Enhanced Sensitivity Using A Direct Flow Capillary HPLC/MS System with Nanoflow Capability

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Overview

- The sensitivity of a direct flow capillary LC/MS system was examined by comparing separations achieved with:
  - 75 and 180 micron i.d. columns
  - flow rates ranging from 400 nl/min to 2 µl/min
  - nanospray and microspray (standard ESI interface fitted with a 60 µm i.d. capillary) ESI interfaces

- An integrated sample focusing configuration was used in the analyses to:
  - concentrate low amounts of sample on a precolumn
  - reduce the gradient delay time associated with low flow rates by minimizing the system volume

Introduction

- The need for high sensitivity separations in sample limited applications has driven the development of systems that couple capillary LC with MS

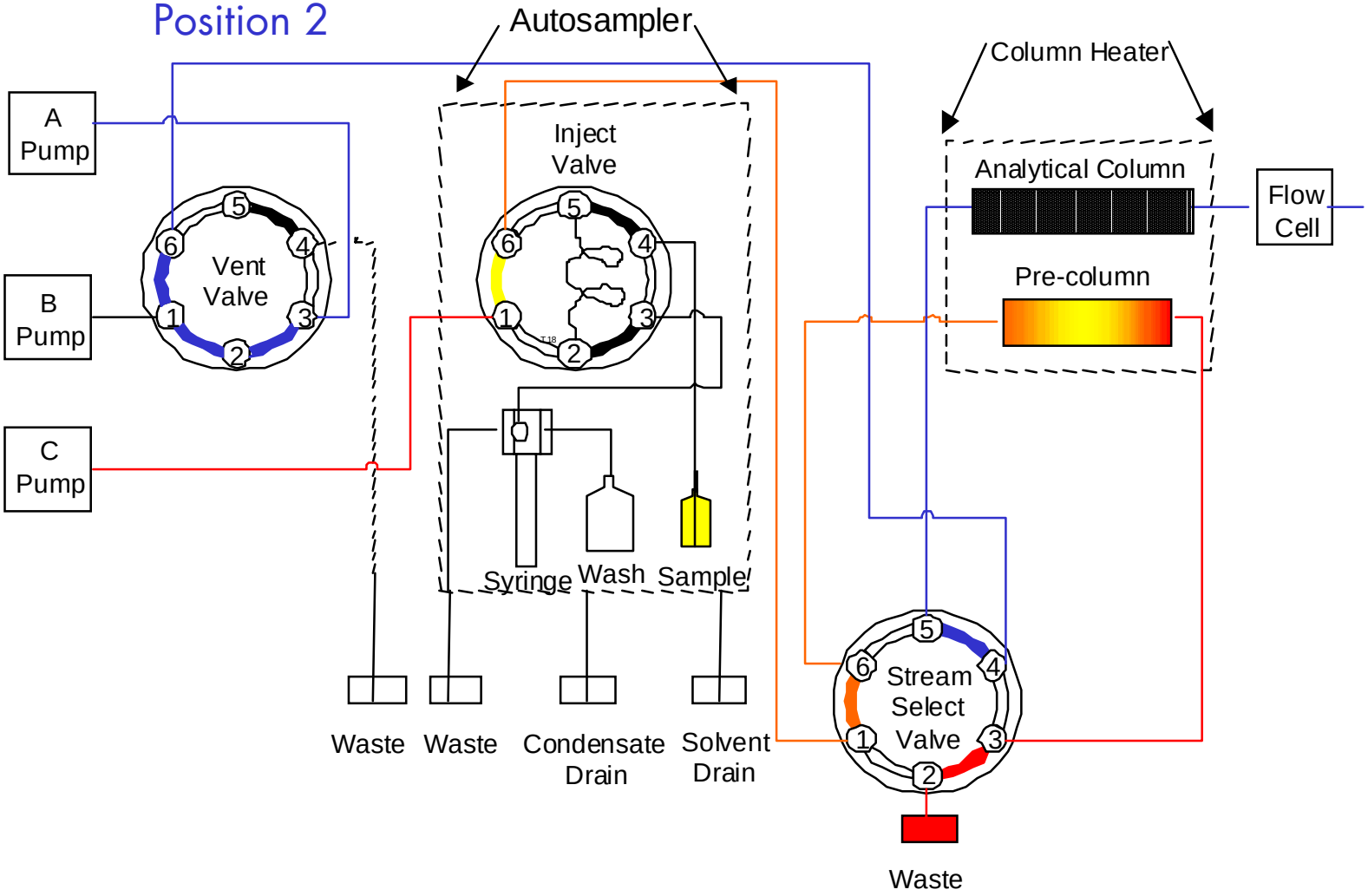
- To increase sensitivity, the trend has been to:
  - use very small internal diameter columns
    - minimizes sample dilution on column
  - scale down flow rates
    - improves ionization efficiency and throughput
  - incorporate nanoflow ESI interfaces on MS/MS systems
    - minimizes band broadening in the interface

- In this study, we used a direct flow capillary LC/MS system operating at low flow rates with various columns and ESI interfaces to maximize sensitivity

Sample Focusing

Sample Loading

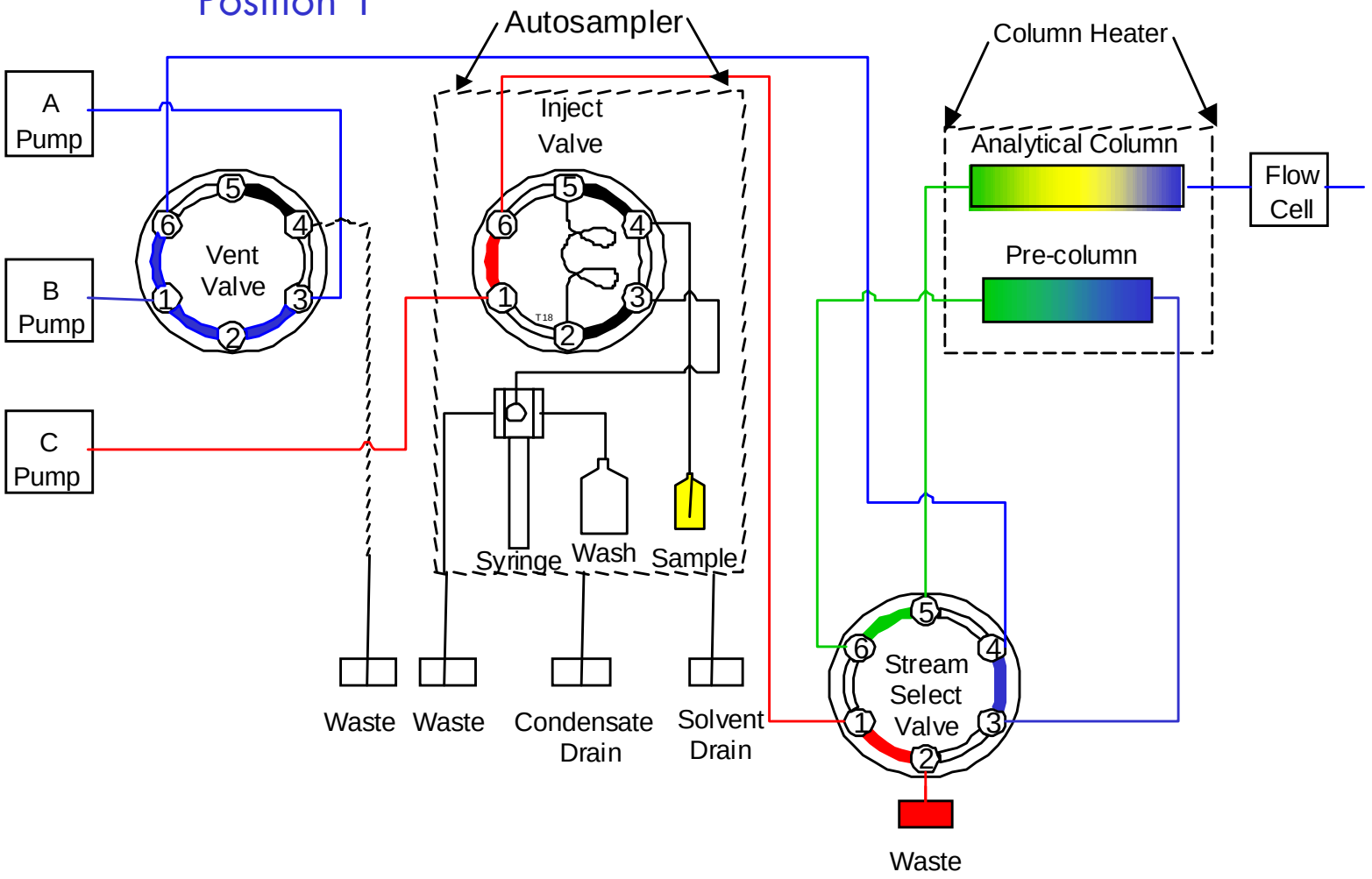
Position 2



Sample is loaded onto the precolumn at a high flow rate using Pump C. Using an integrated six position valve, the sample is trapped on the precolumn, and the loading solvent is directed to waste. Once the sample has been loaded, the stream select valve changes positions and the sample is eluted from the precolumn with the gradient formed by Pumps A and B as shown below. In this instance, the pre-column is backflushed.

Sample Separation

Position 1

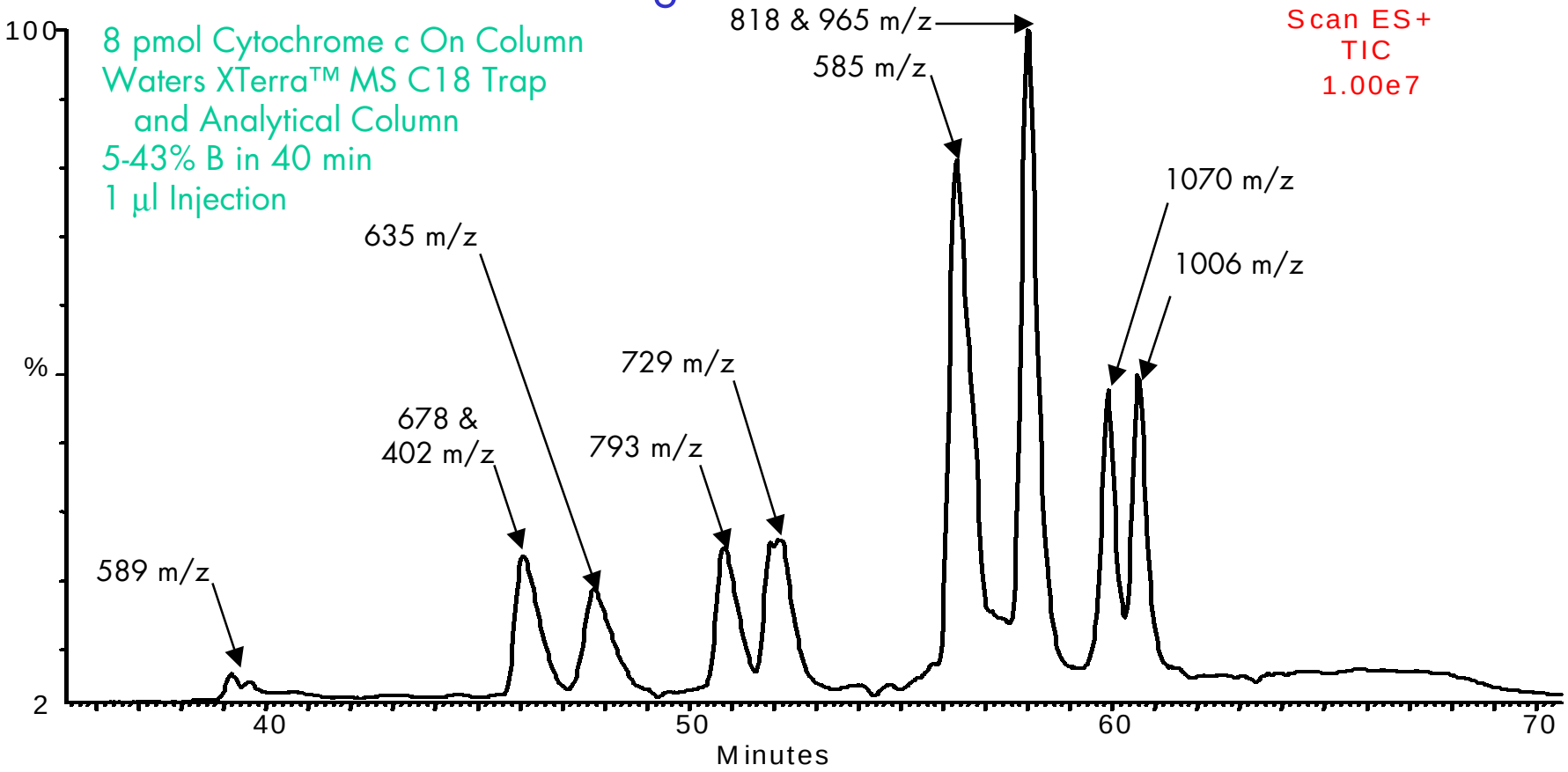


As the sample elutes from the pre-column, it is carried into the analytical column where separation occurs. By plumbing Pumps A and B into the stream select valve and by-passing the injection valve and loop, the system volume is significantly decreased which reduces the gradient delay time.

Experiments with 75 Micron Column

400 nl/min Flow with Nanospray ESI

Total Ion Current Chromatogram

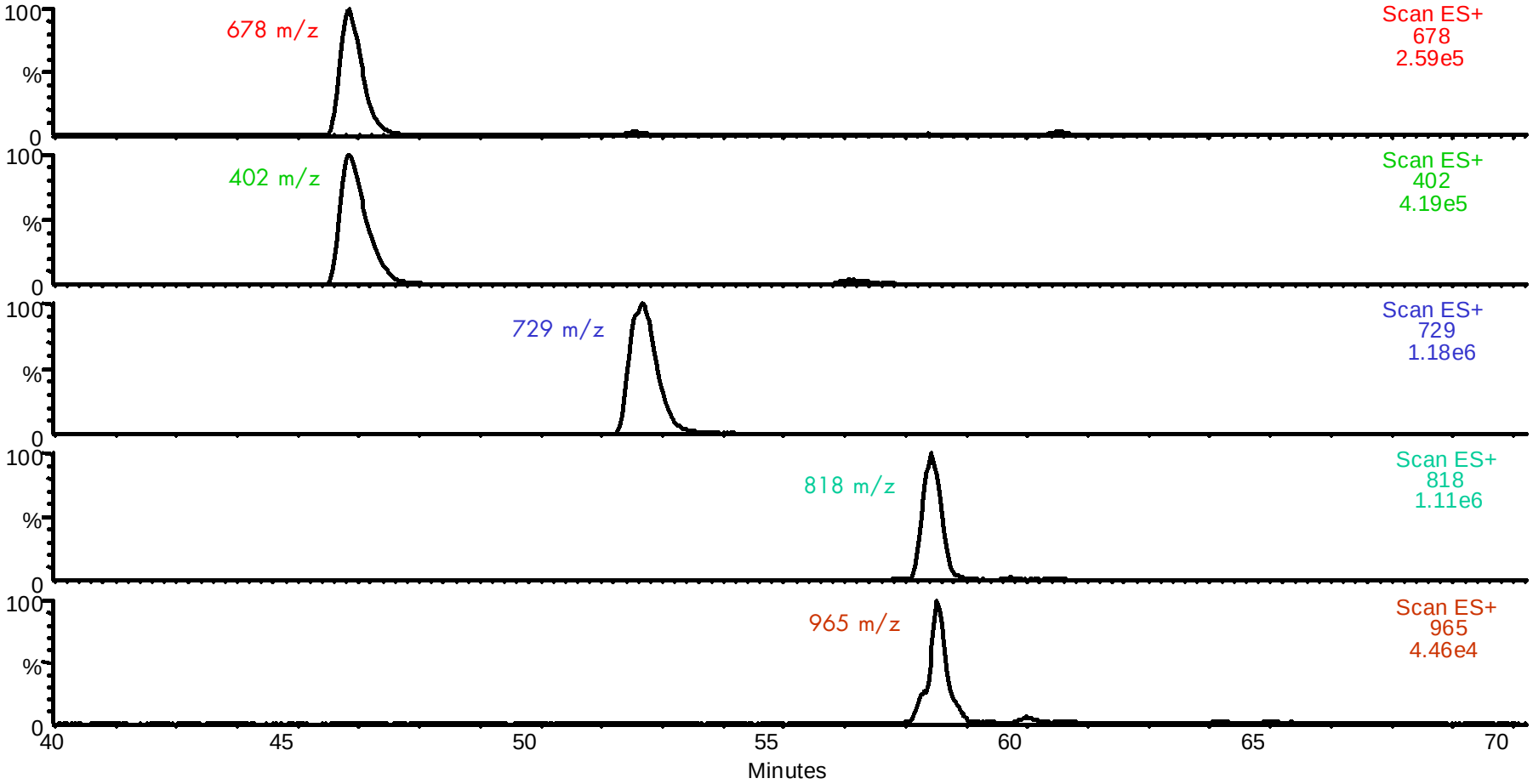


The total ion current chromatogram shown here displays the sensitivity and resolution possible with separations performed on 75 µm columns with nanoliter flow rates. By replacing the more commonly used 320 µm capillary column with a 75 µm column, the column cross sectional area is reduced by a factor of approximately 18. Theoretically, this reduction should translate into an 18-fold enhancement in sensitivity. This improvement in sensitivity is the factor that is enticing to chemists and is driving them toward separations on smaller columns.

400 nl/min Flow with Nanospray ESI

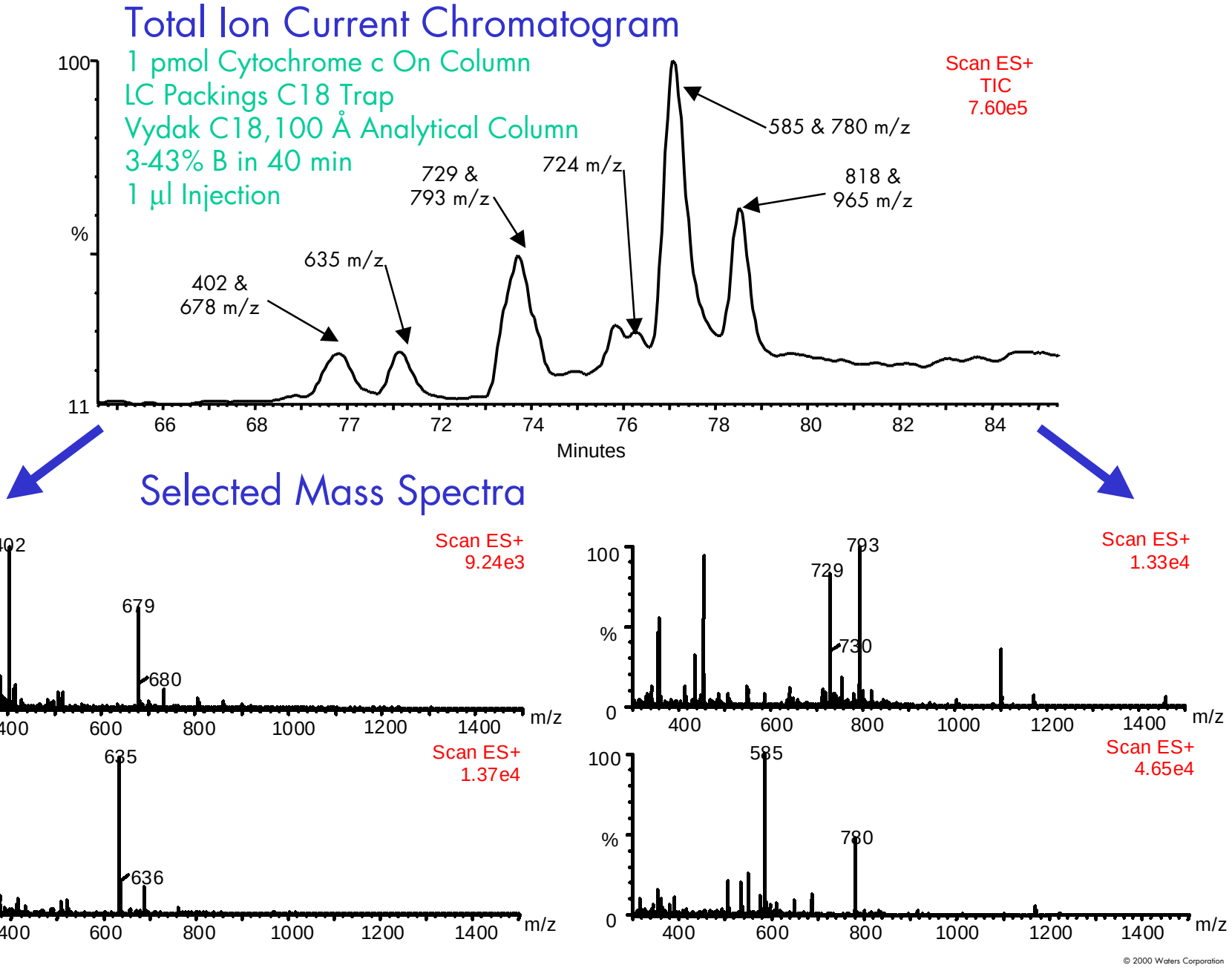
Selected Extracted Mass Chromatograms

8 pmol Cytochrome c On Column



The ability to extract masses from a total ion current chromatogram allows for the resolution of coeluting peaks as shown above. While complete chromatographic resolution is favorable to minimize ion suppression in the source, peak resolution for identification and qualitative analysis of particular ions can be achieved by extracting masses of interest.

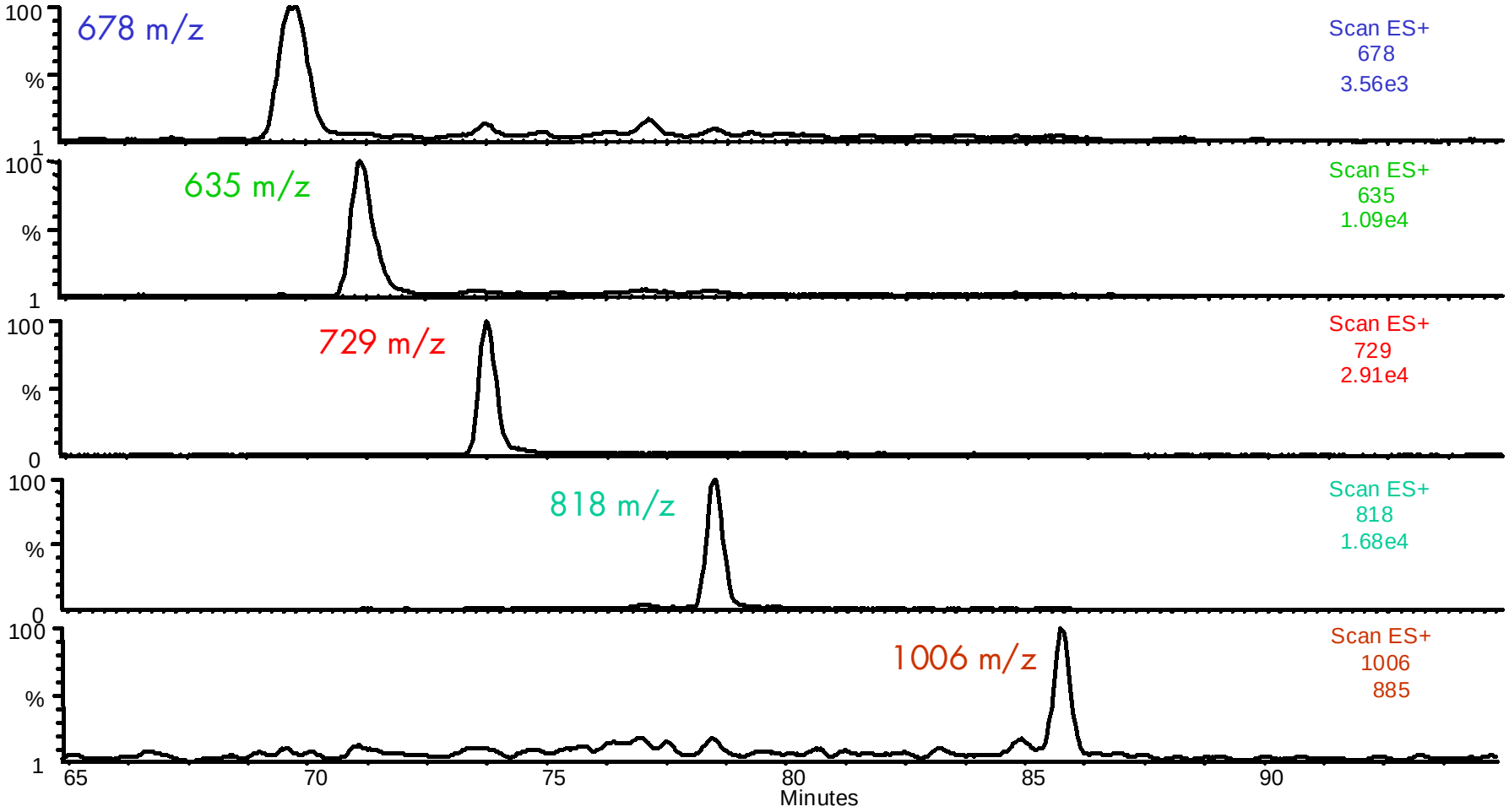
400 nl/min Flow with Nanospray ESI



400 nl/min Flow with Nanospray ESI

Selected Extracted Mass Chromatograms

1 pmol Cytochrome c On Column



Although limit of detection studies were not performed in these initial experiments, it is estimated that the limit of detection for the majority of the peaks is 200 fmol or less. This detection limit could be further improved with detection by MS/MS.

General Experimental LC/MS Conditions
75 Micron Column with Nanospray ESI

Chromatographic Conditions
Instrument: Waters® CapLC™ System
Trapping Column: 0.30x5 mm C18, 5 µm
Analytical Column: 0.075x150 mm C18, 5 µm
Mobile Phase A: 0.01% TFA in MilliQ Water
Mobile Phase B: 0.0085% TFA in MeCN
Loading Solvent: 0.1% TFA in MilliQ Water
Loading Flow Rate: 20 µl/min
Gradient Flow Rate: 400 nl/min
Gradient Slope: 3-43 and 5-43% B in 40 minutes

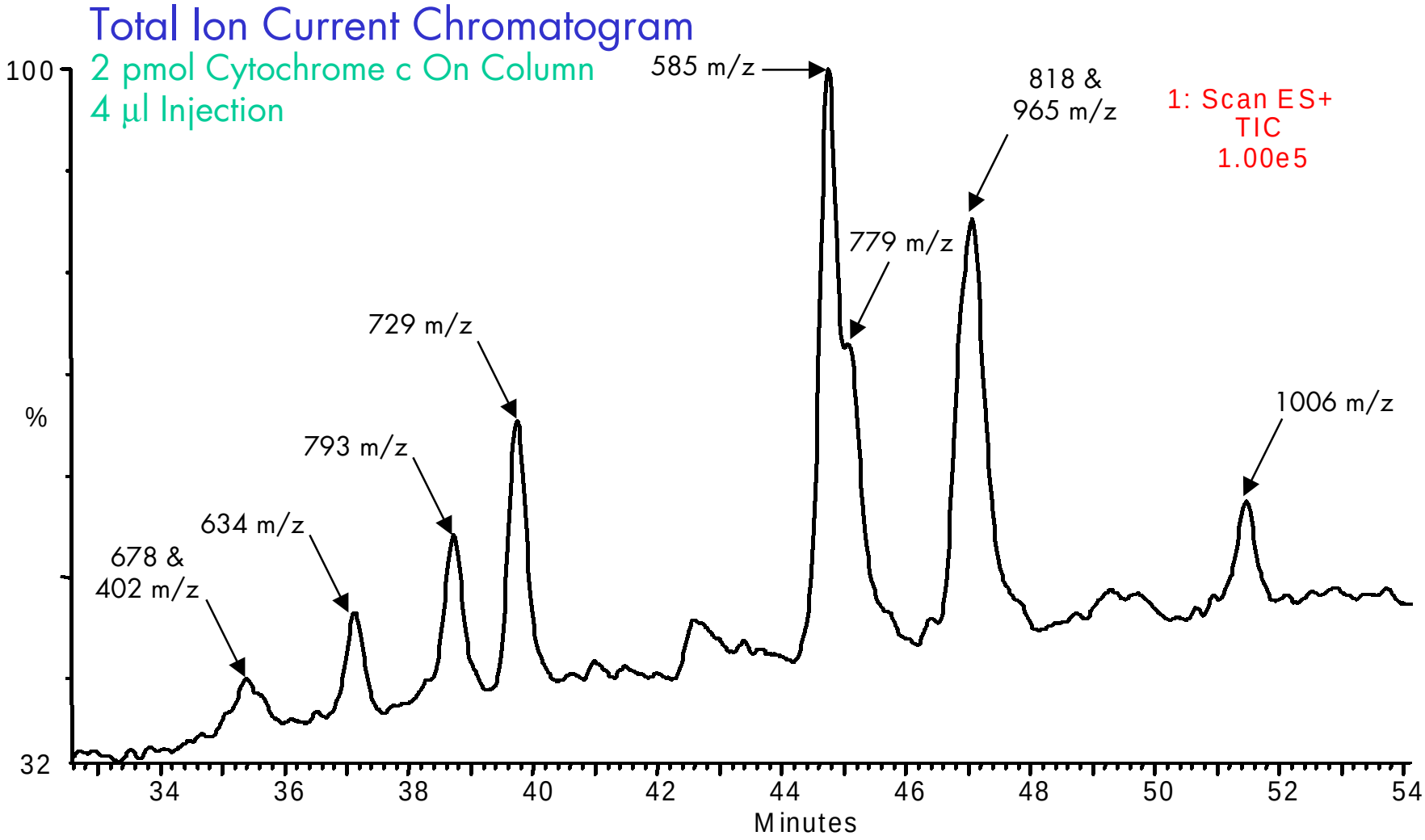
Sample Information
Sample: Tryptic Digest of Bovine Cytochrome c
Sample Concentration: 1-8 pmol/µl
Injection Volume: 1 µl

MS Acquisition Conditions
Instrument: Micromass Platform LCZ
Nanospray ESI Interface
Positive Electrospray
Centroid Data
Scan Mode: 300-1500 m/z
Scan Time: 1.2-2.4 seconds
Interscan Delay Time: 0.08 seconds

MS Tune Page Settings
Capillary: 3.5-3.6 kV
Cone: 40-45 V
Extractor: 5 V
RF Lens: 0.2-0.8 V
Ion Energy: 0.2 V
LM Resolution: 11.0
HM Resolution: 11.0
Source Block Temperature: 100°C

Experiments with 180 Micron Column

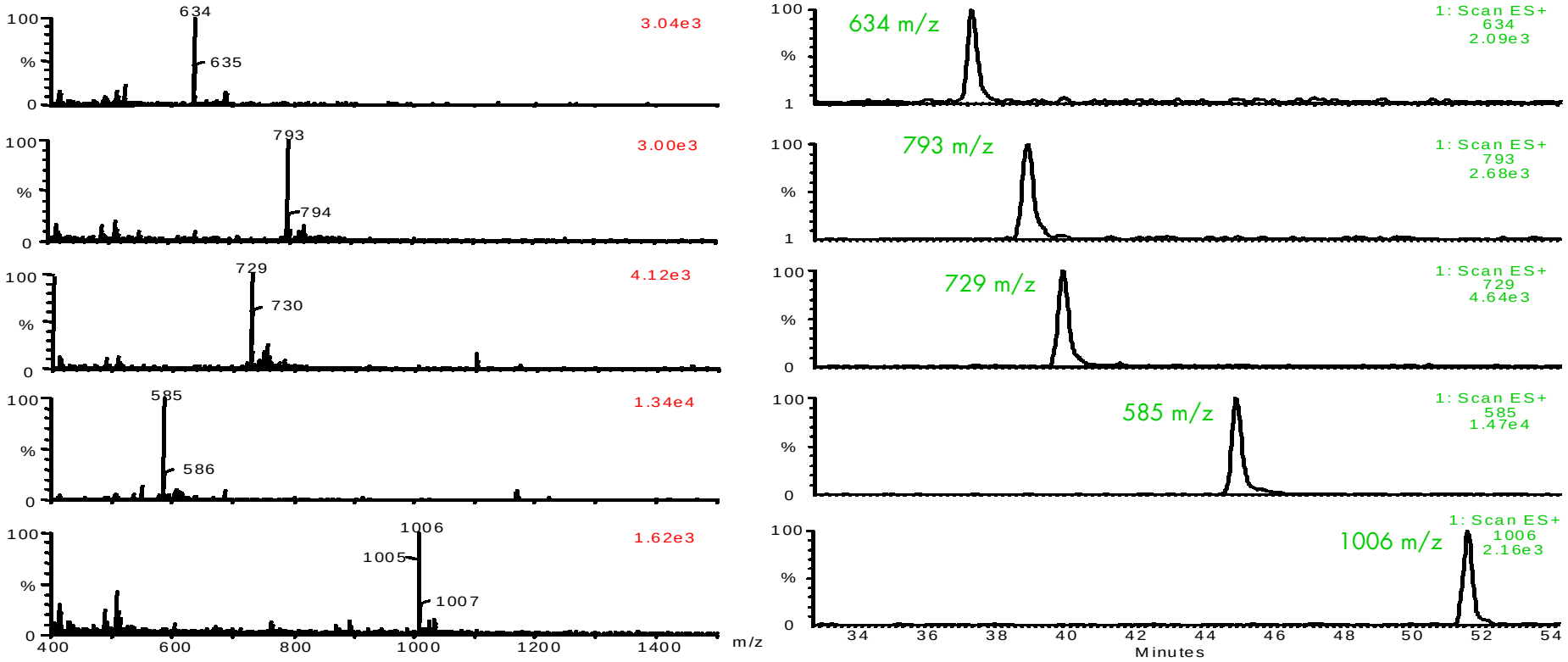
1 µl/min Flow with Nanospray ESI



In this analysis, 4 µl of a 500 fmol/µl sample solution were concentrated on the precolumn and then separated on a 180 µm column. The ability to trap sample on a pre-column could be very useful in situations where large volumes of solution must be loaded to provide an appreciable amount of sample or in instances where on-line sample clean-up must be performed.

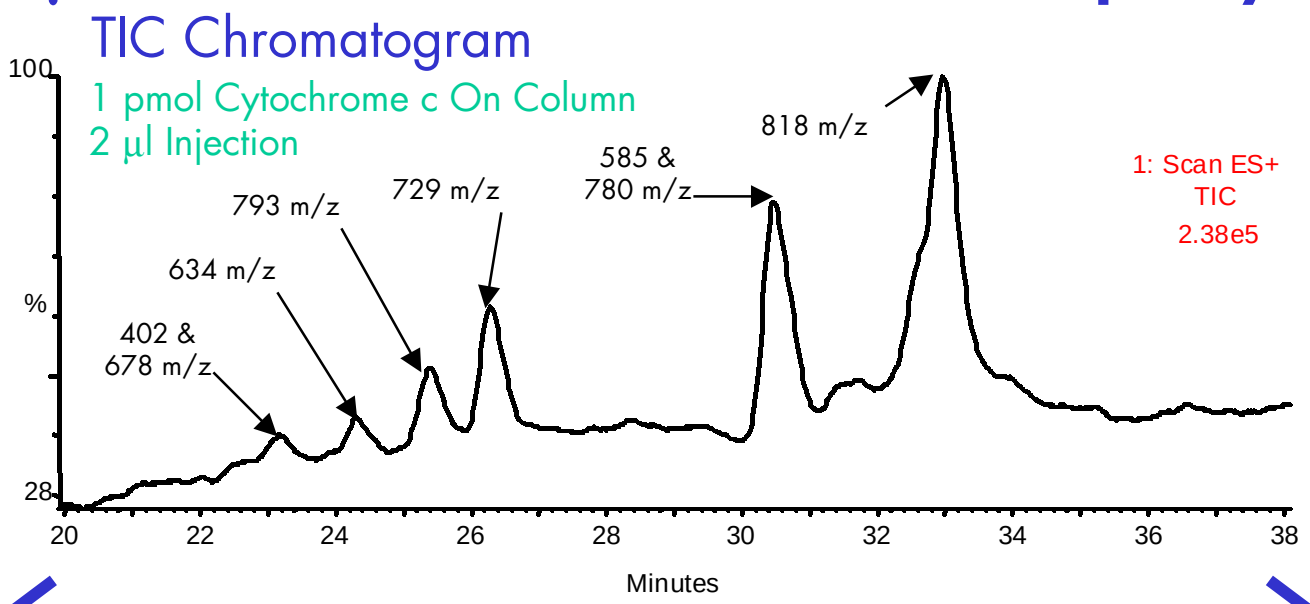
Selected Mass Spectra and Extracted Mass Chromatograms

2 pmol Cytochrome c On Column

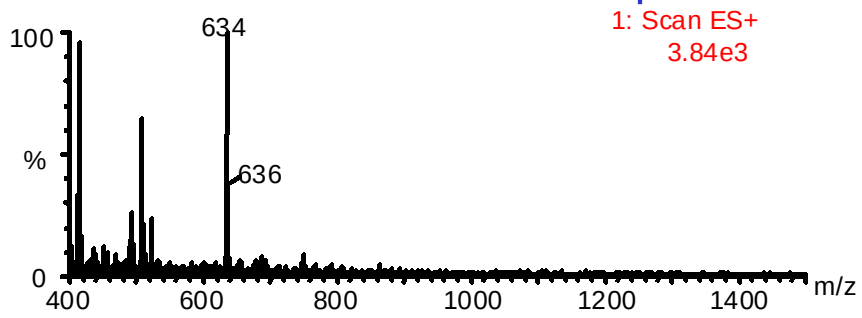


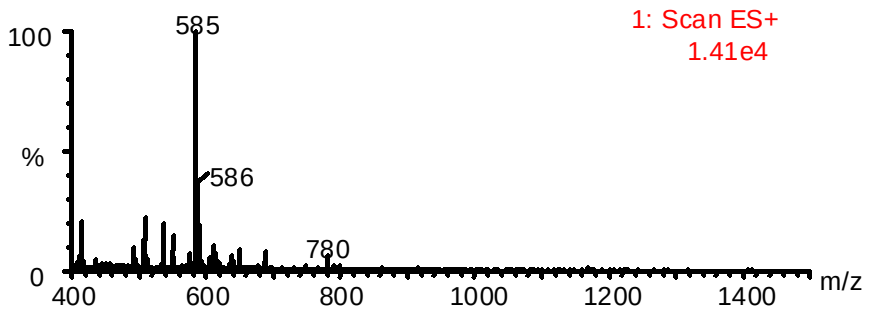
By using a flow rate in the upper range for the nanospray ESI interface, very clean mass spectra and high signal-to-noise extracted mass chromatograms were produced. In addition, the use of a 180 µm column provided a much more robust system than that which used a 75 µm column in terms of flow rate control (leak detection), column equilibration, column packing variability, consistent operating pressure, number of injections per column, and general ease of use. Furthermore, the flow rates used with 180 µm columns permit the use of PDA detection as well as MS detection.

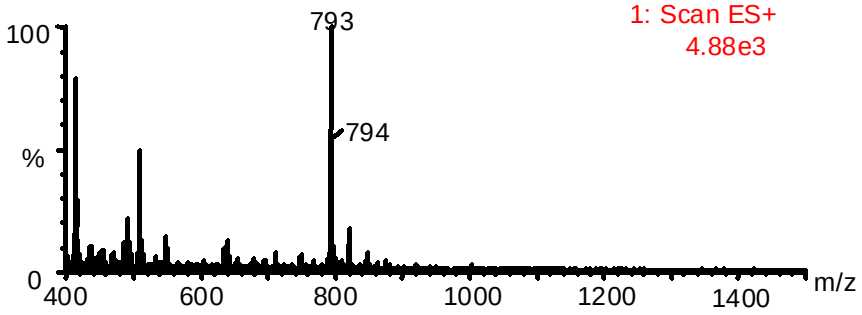
2 µl/min Flow with Microspray ESI

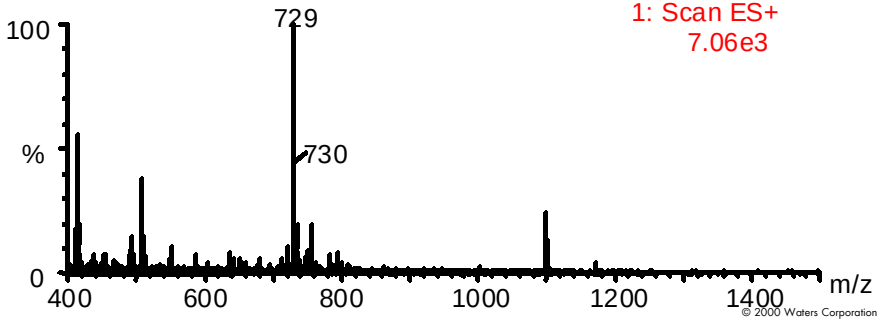


Selected Mass Spectra





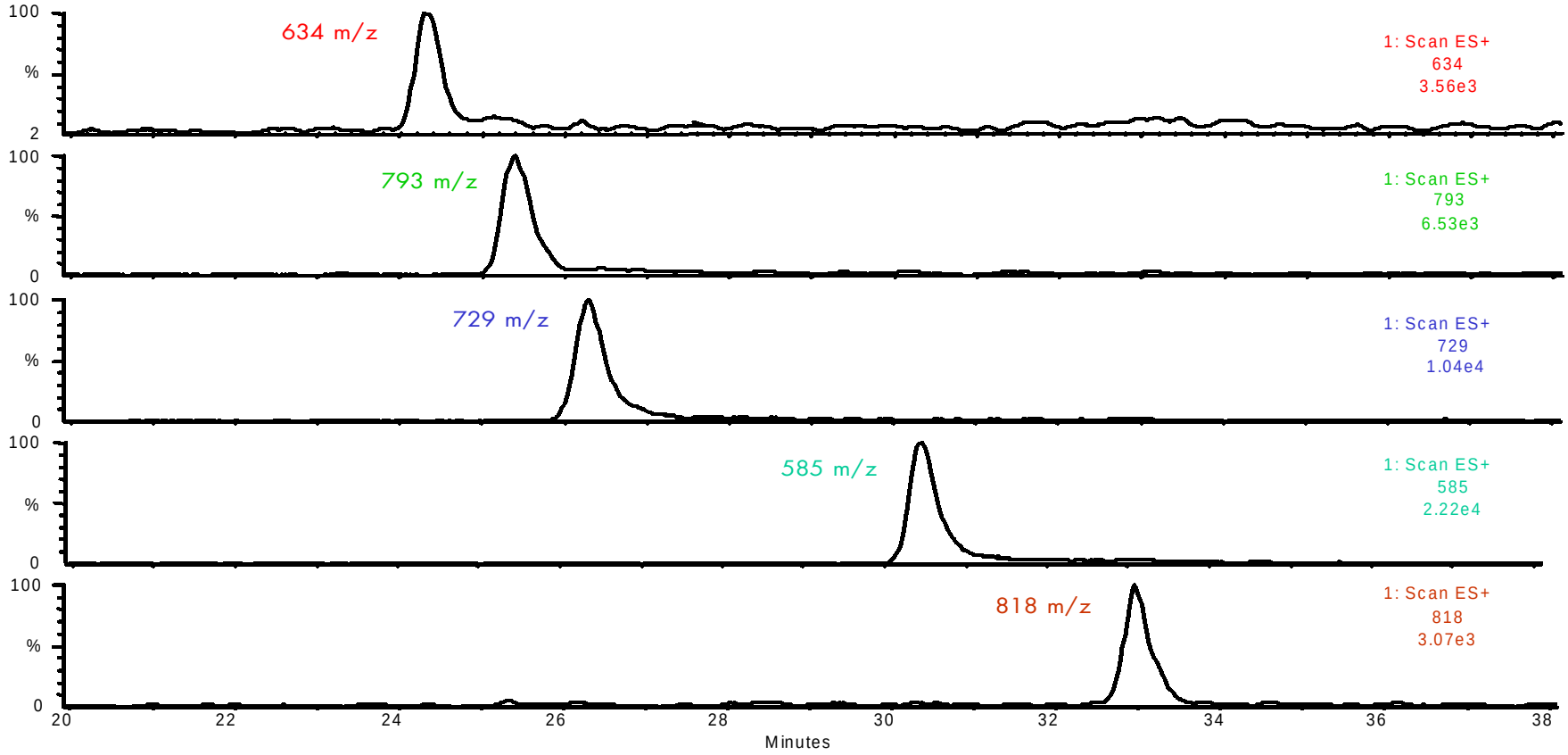




2 µl/min Flow with Microspray ESI

Selected Extracted Mass Chromatograms

1 pmol Cytochrome c On Column



This separation was performed on a 180 µm column with a flow rate of 2 µl/min and a standard ESI source fitted with a 60 µm i.d. capillary (microspray). The increased flow rate decreased the total run time for the analysis by 32 minutes. The use of a microspray ESI interface with a flow rate of 2 µl/min yielded a signal comparable to that given by the nanospray source at 1 µl/min.

General Experimental LC/MS Conditions
180 Micron Column

Chromatographic Conditions

Instrument: Waters® CapLC™ System
Trapping Column: 0.30x5 mm LC Packings C18, 5 µm
Analytical Column: 0.180x150 mm Waters Symmetry C18, 100Å, 5 µm
Mobile Phase A: 0.01% TFA in MilliQ Water
Mobile Phase B: 0.0085% TFA in MeCN
Loading Solvent: 0.1% TFA in MilliQ Water
Loading Flow Rate: 20 µl/min
Gradient Flow Rate: 1-2 µl/min
Gradient Slope: 3-43% B in 40 minutes

Sample Information

Sample: Tryptic Digest of Bovine Cytochrome c
Sample Concentration: 500 fmol/µl
Injection Volume: 2-4 µl

MS Acquisition Conditions

Instrument: Micromass Platform LCZ
Nanospray and Standard (with 60 µm capillary) ESI Interfaces
Positive Electrospray
Centroid Data
Scan Mode: 400-1500 m/z
Scan Time: 2.4 seconds
Interscan Delay Time: 0.08 seconds

MS Tune Page Settings

Capillary: 3.5 kV
Cone: 45 V
Extractor: 5 V
RF Lens: 0.2 V
Ion Energy: 0.2 V
LM Resolution: 11.0
HM Resolution: 11.0
Source Block Temperature: 100°C

Peaks of Interest for Bovine Cytochrome c Tryptic Digest

Peak	#AA	Other		MW	MW/2	MW/3	Observed	AA Sequence
1	5	acetyl	41.0	588.3	294.1	196.1	589	GNVEK
2	6			678.4	339.2	226.1	679	YIPGTK
3	7			807.5	403.7	269.2	402	KYIPGTK
4	5			634.4	317.2	211.5	635	IFVQK
5	15			1585.8	792.9	528.6	793	KTGQAPGFSYTDANK
6	14			1456.7	728.3	485.6	729	TGQAPGFSYTDANK
7	6			722.3	361.2	240.8	724	EDLIAY
8	7			779.4	389.7	259.8	780	MIFAGIK
9	11			1168.6	584.3	389.5	585	TGPNLHGLFGR
10	9	heme	614.5	1633.0	816.5	544.3	818	CAQCHTVEK
11	8			964.5	482.3	321.5	965	EDLIAYLK
12	18			2139.0	1069.5	713.0	1070	GITWGEETLMEYLENPKK
13	17			2009.9	1005.0	670.0	1006	GITWGEETLMEYLENPK

Conclusions

- A direct flow capillary LC system can be used for gradient solvent delivery to columns as small as 75 microns in internal diameter
- 75 and 180 micron columns can be used to enhance sensitivity when used with nanospray ESI interfaces
- Given the high quality of data produced by the 180 μm column with a nanospray interface and the column's ease of use, 180 micron columns may be a very favorable alternative to 75 micron columns for sensitivity enhancement
- Separations can be accelerated, while still enhancing sensitivity, by operating at microliter per minute flow rates on a 180 micron column with a microspray ESI interface
- A large volume of sample can be loaded quickly onto a pre-column using an integrated trapping configuration