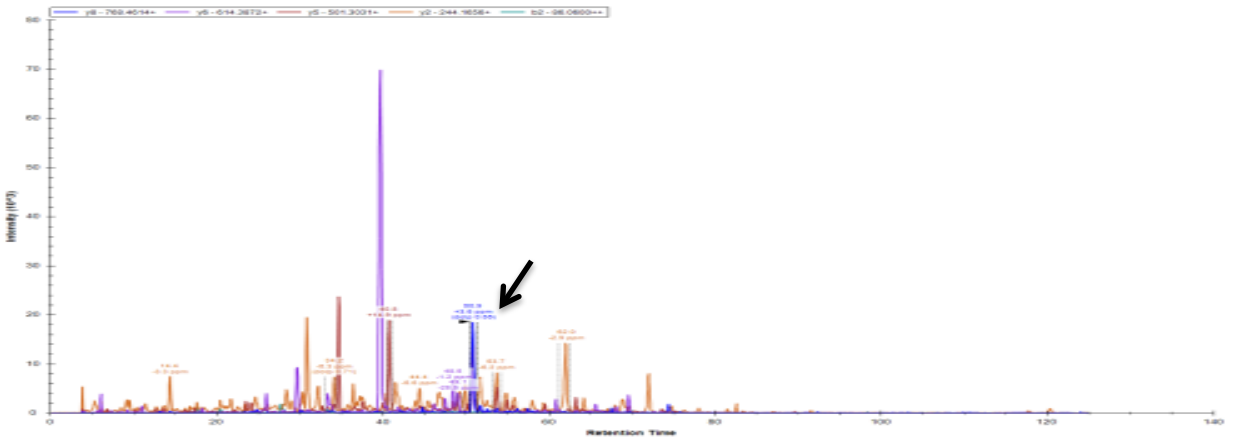

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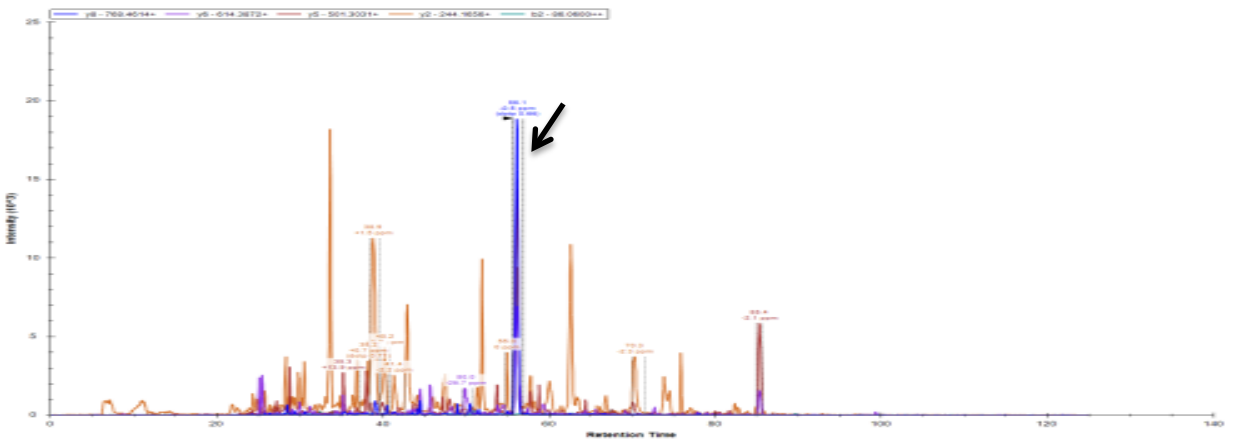

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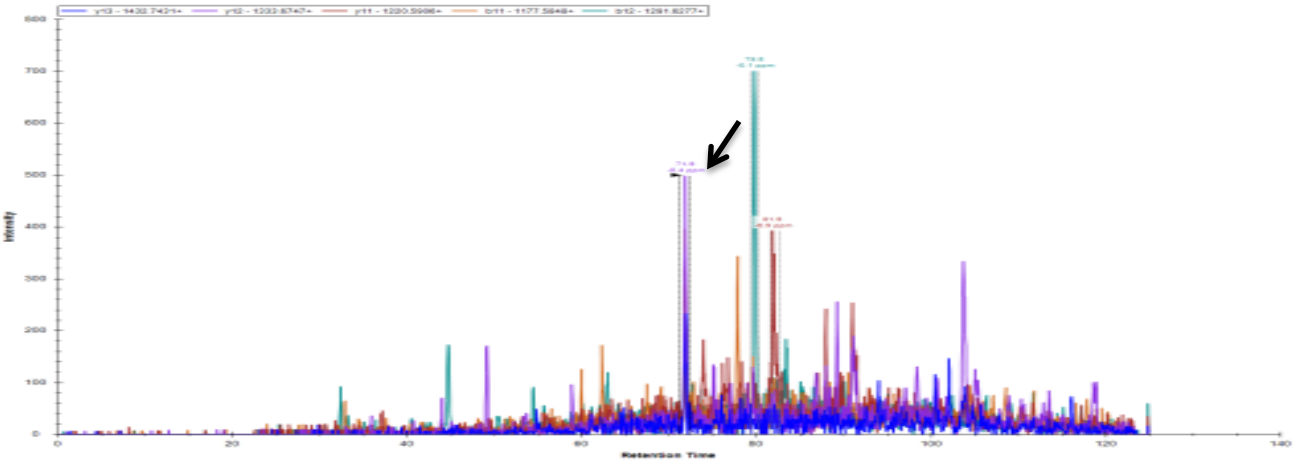

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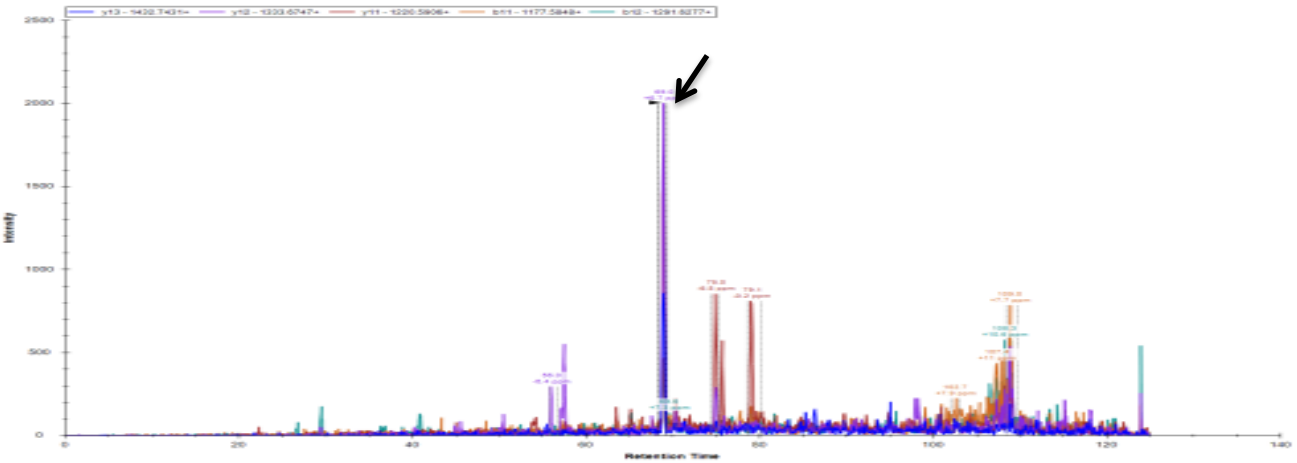

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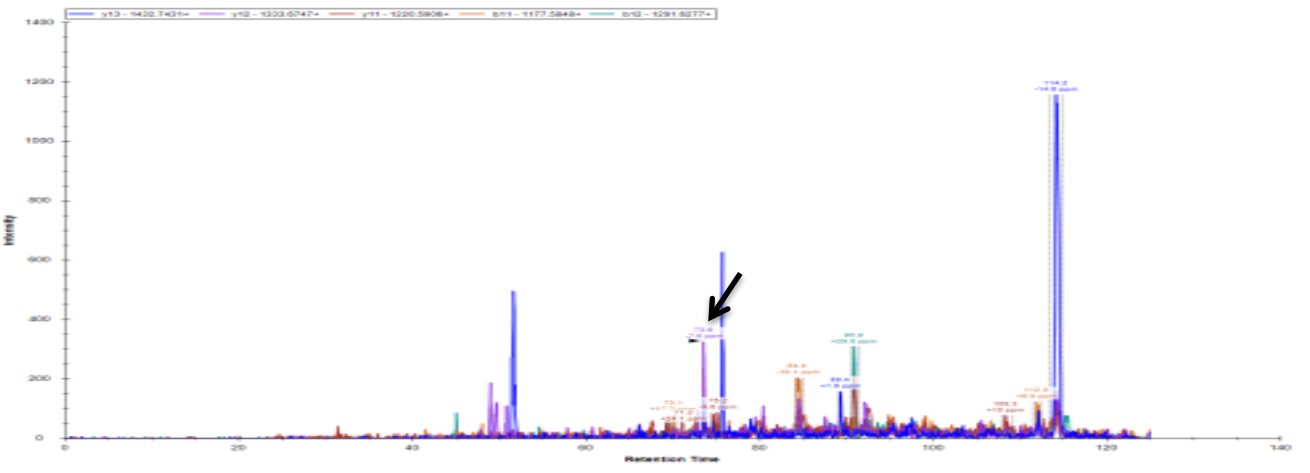

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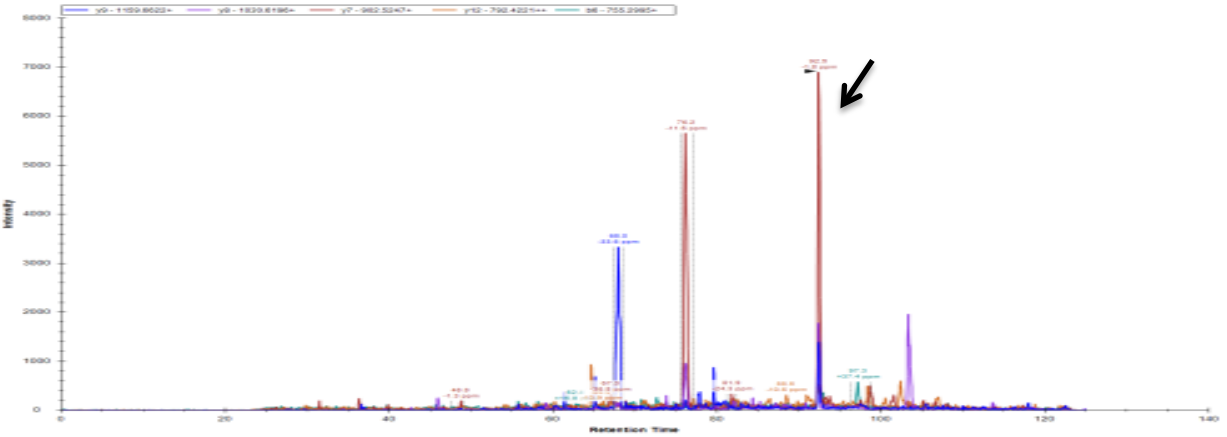

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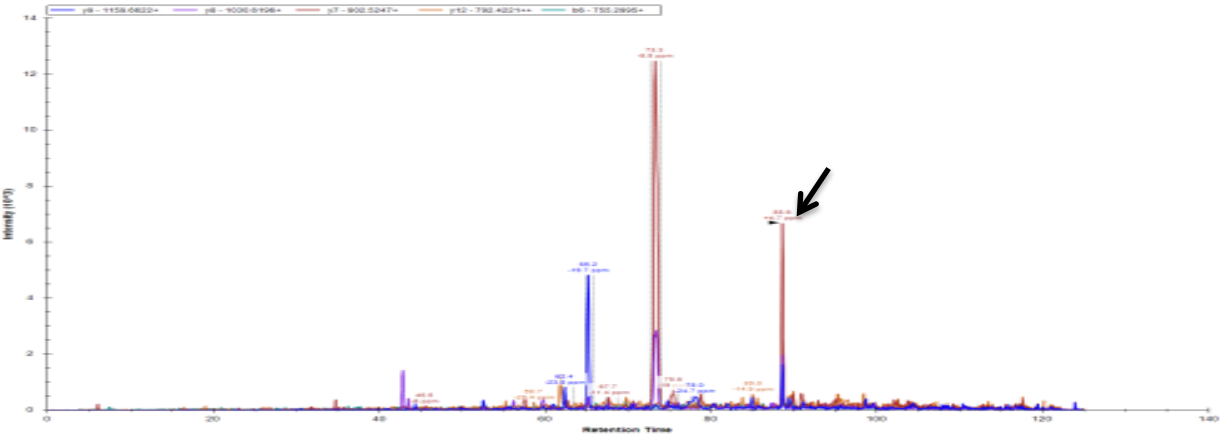

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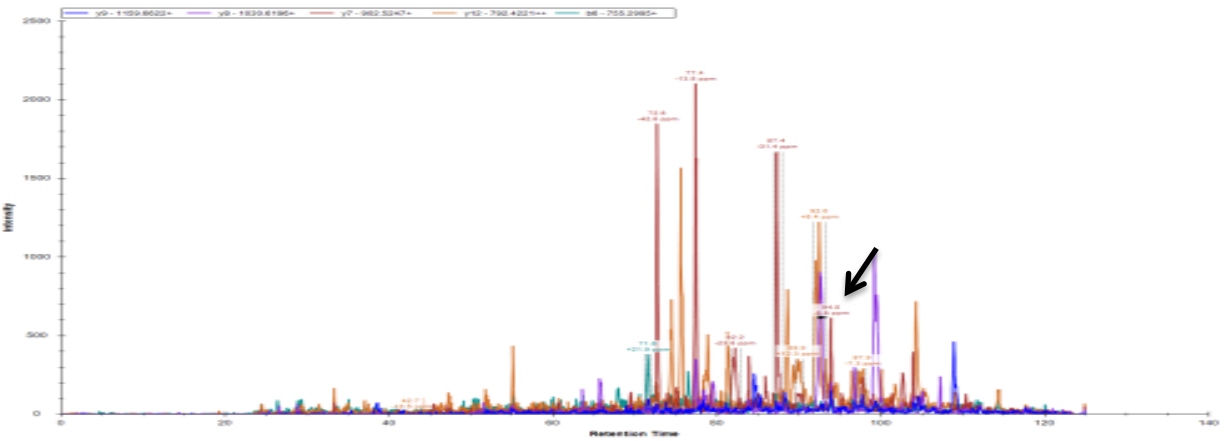

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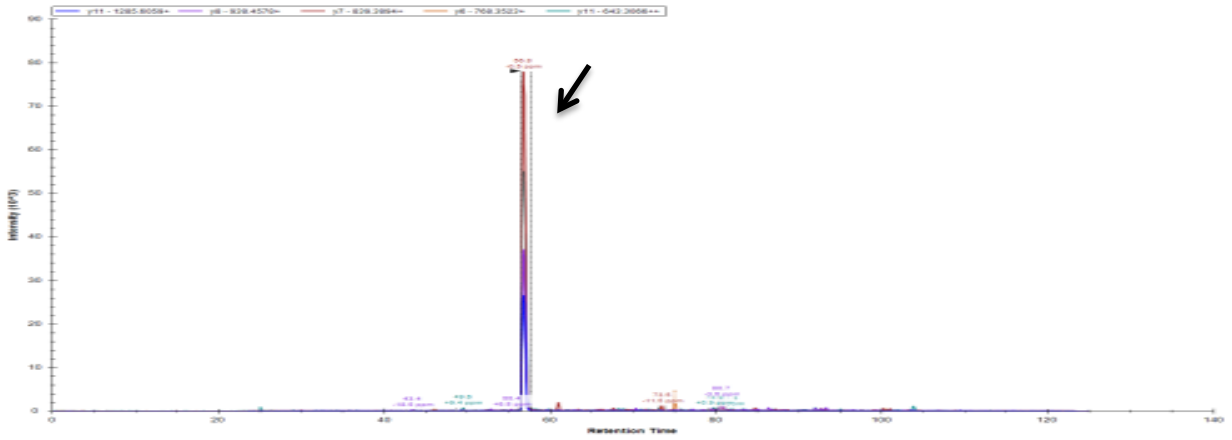

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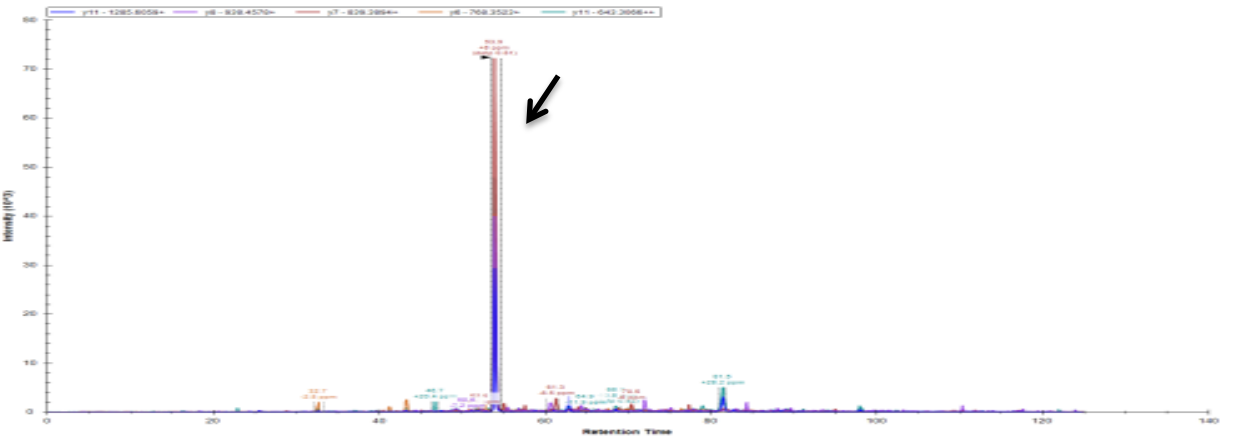

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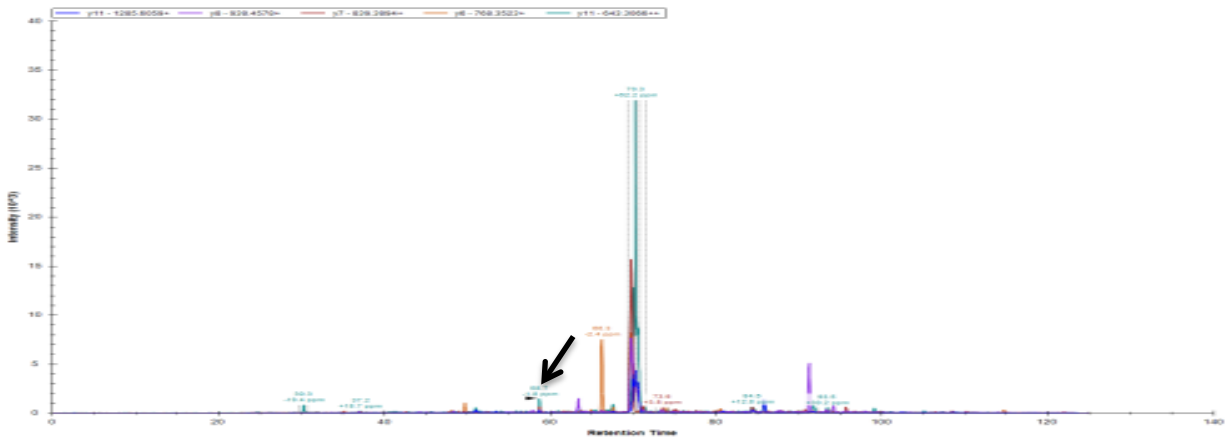

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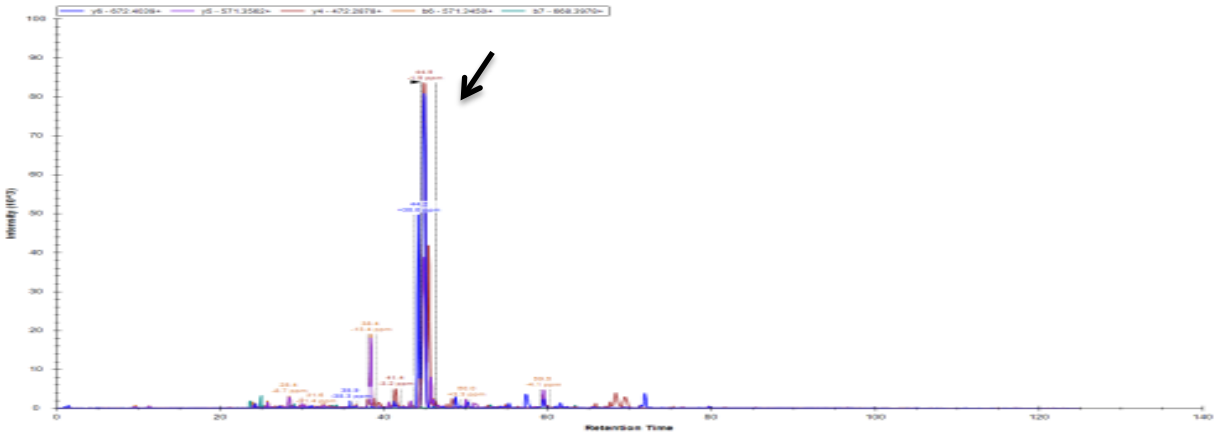

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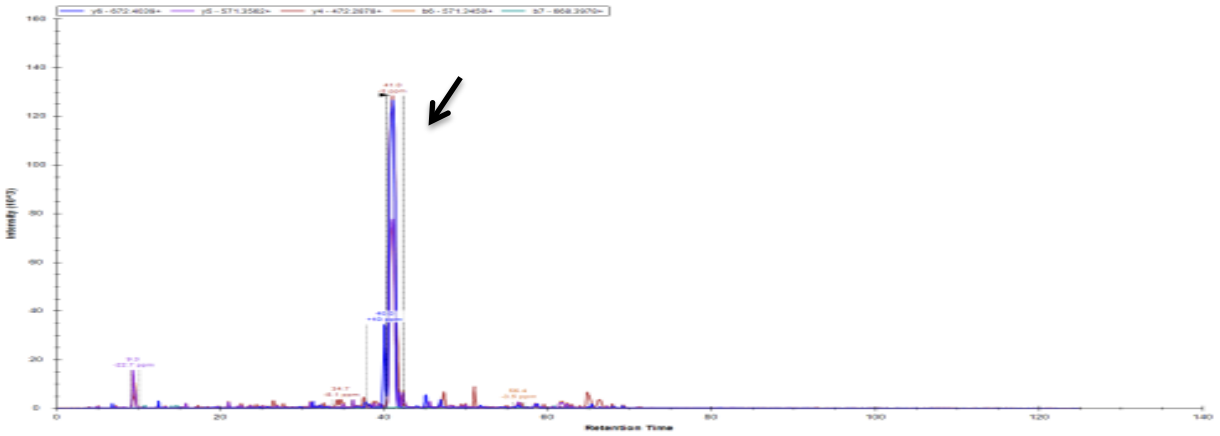

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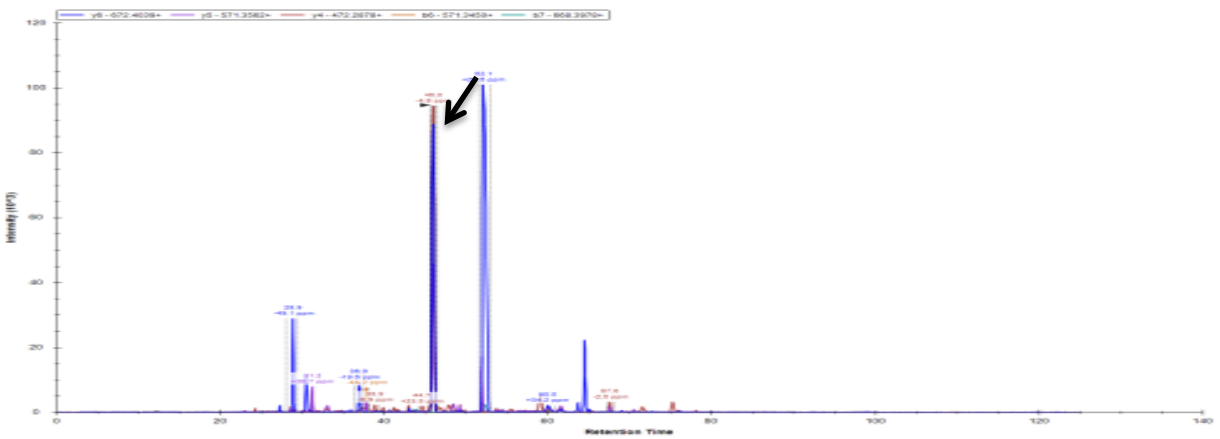

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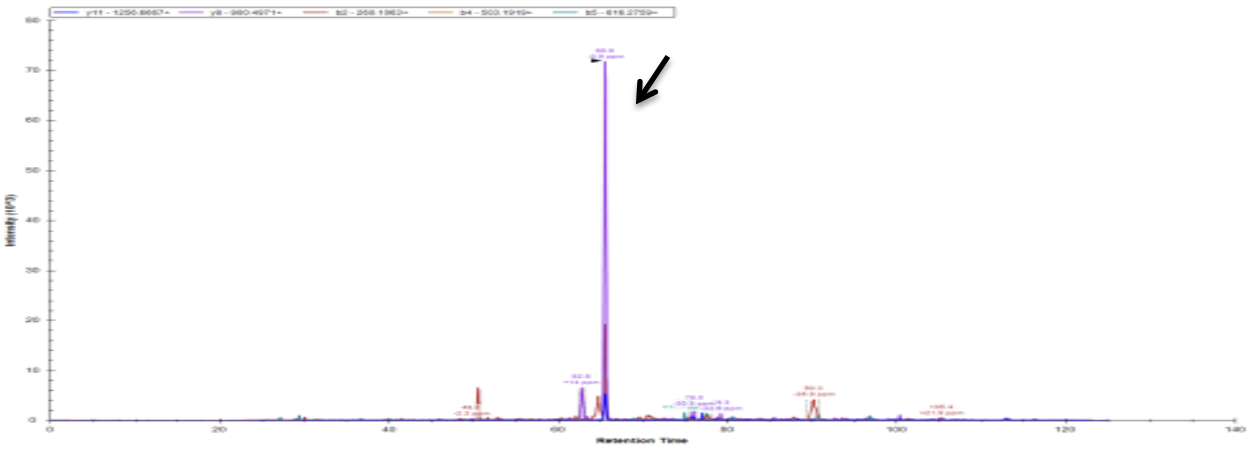

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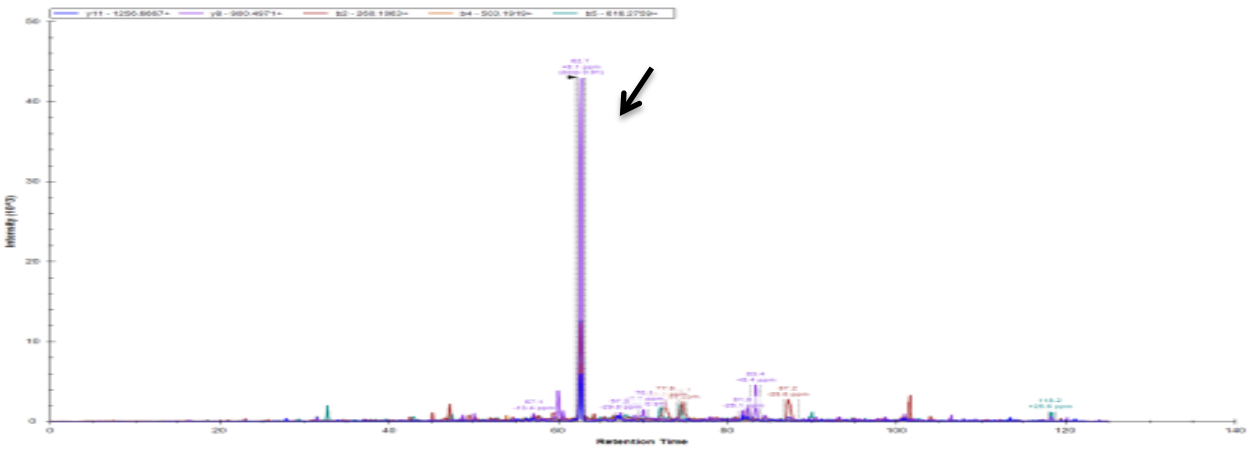

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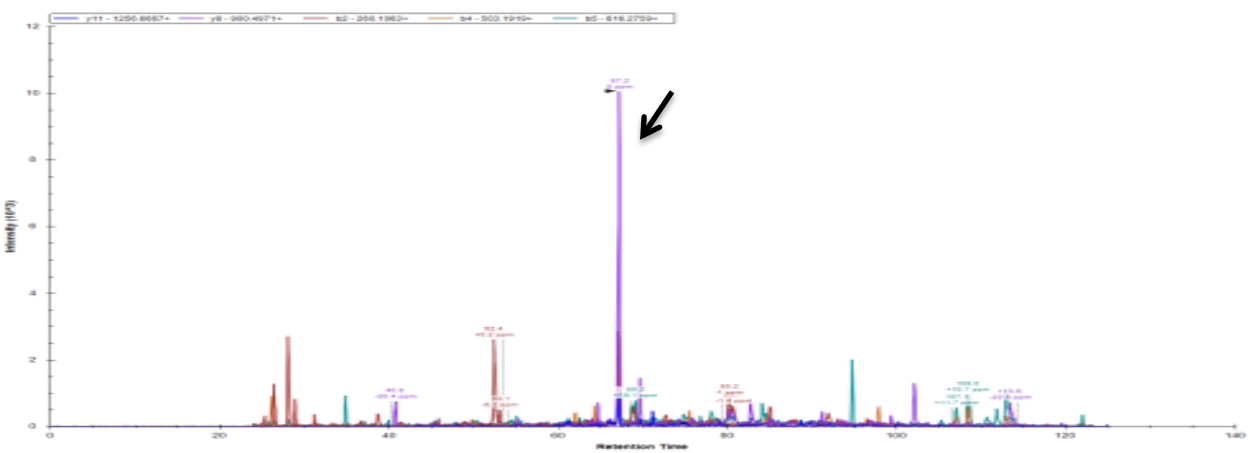

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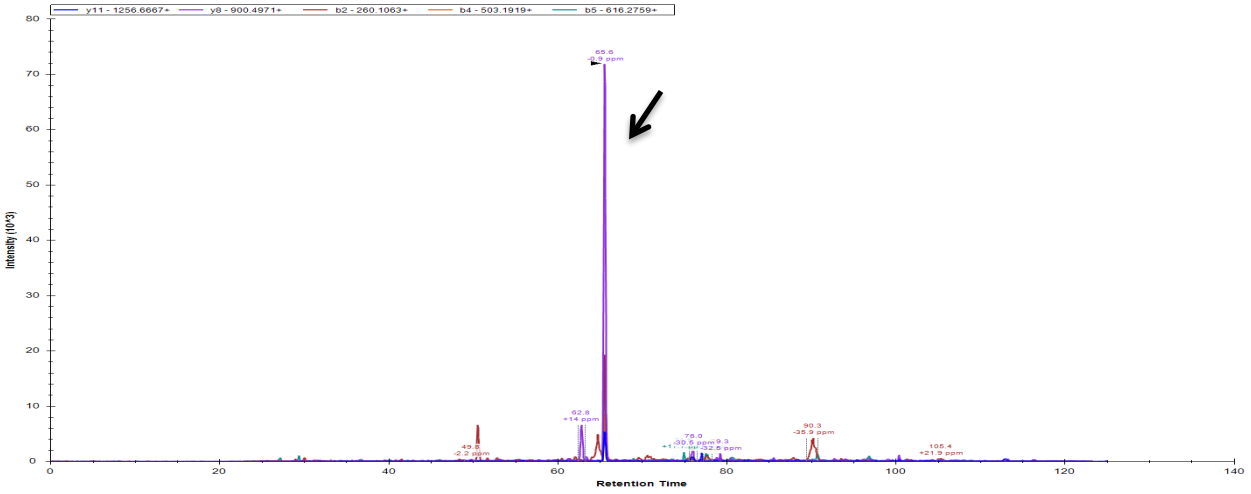

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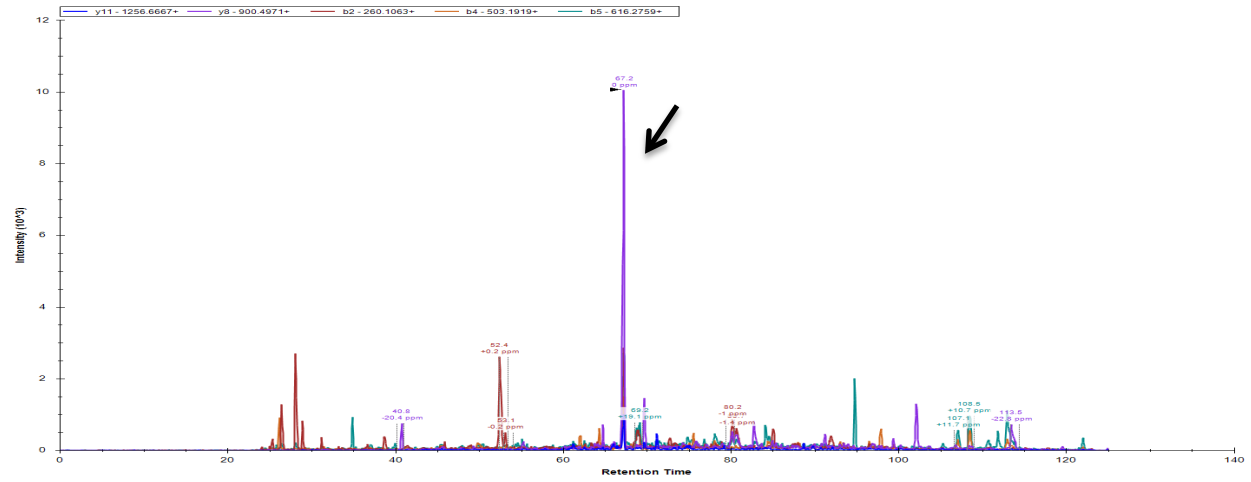

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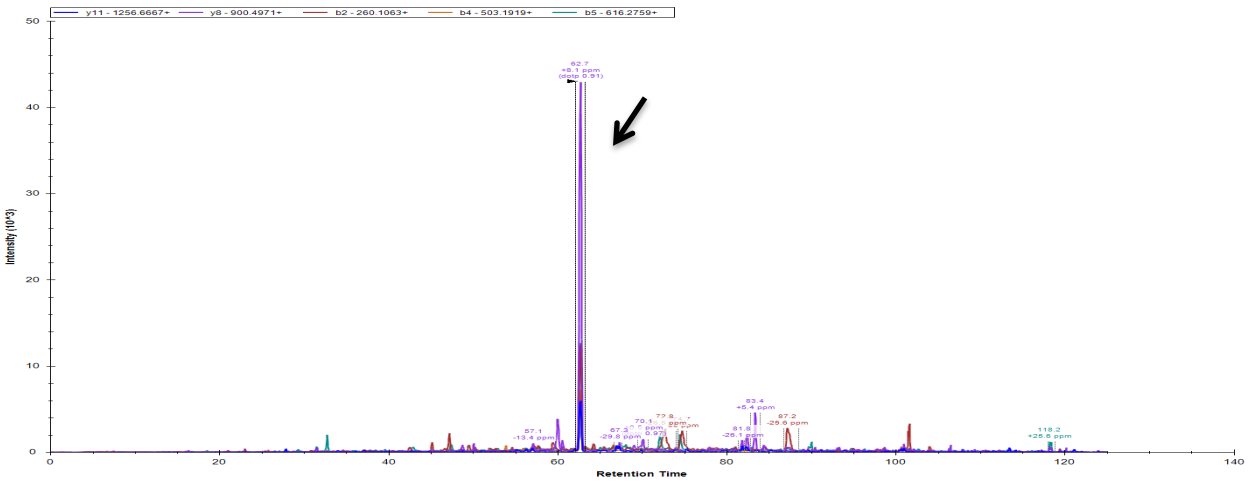

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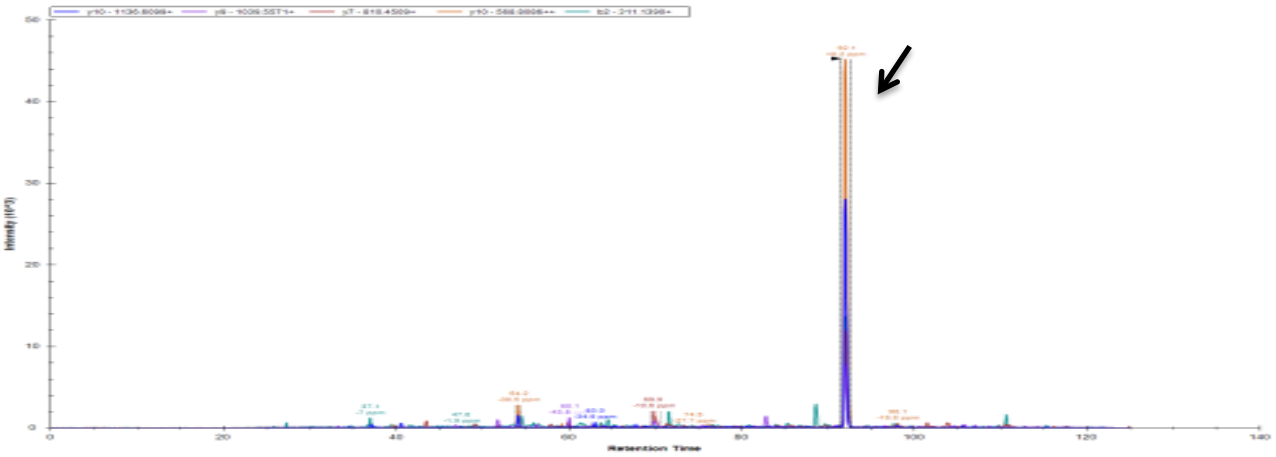

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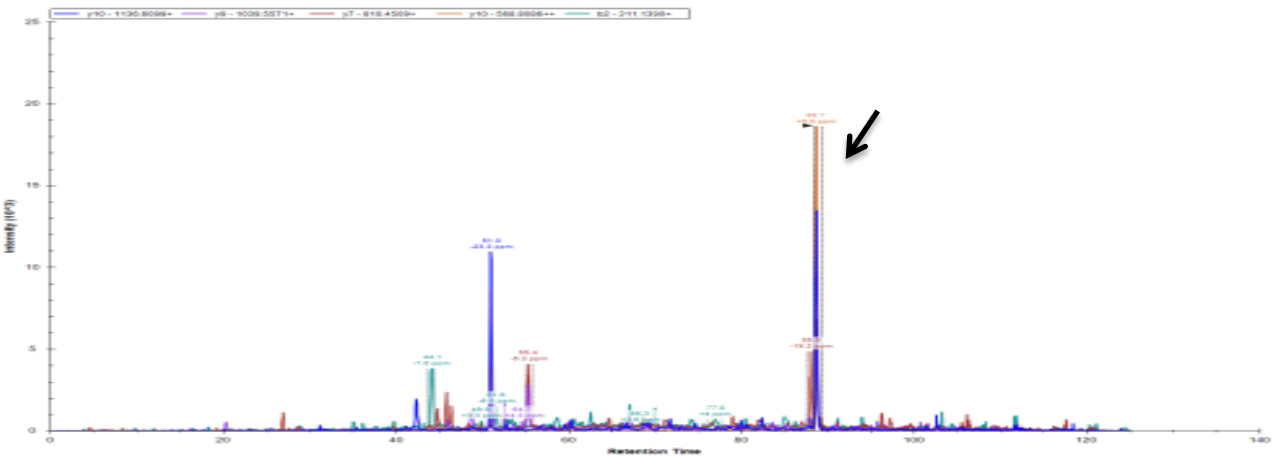

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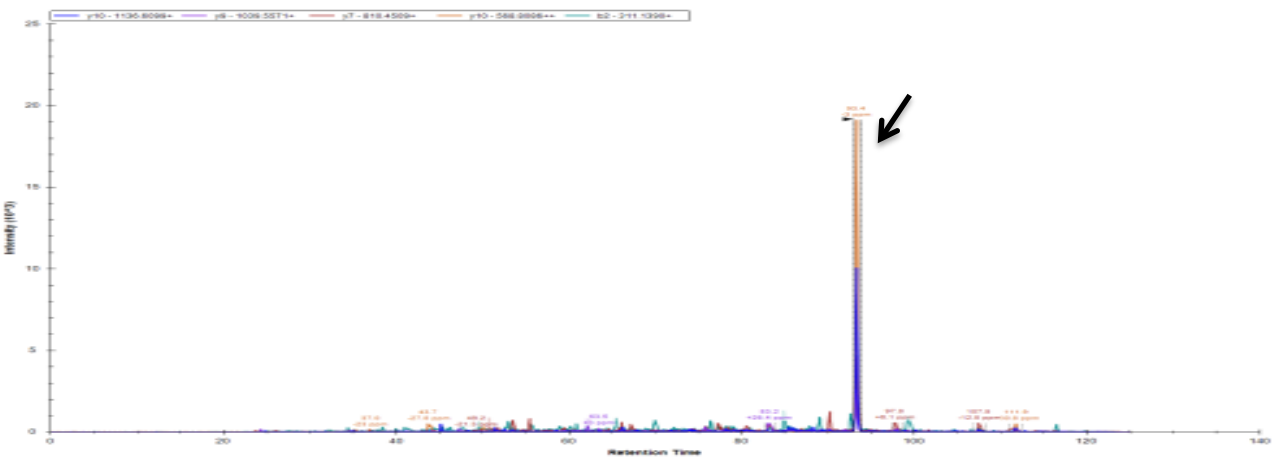

GVLMYGPPGTGK (NOT USED; HIGH NOISE)

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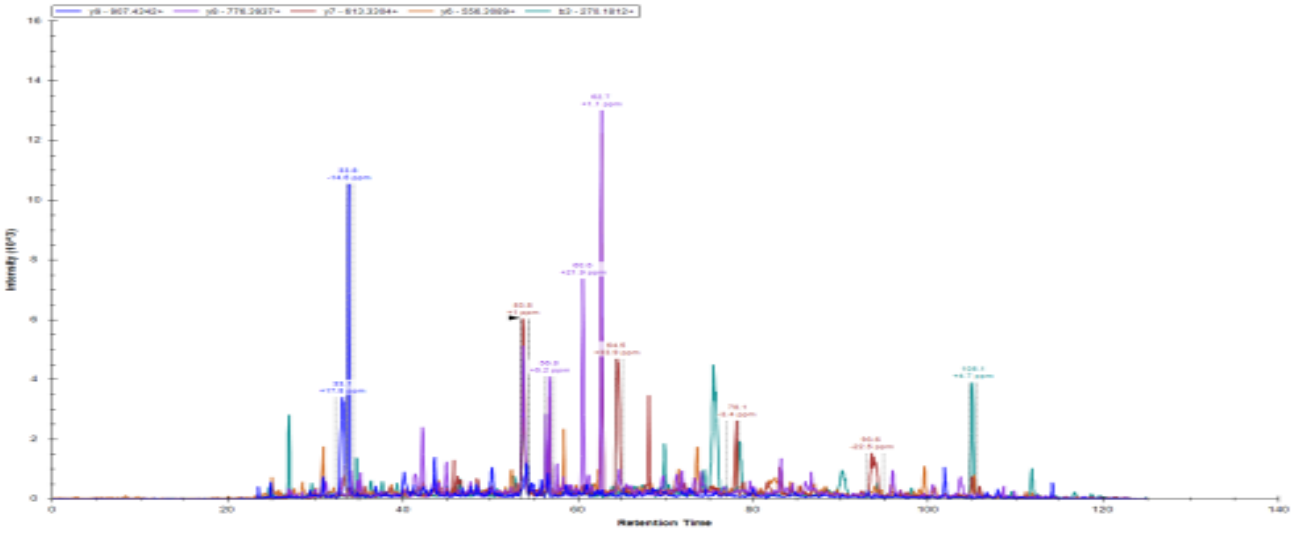

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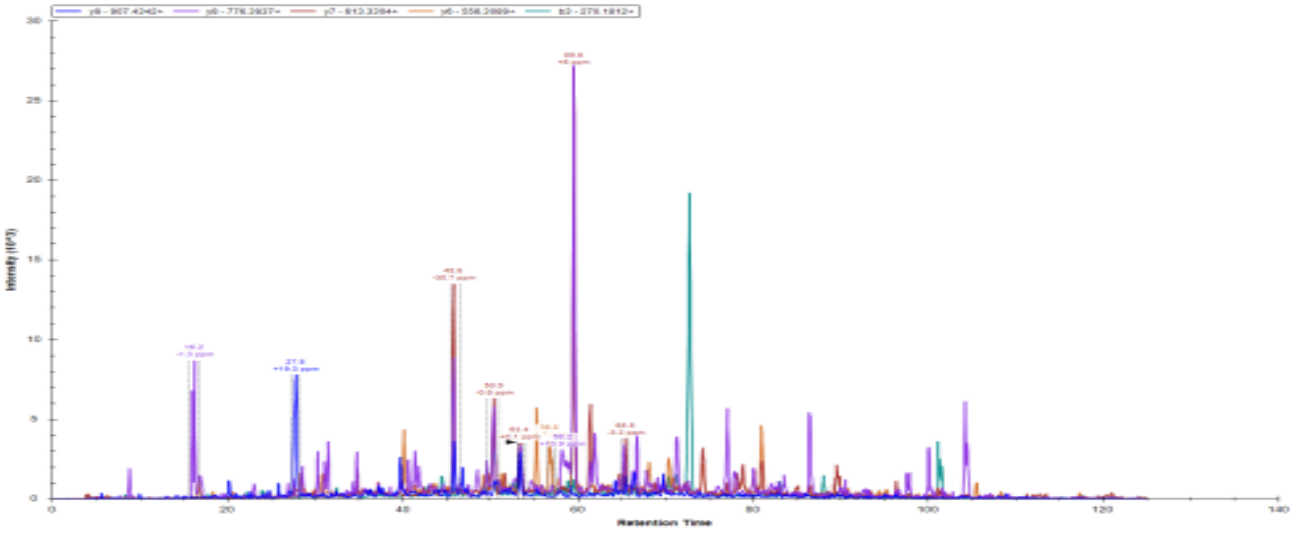

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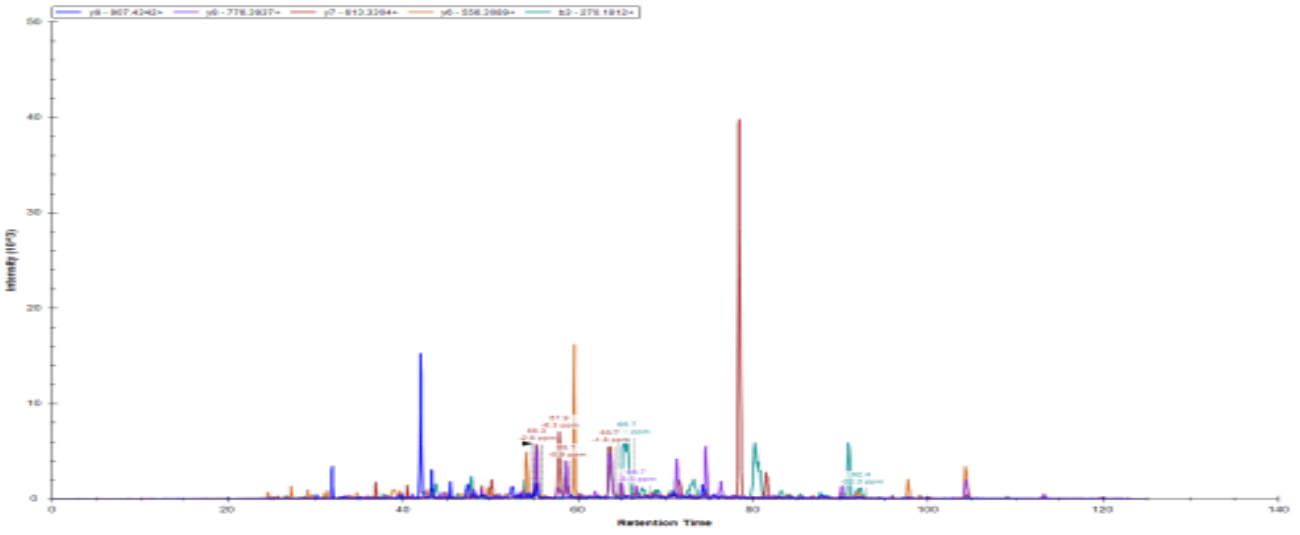

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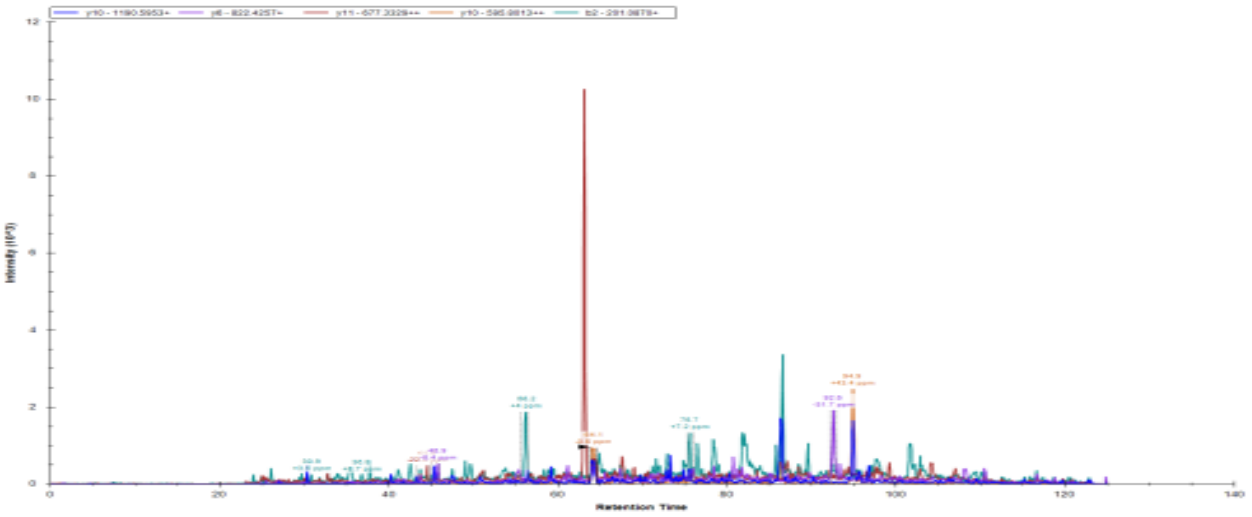

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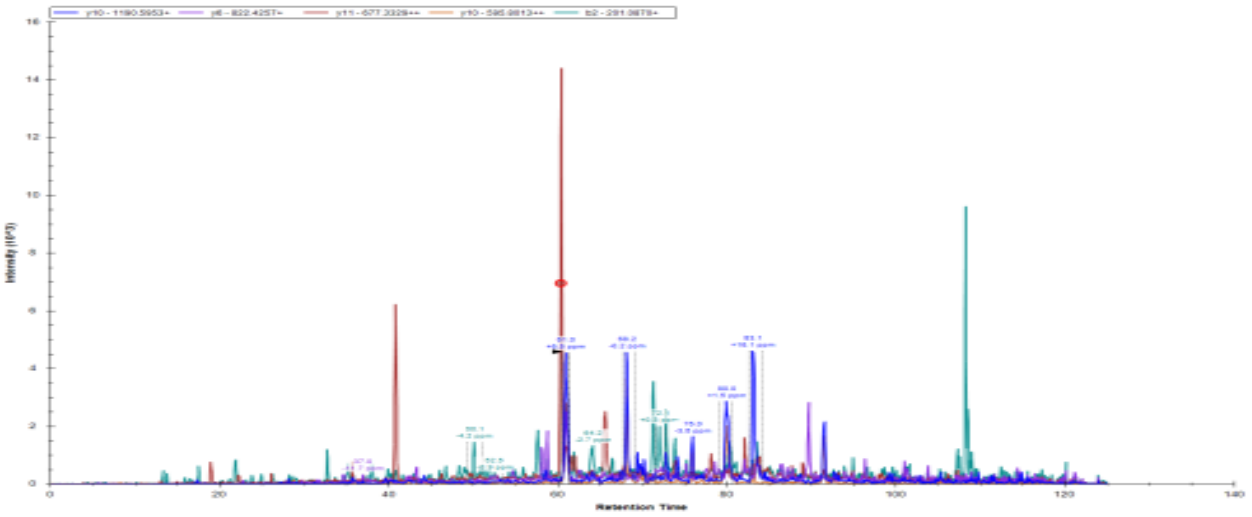

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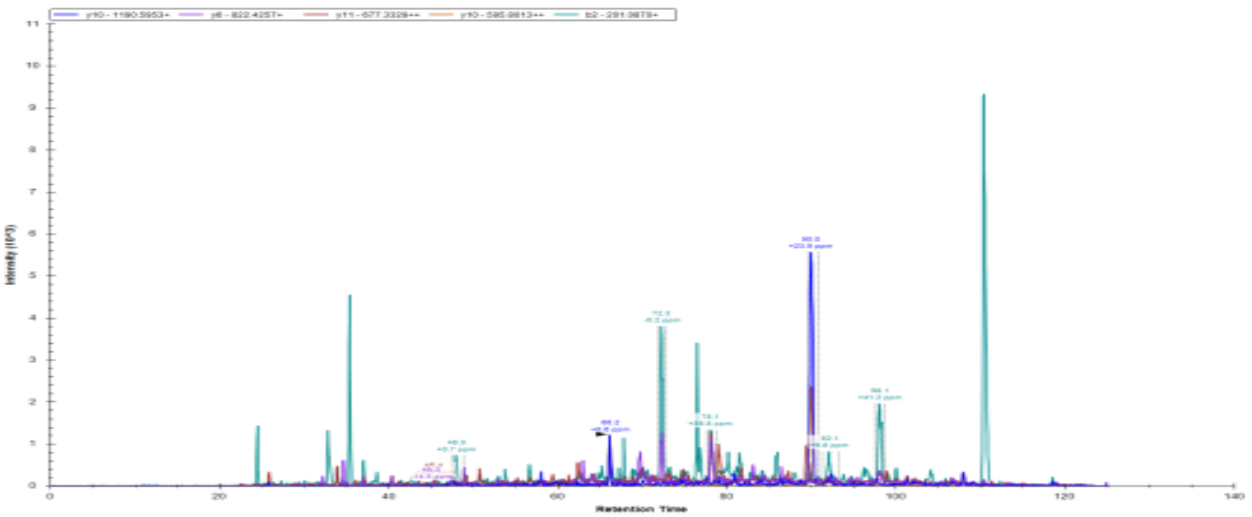

YPIEHGIVTNWDDMEK (NOT USED; LOW SIGNAL:NOISE)

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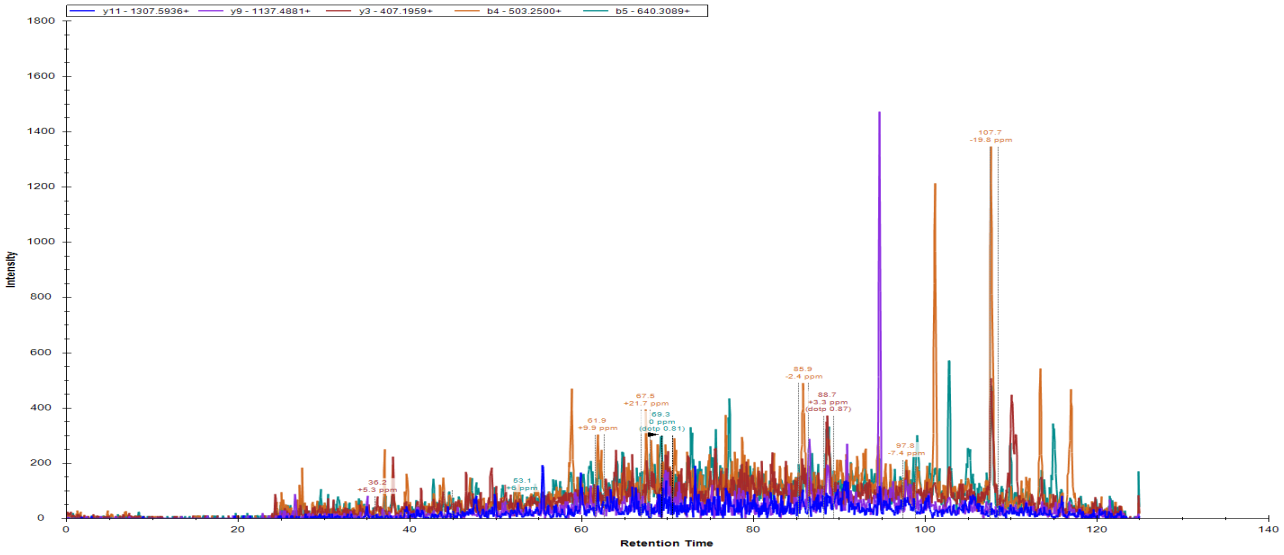

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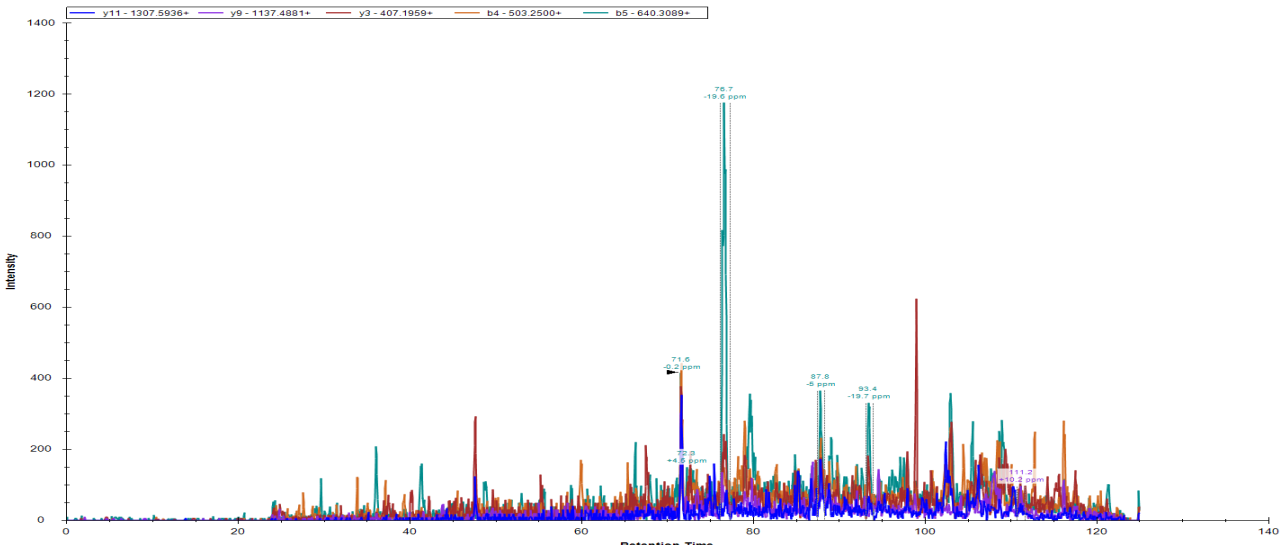

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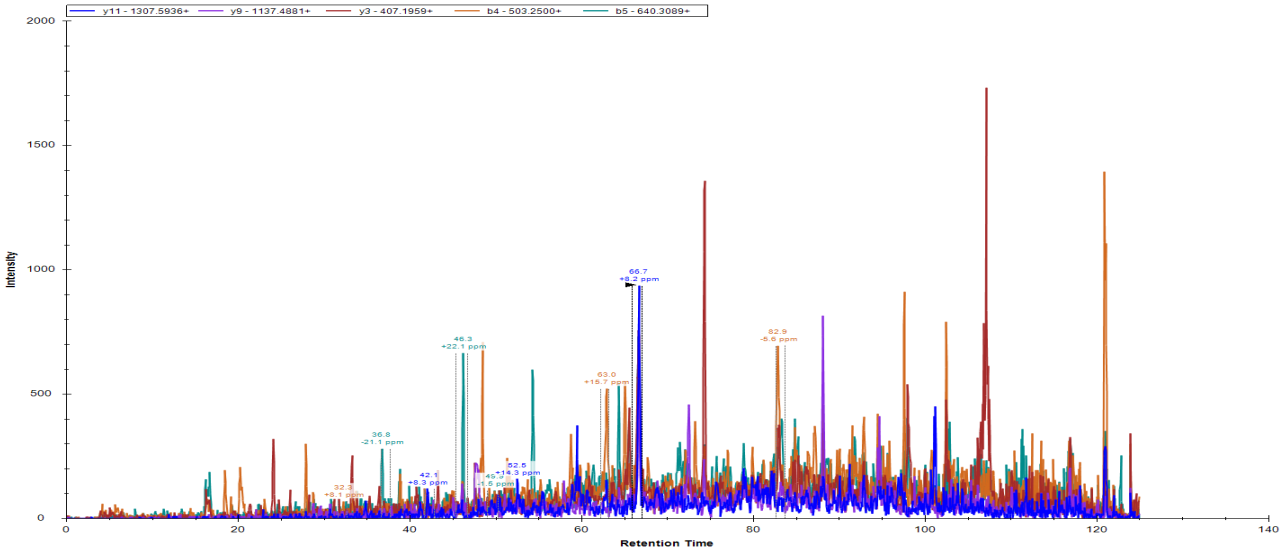

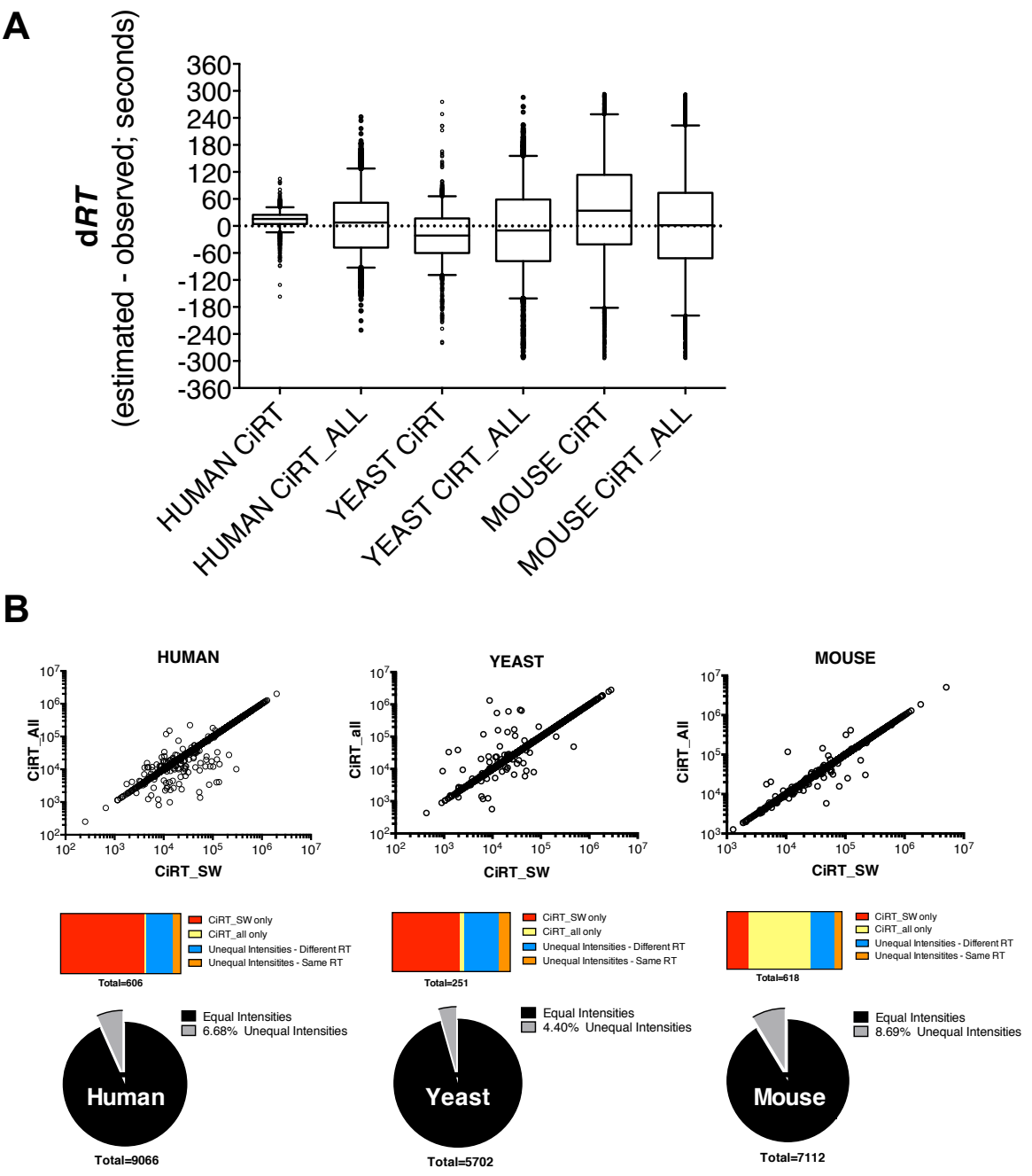
A
dRT
(estimated - observed; seconds)
360
300
240
180
120
60
0
-60
-120
-180
-240
-300
-360
HUMAN CiRT
HUMAN CiRT_ALL
YEAST CiRT
YEAST CiRT_ALL
MOUSE CiRT
MOUSE CiRT_ALL
B
HUMAN
YEAST
MOUSE
CiRT_All
CiRT_all
CiRT_SW
CiRT_SW only
CiRT_all only
Unequal Intensities - Different RT
Unequal Intensitites - Same RT
Total=606
Total=251
Total=618
Equal Intensities
6.68% Unequal Intensities
4.40% Unequal Intensities
8.69% Unequal Intensities
Human
Yeast
Mouse
Total=9066
Total=5702
Total=7112

Supplementary Table 1: CiRT Peptide List

Peptide Sequence	UniProtID*	Gene Name**	iRT Value***
ADTLDPALLRPGR	P33298	PRS6B	36.0
AFEEAEK	P25694	CDC48	-21.4
AFLIEEQK	P40525	RL34B	22.8
AGFAGDDAPR	P60010	ACT	-9.8
AGLQFPVGR	Q12692	H2AZ	37.0
AILGSVER	P04801	SYTC	5.4
APGFGDNR	P19882	HSP60	-15.6
AQIWDTAGQER	P51996	YPT32	16.9
ATAGDTHLGGEDFDNR	P09435	HSP73	3.2
ATIGADFLTK	P32939	YPT7	43.8
AVANQTSATFLR	P40327	PRS4	19.2
AVFPSIVGRPR	P60010	ACT	34.0
CATITPDEAR	P53982	IDHH	-10.1
DAGTIAGLNVLR	P22202	HSP74	59.0
DAHQSLLATR	P32327	PYC2	-3.3
DELTLEGIK	P10081	IF4A	39.1
DLMACAQTGSGK	P06634	DED1	0.3
DLTDYLMK	P60010	ACT	60.0
DNIQGITKPAIR	P02309	H4	12.6
DNTGYDLK	P39976	DLD3	-9.4
DSTLIMQLLR	P34730	BMH2	103.7
DSYVGDEAQSK	P60010	ACT	-15.5
DVQEIFR	Q07478	SUB2	29.6
DWNVDLIPK	Q5SX87	GDI2	70.5
EAYPGDVFYLHSR	P07251	ATPA	46.4
ECADLWPR	J3KTJ3	RPL23	28.7
EDAANNYAR	P09734	TBA3	-23.2
EGIPPDQQR	P0CG63	UBI4P	-15.8
EHAALEPR	P19414	ACON	-22.6
EIAQDFK	P61830	H3	-4.0
EIQTAVR	P02293	H2B1	-17.1
ELIIGDR	K7EK77	ATP5A1	11.6
ELISNASDALDK	P02829	HSP82	23.5
EMVELPLR	P25694	CDC48	48.0
ESTLHLVLR	M0R2S1	UBA52	28.5
EVDIGIPDATGR	P25694	CDC48	37.1
FDDGAGGDNEVQR	P33299	PRS7	-11.3
FDLMYAK	C9J2C0	TUBA8	38.2
FDNLYGCR	P39954	SAHH	9.6

FEELCADLFR	P22202	HSP74	73.5
FELTGIPPAPR	P16474	GRP78	53.1
FELSGIPPAPR	P22202	HSP74	52.5
FPFAANSR	E9PEX6	DLD	18.8
FQSLGVAFYR	P32939	YPT7	60.2
FTQAGSEVSALLGR	P00830	ATPB	61.5
FTVDLPK	I3L492	ETF1	37.9
FVIGGPQGDAGLTGR	P19358	METK2	40.6
GCEVVVSGK	P05750	RS3	-15.5
GEEILSGAQR	P04802	SYDC	-1.8
GILFVGSGVSGGEEGAR	P38720	6PGD1	51.2
GILLYGPPGTGK	P52917	VPS4	45.4
GIRPAINVGLSVSR	K7ENP3	ATP5A1	38.0
GNHECASINR	F8VYE8	PPP1CC	-23.6
GVCTEAGMYALR	Q01939	PRS8	31.2
GVLLYGPPGTGK	Q01939	PRS8	28.1
GVLMYGPPGTGK	P25694	CDC48	28.2
HFSVEGQLEFR	Q86U12	HSP90AA1	41.1
HITIFSPEGR	P21243	PSA1	22.4
HLQLAIR	Q12692	H2AZ	9.4
HLTGEFEK	P32836	GSP2	-13.7
HVFGQAAK	Q06440	CORO	-24.5
ICDFGLAR	P41808	SMK1	28.0
ICGDIHGQYYDLLR	F5H037	PPP1CA	50.3
IETLDPALIRPGR	P40327	PRS4	43.4
IGGIGTVPVGR	P02994	EF1A	21.9
IGLFGGAGVGK	P00830	ATPB	43.3
IGPLGLSPK	P0CX54	RL12B	29.5
IHETNLK	P19414	ACON	-25.5
IINEPTAAAIAYGLDK	E9PN89	HSPA8	65.7
IYGFYDECK	P32598	PP12	31.7
KPLLESGTLGTK	P22515	UBA1	9.1
LAEQAER	P34730	BMH2	-25.1
LGANSLLDLVVFGR	H0Y8X1	SDHA	134.0
LIEDFLAR	P36149	BET3	56.9
LILIESR	P05756	RS13	28.1
LPLQDVYK	P02994	EF1A	29.2
LQIWDTAGQER	H0YDK7	RAB30	36.3
LVIVGDGACGK	E9PQH6	RHOC	10.8
LVLVGDGGTGK	P32836	GSP2	12.0
LYQVEYAFK	P21243	PSA1	46.3
MLSCAGADR	P41805	RL10	-15.5

NILGGTVFR	P21954	IDHP	49.6
NIVEAAAVR	P39939	RS26B	5.7
NLLSVAYK	I3L3T1	YWHAE	34.3
NLQYYDISAK	F5H018	RAN	25.8
NMSVIAHVDHGK	P13639	EF2	-5.4
QAVDVSPLR	M0QZN2	RPS5	11.3
QTVAVGVIK	P02994	EF1A	9.9
SAPSTGGVK	P61830	H3	-27.6
SGQGAFGNMCR	H3BU31	RPL4	0.8
SNYNFEKPFLWLAR	F5H018	RAN	96.0
STELLIR	P61830	H3	18.1
STTTGHLIYK	A6PW80	EEF1A1	-9.5
SYELPDGQVITIGNER	P60010	ACT	67.3
TIAMDGTEGLVR	P00830	ATPB	32.8
TIVMGASFR	P15019	TAL1	29.5
TLSDYNIQK	P0CG63	UBI4P	4.4
TTIFSPEGR	P23638	PSA3	15.2
TTPSYVAFTDTER	E9PPY6	HSPA8	33.8
TTVEYLIK	Q00955	ACAC	30.2
VAVVAGYGDVGK	P39954	SAHH	15.3
VCENIPIVLCGNK	P32836	GSP2	49.1
VLPSIVNEVLK	P50085	PHB2	83.8
VPAINVNDSVTK	P39954	SAHH	17.7
VSTEVDAR	P53228	TAL2	-20.1
VVPGYGHAVLR	P00890	CISY1	8.6
WPFWLSPR	P04801	SYTC	98.4
YAWVLDK	P02994	EF1A	41.6
YDSTHGR	P00358	G3P2	-57.1
YFPTQALNFAFK	P18238	ADT3	95.4
YLVLDEADR	P06634	DED1	27.7
YPIEHGIVTNWDDMEK	P60010	ACT	56.9
YTQSNSVCYAK	P54113	PUR91	-12.8

C - Carbamidomethylation, * Human or Yeast Uniprot Entry; ** Most common gene name; ***Negative iRTs are a function of how the scale is defind, as described in (15)

Supplementary Table 2: CiRT peptides across basic reverse phase fractions

	Fraction													
Peptide Sequence	1	2	3	4	5	6	7	8	9	10	11	12	Total Fractions Observed	
AGFAGDDAPR	■	■	■	■	■	■	■	■	■	■	■	■	12	76 used
AGLQFPVGR	■	■	■	■	■	■	■	■	■	■	■	■	12	
AVFPSIVGRPR	■	■	■	■	■	■	■	■	■	■	■	■	12	
DSYVGDEAQSK	■	■	■	■	■	■	■	■	■	■	■	■	12	
IGGIGTVPVGR	■	■	■	■	■	■	■	■	■	■	■	■	12	
IINEPTAAAIAYGLDK	■	■	■	■	■	■	■	■	■	■	■	■	12	
SYELPDGQVITIGNER	■	■	■	■	■	■	■	■	■	■	■	■	12	
TTPSYVAFTDTER	■	■	■	■	■	■	■	■	■	■	■	■	12	
DAGTIAGLNVLR	■	■	■	■	■		■	■	■	■	■	■	11	
EIQTAVR	■		■	■	■	■	■	■	■	■	■	■	11	
QTVAVGVIK	■	■	■	■	■	■	■	■	■		■	■	11	
TIAMDGTEGLVR	■	■	■	■	■	■	■	■		■	■	■	11	
LVLVGDGGTGK	■			■	■	■	■	■	■	■	■	■	10	
STELLIR	■	■	■	■	■	■		■	■	■		■	10	
STTTGHLIYK		■		■	■	■				■	■	■	7	
IGLFGGAGVGK							■	■	■	■	■	■	6	
IGPLGLSPK	■						■	■	■	■			5	
ELISNASDALDK								■	■	■	■		4	
GILFVGSGVSGGEEGAR						■		■	■				3	
NIVEAAAVR									■	■	■		3	
NMSVIAHVDHGK				■	■	■							3	
SGQGAFGNMC[160]R							■	■	■				3	
AQIWDTAGQER										■	■		2	
AVANQTSATFLR		■	■										2	
DLMAC[160]AQTGSGK							■	■					2	
DLTDYLMK		■	■										2	
DNIQGITKPAIR					■	■							2	
EC[160]ADLWPR											■	■	2	
FTQAGSEVSALLGR											■	■	2	
FVIGGPQGDAGLTGR							■	■					2	
GNHEC[160]ASINR				■	■								2	
HFSVEGQLEFR				■			■						2	
HITIFSPEGR	■	■											2	
HLQLAIR				■	■								2	
HLTGEFEK										■	■		2	
IHETNLK									■		■		2	
LGANSLLDLVVFGR				■	■								2	
LPLQDVYK				■	■								2	
LVIVGDGAC[160]GK	■	■											2	
LYQVEYAFK							■	■					2	
MLSC[160]AGADR							■	■					2	
SNYNFEKPFLWLAR			■	■									2	
VLPSIVNEVLK			■	■									2	
VPAINVNDSVTK				■	■								2	
VSTEVDAR			■	■									2	
VVPGYGHAVLR	■						■						2	

AFEEAEK					■								1
AFLIEEQK					■								1
AILGSVER												■	1
APGFGDNR							■						1
ATIGADFLTK			■										1
C[160]ATITPDEAR		■											1
DSTLIMQLLR						■							1
EAYPGDVFYLHSR			■										1
EDAANNYAR						■							1
EHAALEPR			■										1
EIAQDFK							■						1
FDDGAGGDNEVQR					■								1
FQSLGVAFYR										■			1
GC[160]EVVVSGK		■											1
GEEILSGAQR										■			1
GVC[160]TEAGMYALR				■									1
GVLMYGPPGTGK					■								1
KPLLESGTLGTK				■									1
LIEDFLAR								■					1
LQIWDTAGQER				■									1
NLLSVAYK		■											1
NLQYYDISAK				■									1
QAVDVSPLR										■			1
TIVMGASFR			■										1
TLSDYNIQK									■				1
TTIFSPEGR												■	1
VAVVAGYGDVGK				■									1
VC[160]ENIPIVLC[160]GNK								■					1
YPIEHGIVTNWDDMEK				■									1
YTQSNSVC[160]YAK										■			1
TotalPeptidesUsed	**18**	**20**	**22**	**31**	**26**	**19**	**24**	**25**	**21**	**24**	**22**	**20**	

Supplementary Table 3: CiRT peptides across basic reverse phase fractions

	Fraction											
Peptide Sequence	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
AGFAGDDAPR	■	■	■	■	■	■	■	■	■	■	■	■
AGLQFPVGR	■	■	■	■	■	■	■	■	■	■	■	■
AVFPSIVGRPR	■	■	■	■	■	■	■	■	■	■	■	■
DSYVGDEAQSK	■	■	■	■	■	■	■	■	■	■	■	■
IGGIGTVPVGR	■	■	■	■	■	■	■	■	■	■	■	■
IINEPTAAAIAYGLDK	■	■	■	■	■	■	■	■	■	■	■	■
SYELPDGQVITIGNER	■	■	■	■	■	■	■	■	■	■	■	■
TTPSYVAFTDTER	■	■	■	■	■	■	■	■	■	■	■	■
DAGTIAGLNVLR	■	■	■	■	■		■	■	■	■	■	■
EIQTAVR	■		■	■	■	■	■	■	■	■	■	■
QTVAVGVIK	■	■	■	■	■	■	■	■	■		■	■
TIAMDGTEGLVR	■	■	■	■	■	■	■	■		■	■	■
LVLVGDGGTGK	■			■	■	■	■	■	■	■	■	■
STELLIR	■	■	■	■	■	■		■	■	■		■
STTTGHLIYK		■		■	■	■				■	■	■
IGLFGGAGVGK							■	■	■	■	■	■
IGPLGLSPK	■						■	■	■	■		
ELISNASDALDK								■	■	■	■	
GILFVGSGVSGGEEGAR						■		■	■			
NIVEAAAVR									■	■	■	
NMSVIAHVDHGK				■	■	■						
SGQGAFGNMC[160]R							■	■	■			
AQIWDTAGQER										■	■	
AVANQTSATFLR		■	■									
DLMAC[160]AQTGSGK							■	■				
DLTDYLMK		■	■									
DNIQGITKPAIR					■	■						
EC[160]ADLWPR											■	■
FTQAGSEVSALLGR											■	■
FVIGGPQGDAGLTGR							■	■				
GNHEC[160]ASINR				■	■							
HFSVEGQLEFR				■			■					
HITIFSPEGR	■	■										
HLQLAIR				■	■							
HLTGEFEK										■	■	
IHETNLK									■		■	
LGANSLLDLVVFGR				■	■							

LPLQDVYK				■	■							
LVIVGDGAC[160]GK	■	■										
LYQVEYAFK							■	■				
MLSC[160]AGADR							■	■				
SNYNFEKPFLWLAR			■	■								
VLPSIVNEVLK			■	■								
VPAINVNDSVTK				■	■							
VSTEVDAR			■	■								
VVPGYGHAVLR	■						■					
AFEEAEK					■							
AFLIEEQK					■							
AILGSVER												■
APGFGDNR							■					
ATIGADFLTK			■									
C[160]ATITPDEAR		■										
DSTLIMQLLR						■						
EAYPGDVFYLHSR			■									
EDAANNYAR						■						
EHAALEPR			■									
EIAQDFK							■					
FDDGAGGDNEVQR					■							
FQSLGVAFYR										■		
GC[160]EVVVSGK		■										
GEEILSGAQR										■		
GVC[160]TEAGMYALR				■								
GVLMYGPPGTGK					■							
KPLLESGTLGTK				■								
LIEDFLAR								■				
LQIWDTAGQER				■								
NLLSVAYK		■										
NLQYYDISAK				■								
QAVDVSPLR										■		
TIVMGASFR			■									
TLSDYNIQK									■			
TTIFSPEGR												■
VAVVAGYGDVGK				■								
VC[160]ENIPIVLC[160]GNK								■				
YPIEHGIVTNWDDMEK				■								
YTQSNSVC[160]YAK										■		
TotalPeptidesUsed	**18**	**20**	**22**	**31**	**26**	**19**	**24**	**25**	**21**	**24**	**22**	**20**

Supplementary Table 4: Software and Analytical Scripts for using CiRT peptides in openSWATh workflow

Task	Software or Script	Hosting Program	Standard Workflow	CiRT adjustment
Peptide Assay Library Assembly and Alignment				
Initial peptide assay library build	SpectraST - Import Library	Trans Proteomic Pipeline (TPP)	spectrast -c_BIN! -cf'Protein!~DECOY' -cP[Minimum probability to include] -cI[fragmentation method_instrument, e.g, CID_TOF] -cN[Output_name] [input_interact.pep.xml]	None
Library RT alignment to iRT space	spectrast2spectrast_iRT.py	Python, code available from code.google.com/p/msproteomicstools	spectrast2spectrast.py -r -i inputfile.splib -o outputfile_iRT.splib	add the option -k Peptide1:iRT, peptide2:iRT, … peptide[Last]:iRT to stipulate the use of CiRT internal peptides* rather than the default iRTs
Library Consolidation	SpectraST	TPP	spectrast -cAC -c_BIN! -cf'Protein!~iRT' -cNFractionsLib_14Oct13_iRT.splib_con FractionsLib_14Oct13_iRT.splib	none
Library conversion to .tsv	spectrast2tsv.py	Python, code available from code.google.com/p/msproteomicstools	spectrast2tsv.py -l [ms/ms mass range] -s [ms/ms ions to include] -x[charge states] -o [min transitions] -n [max transitions] -p 0.05 -d -e -g [modifications allowed] -w [swath paramter file].txt -k [specify output format, i.e., openswath] -a [output name].tsv [input file].splib	none
Library conversion to .TraML	ConvertTSVToTraML.py	Python, code available from code.google.com/p/msproteomicstools	ConvertTSVToTraML -in [input_filename].tsv -out [output_filename].TraML	none
Append decoy transitions to peptide assay library	OpenSwathDecoyGenerator	OpenMS	OpenSwathDecoyGenerator -in [input_File].TraML -out [output_file]_decoy.TraML -min_transitions 5 -max_transitions 5 -method shuffle -append -exclude_similar	none
OpenSWATH Data Analysis				
Extract iRT chromatograms	OpenSwathChromatogramExtractor	openMS	See paper by Rost et al, Reference #15	-tr CiRT_SW.TraML* (rather than standard SiRT .TraML)
Align RT-to-iRT for SiRT or CiRT reference peptides	OpenSWATHRTNormalizer	openMS	See paper by Rost et al, Reference #15	-best_peptides
Extract and score full dataset	OpenSwathAnalyzer	openMS	See paper by Rost et al, Reference #15	none

*Available as supplemental downloads to this manuscript