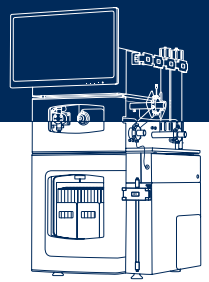
Purification of peptide (1927g/moL) during a live demonstration

Application Note #076



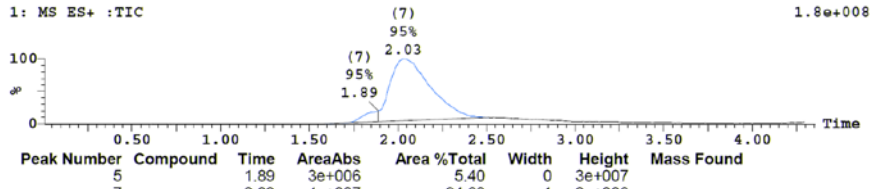
1. Analytical conditions

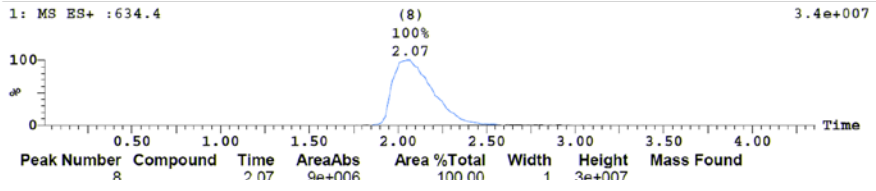
Solvents: A: Water + 0.1% Formic acid
B: Acetonitrile

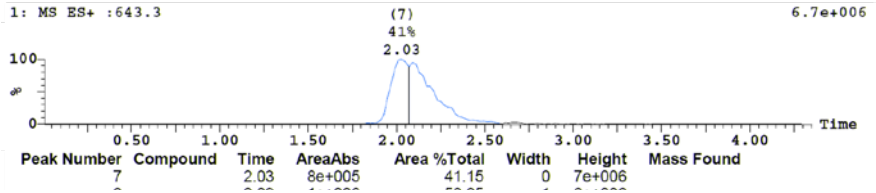
HPLC Column: Onyx Monolithic C18 50x2mm
Flow rate: 0.6mL/min to 1.2mL/min
Injection mode: Liquid
Injection volume: 5µL
Concentration: 1mg/mL
Detection: MS TIC = 100-1200m/z
Mass XIC1: 634,4m/z
Mass XIC2: 643,3m/z
Mass XIC3: 964,5m/z
UV: 254nm

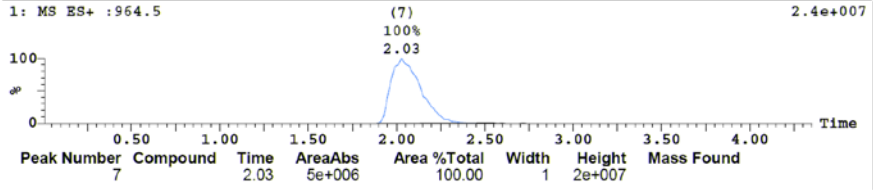
Elution conditions:

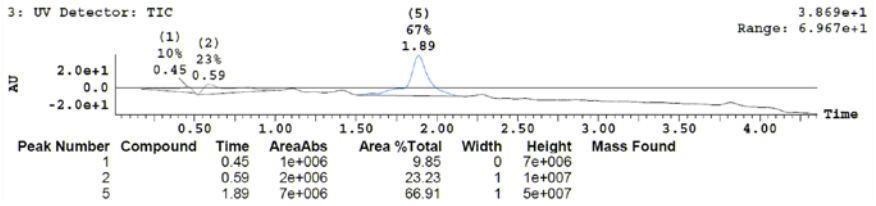
t (min)	A (%)	B (%)
00:00	95	5
03:48	0	100
03:49	0	100
04:00	0	100
04:04	95	5
04:12	95	5
04:18	95	5

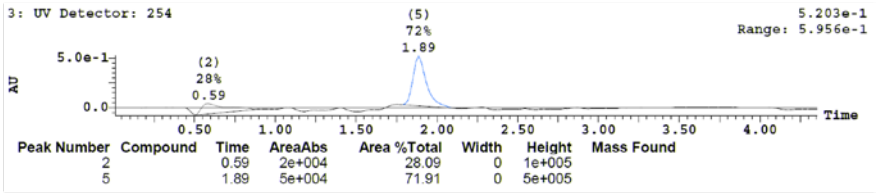






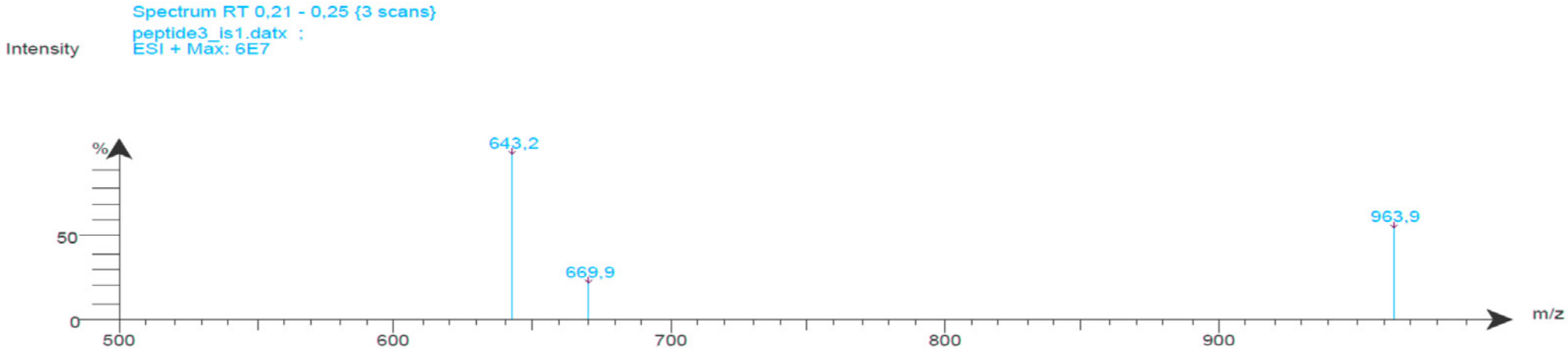






2. Direct mass injection of crude sample

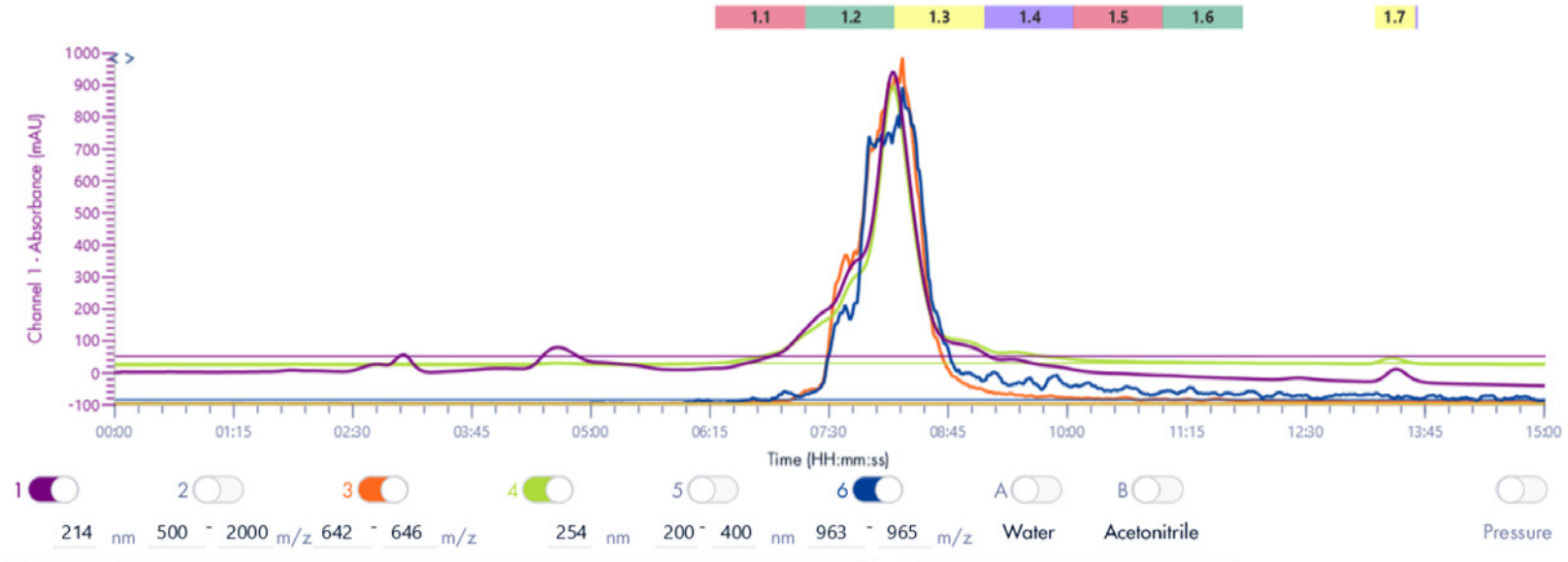
Source type: ESI
Mode: Positif
Capillary temperature: 200°C
Capillary voltage: 160V
Source voltage offset: 30V
Source voltage span: 5V
Source gas temperature: 250°C
ESI Voltage: 3500V
Mass of the spectrum: 643.2 $[M + 2H^+]/2$
669.9$[M + 3H^+]/3$
963.9 $[M - N_2 + 3H^+]/3$



3. Prep conditions

Device: puriFlash® 5.250P + MS
Solvents: A: Water + 0.1% FA
B: Acetonitrile
Column: PFB5C18T-150/212
Flow rate: 16mL/min
Injection mode: Liquid
Injection volume: 1mL
Concentration: 25mg/mL
Crude sample: 25mg
Detection: UV 214nm (Purple),
UV 254nm (Green)
Mass XIC 644m/z (Orange),
Mass XIC 964m/z (Blue)
MS split parameters
Valve rotation: 0.5s
Make-up flow-rate: 0.5mL/min
Dilution flow-rate: 0.5mL/min
Solvents for make-up and dilution:
Acetonitrile + 0.1%FA
Pressure: 60bar
Elution conditions:

t (min)	A (%)	B (%)
00:00	95	5
20:00	0	100



To achieve this purification:

You will need

- puriFlash® 5.250P
  Discover it Add to card
- puriFlash® MS
  Discover it Add to card
- puriFlash® column PFB5C18T-150/212
  Discover it Add to card

We highly recommend

- Uptidisc PTFE 1,3mm 0,45µm PP synringe filter
  P00530 Add to card
- Manometer
  B4VBK0 Add to card
- Safety solvent caps kit
  B0DAN0 Add to card