ADDITIONAL FILES 4.

- Full labeling scheme of iTRAQ labels
- Waters-Micromass CapLC settings
- Depletion repeatability
- Sample comparison in depletion runs
- Example MS chromatogram

Full labeling scheme of iTRAQ labes:

	1st iTRAQ experiment, iTRAQ labels	2nd iTRAQ experiment, iTRAQ labels	3rd iTRAQ experiment, iTRAQ labels
Patient 1. Arteria	113	113	
Patient 1. Porta	114	114	
Patient 1. Hepatica	115	115	
Patient 2. Arteria	116		118
Patient 2. Porta	117		119
Patient 2. Hepatica	118		121
Patient 3. Arteria		116	115
Patient 3. Porta		117	116
Patient 3. Hepatica		118	117
Control sample	119	121	113

Three iTRAQ labeling experiments were performed followed by MS analysis. All patients sample were analyzed twice.

Waters-Micromass CapLC settings:

Waters CapLC Initial Conditions:

Method Type=Normal

Percentage A = 95.0
Percentage B = 5.0
Solvent Select = A1
Solvent Name A1 = 0.1% FA
Solvent Name A2 = Solvent A2
Solvent Name A3 = Solvent A3
Solvent Name B = 95% ACN, 0.1% FA

Total Flow(ul) = 5.000
Run Time (min) = 91.00
Low Pressure(psi) = 0.0
High Pressure(psi) = 4300.0

Aux Flow(ul) =1.000
Aux Low Pressure(psi) = 0.0
Aux High Pressure Aux(psi) = 4300.0

Column Temperature (°C) = 20.0
Temperature Range(°C) = 20.0
Column Name = Column

Pump Synchronisation = Off

Initial Switch 1 = Off
Initial Switch 2 = Off
Initial Stream Select = Position 1
Initial Vent Valve = System

Chart Recorder 1 = Percent B
Chart Recorder 2 = None

Waters CapLC Gradient Timetable:

Time	Percent A	Percent B	Flow	Curve
0.10	95.0	5.0	5.000	6
5.00	95.0	5.0	5.000	6
40.00	70.0	30.0	5.000	6
70.00	10.0	90.0	5.000	6
75.00	10.0	90.0	5.000	6

76.00	95.0	5.0	5.000	6
91.00	95.0	5.0	5.000	6

Waters CapLC Auxiliary C Flow Timetable:

Time	Flow	Curve
0.10	10.000	6
5.00	10.000	6
6.00	1.000	6

Waters CapLC Event Table:

Time(min)	Event	Action	Parameter
5.00	Stream Select	Position 2	
90.00	Stream Select	Position 1	

Waters CapLC Autosampler Parameters:

Draw Height(mm) = 1
Draw Speed = Slow
Inject Type = uL Pickup
Flush Volume(ul) = 10.00
Use Head Space Pressure = No
Use Air Space Segment = No
Use Low Dispersion Mode = No
Wash Volume(ul) = 10.00
Sample Temperature(°C) = 15.00
Temperature Limit(°C) = 20.00

Waters CapLC Mix Method:

Mix Delay(mins) = 0.00
Mix Cycles = 1
Reagent A Position = 0
Reagent A Volume(ul) = 1.00
Reagent B Position = 0
Reagent B Volume(ul) = 1.00

Mircomass QToF Ultima Global mass spectrometer parameters:

Parameters for C:\MassLynx\2009.PRO\ACQUDB\Ville_90min_NormalCE.EXP

Temperature Correction	Enabled
Temperature Coefficient	71.700000
TDC Gain Control	0.0
TDC Amp Edge Control	0.0
Using 4 GHz TDC	YES
Using TTP 4 GHz TDC	YES
Maldi Plate File	
Maldi ESI Position	3200.0
Maldi Laser Type	337SI
Maldi Laser Firing Rate	1.0
Maldi Plate Speed	1.0
Maldi Pattern Filename	C:\MassLynx\Qtof\line.ptn
Maldi Probe Motion Type	0.0

Instrument Parameters - Function 1:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 1	
Capillary	2.40	2.40
Cone1	20	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.9
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115
Offset1	-0.3	-65.0
Offset2	0.0	-65.3

Pusher	980	880
TOF (kV)	9.10	-9.25
Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	16129.03	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	
Pirani Pressure(mbar)	4.07e0	
Penning Pressure(mbar)	OFF	
Tof Penning Pressure(mbar)	4.12e-7	

Function Parameters - Function 1 - TOF SURVEY FUNCTION

[ACQUISITION]	
Survey Start Time	5.0
Survey End Mass	90.0
Survey Ion Mode	ES Mode
Survey Polarity	Positive
[MS SURVEY]	
Survey Start Mass	400.0
Survey End Mass	2000.0
Intensity Threshold	10.0
Survey Scan Time	2.4
Survey Interscan Time	0.1
Survey Data Format	Continuum
Survey Use Tune Page CV	YES
Survey Cone Voltage (V)	35.0
[MS/MS]	

MSMS Start Mass	50.0
MSMS End Mass	2500.0
Number of components	6
MSMS to MS Switch Criteria Intensity Falling Below Threshold	
Switchback threshold (counts/second)	5.0
Use MSMS to MS Switch After Time	YES
MSMS Switch After Time (sec)	10.0
MSMS Scan Time (sec)	2.4
MSMS Interscan Time (sec)	0.1
MSMS Data Format	Continuum
Use Tune Page Cone Voltage	YES
MSMS Cone Voltage (V)	35.0
Use MS/MS ipr File	NO
MSMS Instrument Parameter Filename	

[PEAK DETECTION]

Peak Detection Window	1.0
Use Include By Charge State	YES
Charge State(s)	2,3,4
Number Of Include Components	60
Charge State Tolerance Window	3.0
Charge State Extraction Window	2.0
Discard survey data	NO

[COLLISION ENERGY]

Use Charge State Recognition	YES
Maximum Charge State	4
Charge State 1 Filename	C:\MassLynx\Service.PRO\ACQUDB\Default_CS_1_CE.txt
Charge State 2 Filename	C:\MassLynx\Service.PRO\ACQUDB\Default_CS_1_CE.txt
Charge State 3 Filename	C:\MassLynx\Default.pro\Acqudb\Default_CS_2_CE.txt
Charge State 4 Filename	C:\MassLynx\Default.pro\Acqudb\Default_CS_3_CE.txt
Charge State 5 Filename	C:\MassLynx\Default.pro\Acqudb\Default_CS_4_CE.txt
Charge State 6 Filename	
CS Recognition Components	60.0
CS Recognition Tolerance Window	3.0
CS Recognition Extraction Window	2.0

[INCLUDE]

Precursor Selection	Everything

[EXCLUDE]		
Use Exclude Masses List		NO
Exclude Mass Range		
Use Exclude File Masses		NO
Exclude Mass Filename		
Exclude Window +/- (mDa)		100.0
Exclude Retention Time Window		10.0
[ADDUCTS]		
Use Adduct File Masses		NO
Adduct Filename		
[VARIABLE FLOW]		
Use Variable Flow		NO
Port Number		1.0

Instrument Parameters - Function 2:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 2	
Capillary	2.40	2.40
Cone	120	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.9
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115
Offset1	-0.3	-65.0
Offset2	0.0	-65.3
Pusher	980	880
TOF (kV)	9.10	-9.25

Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	11363.64	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	
Pirani Pressure(mbar)	4.07e0	
Penning Pressure(mbar)	OFF	
Tof Penning Pressure(mbar)	4.12e-7	

Function Parameters - Function 2 - TOF SURVEY FUNCTION

Set Mass	0.0
Start Mass	50.0
End Mass	2500.0
Start Time (mins)	5.0
End Time (mins)	90.0
Data Format	Continuum
Ion Mode	ES Mode
Polarity	Positive
Instrument Parameter Filename	C:\MassLynx\2009.PRO\ACQUDB\jdwvpos_Q3.ipr
Scans To Sum	16960
Scan Time (sec)	2.4
Interscan Time (sec)	0.1
Use Tune Page Collision Energy	YES
Collision Energy (eV)	429.2
Use Tune Page Cone Voltage	YES
Cone Voltage (V)	35.0

Instrument Parameters - Function 3:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 2	
Capillary	2.40	2.40

Cone	120	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.9
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115
Offset1	-0.3	-65.0
Offset2	0.0	-65.3
Pusher	980	880
TOF (kV)	9.10	-9.25
Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	11363.64	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	
Pirani Pressure(mbar)	4.07e0	
Penning Pressure(mbar)	OFF	
Tof Penning Pressure(mbar)	4.12e-7	

Function Parameters - Function 3 - TOF SURVEY FUNCTION

Set Mass	0.0
Start Mass	50.0
End Mass	2500.0
Start Time (mins)	5.0
End Time (mins)	90.0
Data Format	Continuum
Ion Mode	ES Mode
Polarity	Positive
Instrument Parameter Filename	C:\MassLynx\2009.PRO\ACQUDB\jdwvpos_Q3.ipr
Scans To Sum	16960
Scan Time (sec)	2.4
Interscan Time (sec)	0.1
Use Tune Page Collision Energy	YES
Collision Energy (eV)	451.3
Use Tune Page Cone Voltage	YES
Cone Voltage (V)	35.0

Instrument Parameters - Function 4:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 2	
Capillary	2.40	2.40
Cone	120	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.9
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115
Offset1	-0.3	-65.0
Offset2	0.0	-65.3

Pusher	980	880
TOF (kV)	9.10	-9.25
Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	11363.64	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	
Pirani Pressure(mbar)	4.07e0	
Penning Pressure(mbar)	OFF	
Tof Penning Pressure(mbar)	4.12e-7	

Function Parameters - Function 4 - TOF SURVEY FUNCTION

Set Mass	0.0
Start Mass	50.0
End Mass	2500.0
Start Time (mins)	5.0
End Time (mins)	90.0
Data Format	Continuum
Ion Mode	ES Mode
Polarity	Positive
Instrument Parameter Filename	C:\MassLynx\2009.PRO\ACQUDB\jdwvpos_Q3.ipr
Scans To Sum	16960
Scan Time (sec)	2.4
Interscan Time (sec)	0.1
Use Tune Page Collision Energy	YES
Collision Energy (eV)	434.2
Use Tune Page Cone Voltage	YES
Cone Voltage (V)	35.0

Instrument Parameters - Function 5:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 2	

Capillary	2.40	2.40
Cone	120	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.9
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115
Offset1	-0.3	-65.0
Offset2	0.0	-65.3
Pusher	980	880
TOF (kV)	9.10	-9.25
Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	11363.64	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	
Pirani Pressure(mbar)	4.07e0	
Penning Pressure(mbar)	OFF	
Tof Penning Pressure(mbar)	4.12e-7	

Function Parameters - Function 5 - TOF SURVEY FUNCTION

Set Mass	0.0
Start Mass	50.0
End Mass	2500.0
Start Time (mins)	5.0
End Time (mins)	90.0
Data Format	Continuum
Ion Mode	ES Mode
Polarity	Positive
Instrument Parameter Filename	C:\MassLynx\2009.PRO\ACQUDB\jdwvpos_Q3.ipr
Scans To Sum	16960
Scan Time (sec)	2.4
Interscan Time (sec)	0.1
Use Tune Page Collision Energy	YES
Collision Energy (eV)	413.8
Use Tune Page Cone Voltage	YES
Cone Voltage (V)	35.0

Instrument Parameters - Function 6:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 2	
Capillary	2.40	2.40
Cone	120	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.9
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115

Offset1	-0.3	-65.0
Offset2	0.0	-65.3
Pusher	980	880
TOF (kV)	9.10	-9.25
Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	11363.64	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	
Pirani Pressure(mbar)	4.07e0	
Penning Pressure(mbar)	OFF	
Tof Penning Pressure(mbar)	4.12e-7	

Function Parameters - Function 6 - TOF SURVEY FUNCTION

Set Mass	0.0
Start Mass	50.0
End Mass	2500.0
Start Time (mins)	5.0
End Time (mins)	90.0
Data Format	Continuum
Ion Mode	ES Mode
Polarity	Positive
Instrument Parameter Filename	C:\MassLynx\2009.PRO\ACQUDB\jdwvpos_Q3.ipr
Scans To Sum	16960
Scan Time (sec)	2.4
Interscan Time (sec)	0.1
Use Tune Page Collision Energy	YES
Collision Energy (eV)	427.8
Use Tune Page Cone Voltage	YES
Cone Voltage (V)	35.0

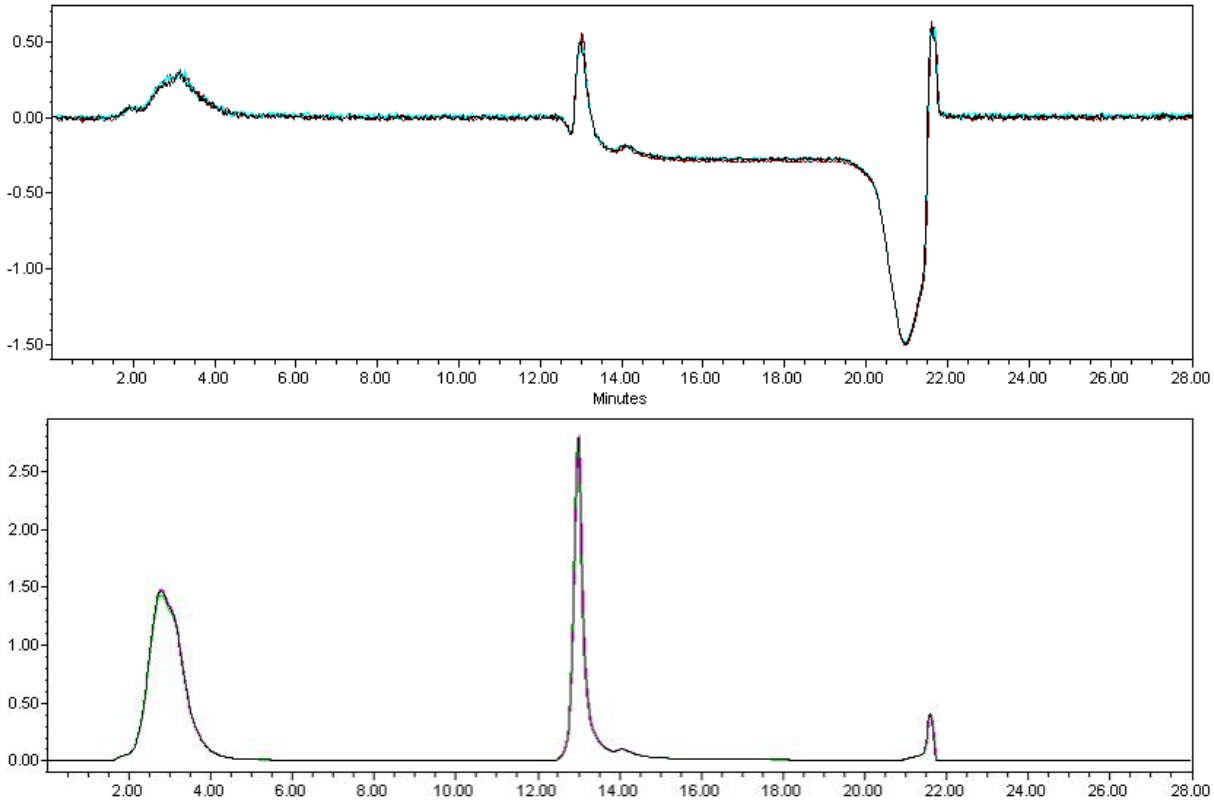
Instrument Parameters - Function 7:	Set point	Actual
Polarity	ES+	
Calibration	Dynamic 2	
Capillary	2.40	2.40
Cone	120	258
RF Lens1 Energy	120.0	
Aperture 1	0.0	
RF Lens2 Energy	1.0	
Source Temp (°C)	80	59
Desolvation Temp (°C)	20	1020
Cone Gas Flow (L/Hr)	0	OFF
Desolvation Gas Flow (L/Hr)	0	OFF
LM Resolution	7.0	
HM Resolution	7.0	
Collision Energy	10.0	10.6
Ion Energy	2.0	
Steering	-0.64	-0.78
Entrance	65.0	-66.2
Pre-filter	6.0	3.8
Transport	4.0	-5.5
Aperture3	10.0	-10.8
Acceleration	200	-197
Focus	0	-2
Tube Lens	110	-115
Offset1	-0.3	-65.0
Offset2	0.0	-65.3
Pusher	980	880
TOF (kV)	9.10	-9.25
Reflectron	35.35	2.08
Pusher Cycle Time (µs)	Auto	
Pusher Frequency (Hz)	11363.64	
Multiplier	650	-648
MCP	2250	2239
Centroid Threshold	0.0	
Min Points	2.0	
Np Multiplier	0.70	
Resolution	4000.0	
Lock Mass	0.0000	
Mass Window +/-	1.0000	
Lteff	1807.8000	
Veff	9100.0000	
	1.0000	
TDC Start (mV)	700.0000	
TDC Stop (mV)	35.0000	
TDC Threshold	0.0000	

Pirani Pressure(mbar)	4.07e0
Penning Pressure(mbar)	OFF
Tof Penning Pressure(mbar)	4.15e-7

Function Parameters - Function 7 - TOF SURVEY FUNCTION

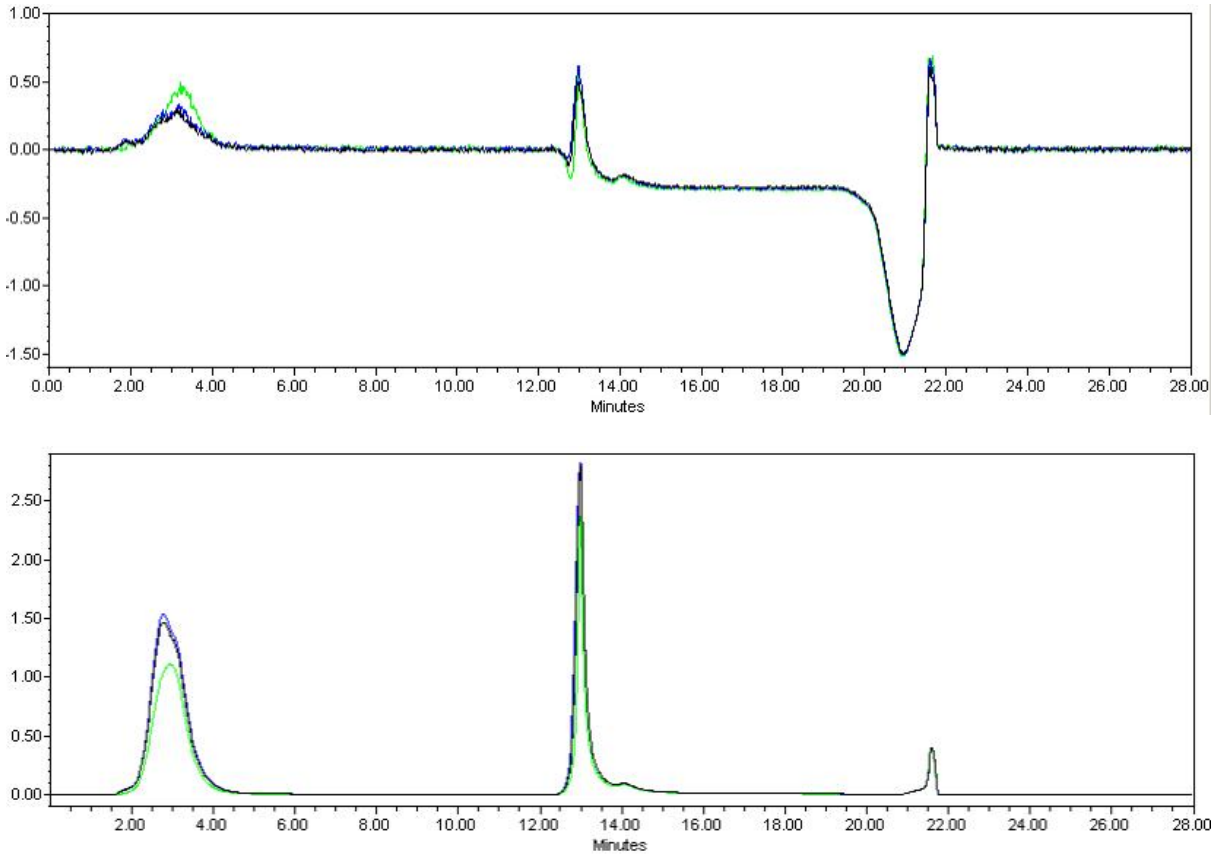
Set Mass	0.0
Start Mass	50.0
End Mass	2500.0
Start Time (mins)	5.0
End Time (mins)	90.0
Data Format	Continuum
Ion Mode	ES Mode
Polarity	Positive
Instrument Parameter Filename	C:\MassLynx\2009.PRO\ACQUDB\jdwvpos_Q3.ipr
Scans To Sum	16960
Scan Time (sec)	2.4
Interscan Time (sec)	0.1
Use Tune Page Collision Energy	YES
Collision Energy (eV)	589.3
Use Tune Page Cone Voltage	YES
Cone Voltage (V)	35.0

Depletion repeatability:



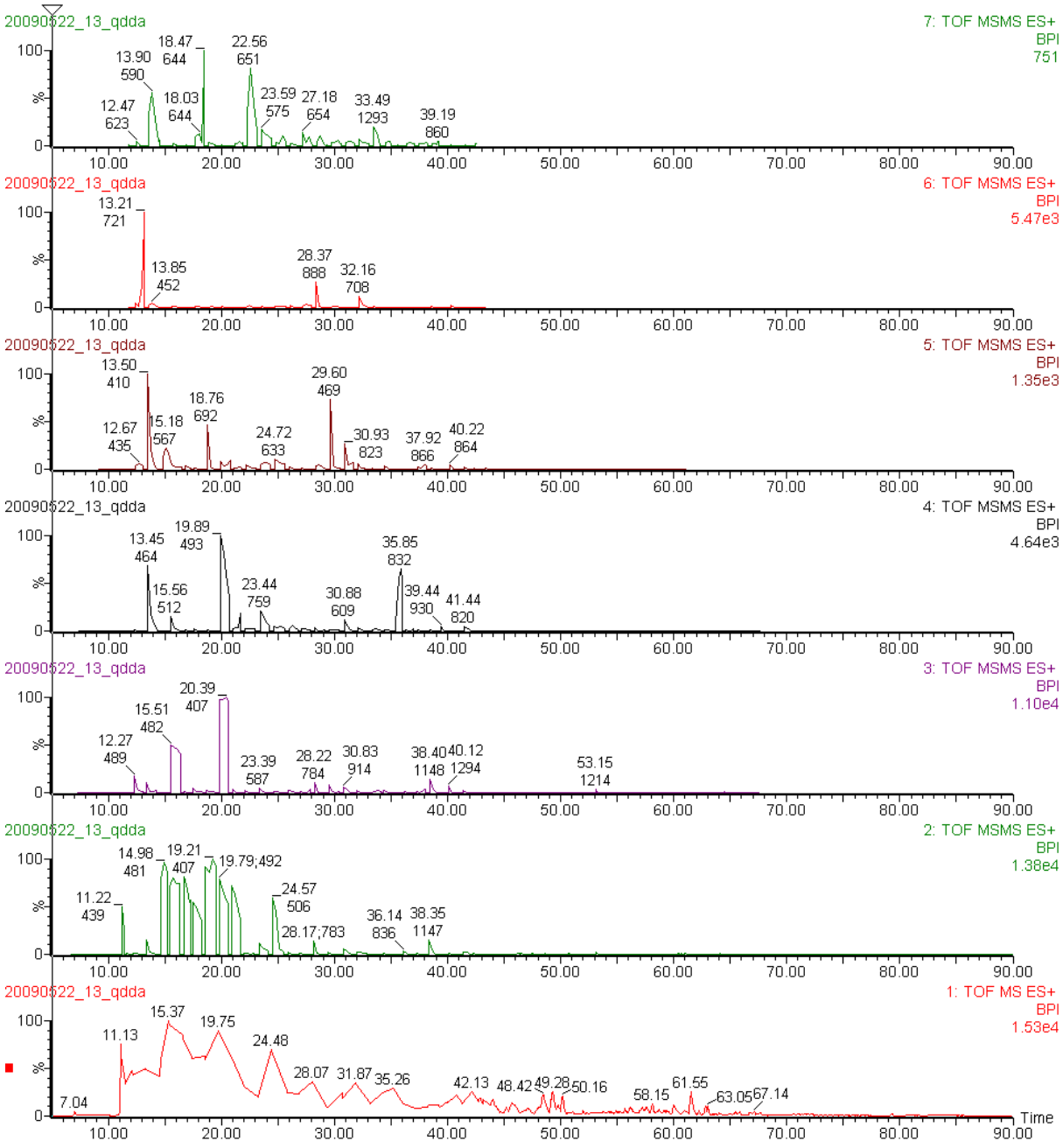
Overlaid depletion chromatograms at absorbance wavelength 204 (upper chromatogram) and at 280 (lower chromatogram) from three consecutive plasma high abundance protein depletion runs of one patient arterial sample. Each run is represented as different color. Low-to-medium abundance proteins collected from 1.5min to 5 minutes

Sample to sample comparison in depletion runs:



Overlaid depletion chromatograms at absorbance wavelength 204 (upper chromatogram) and at 280 (lower chromatogram) from plasma high abundance protein depletion runs of one patient arterial, portal and hepatic samples. Arterial sample in black, portal in blue and hepatic in green. Low-to-medium abundance proteins collected from 1.5min to 5 minutes.

Example MS chromatogram:



Representative MS-chromatogram of one SCX fraction of one iTRAQ experiment. The six MSMS functions are represented in upper five chromatograms and the MS survey scan in the bottom chromatogram. All chromatograms are represented as base peak intensity values.