

Application News

Liquid Chromatography Mass Spectrometry

SSI LCMS 001

LCMS Sample Evaluation Report

Roark Galloway & Miguel Gallego

Introduction

Two samples were submitted to the Shimadzu Southern California (SCA) regional laboratory to demonstrate chromatographic performance and LCMS analysis for components of those samples. The samples were prepared and provided by representatives from CONFIDENTIAL Research Laboratories for this evaluation. The samples were identified as:

- 1) Test mix (5 components)
- 2) Sample ID- A001



OBJECTIVE - Attain resolution of 5 components from test mix in a total method runtime of 2 minutes or less, including MS spectral peaks.

Materials and Methods

System Hardware: The complete LCMS system is comprised of the following components:

CBM-20A:	Prominence communications module
LC-20:	Reservoir tray
LC-20 A3:	Prominence on-line degasser
LC-20ADXR:	Prominence pumps [2], (A=0.05% Formic/H ₂ O; B = ACN) binary gradient elution [BGE] system with 20uL mixer
SIL-20AXR:	Prominence UFLCXR autosampler
CTO-20A:	Prominence column oven
SPD-M20A:	Prominence diode array detector
LCMS-2020:	LCMS with electrospray [ESI]
LCMSsolution:	LCMS system control software, version 5.10.153
Open Solution:	Walk-up user interface software, version 1.0.29.0
Columns:	50mm X 2.0mm ID X 2.5u Phenomenex LUNA C18 HST 75mm X 2.0mm ID X 2.2u Shimadzu ODS, XR-II
Data Station:	Dell Windows XP Professional, SP3

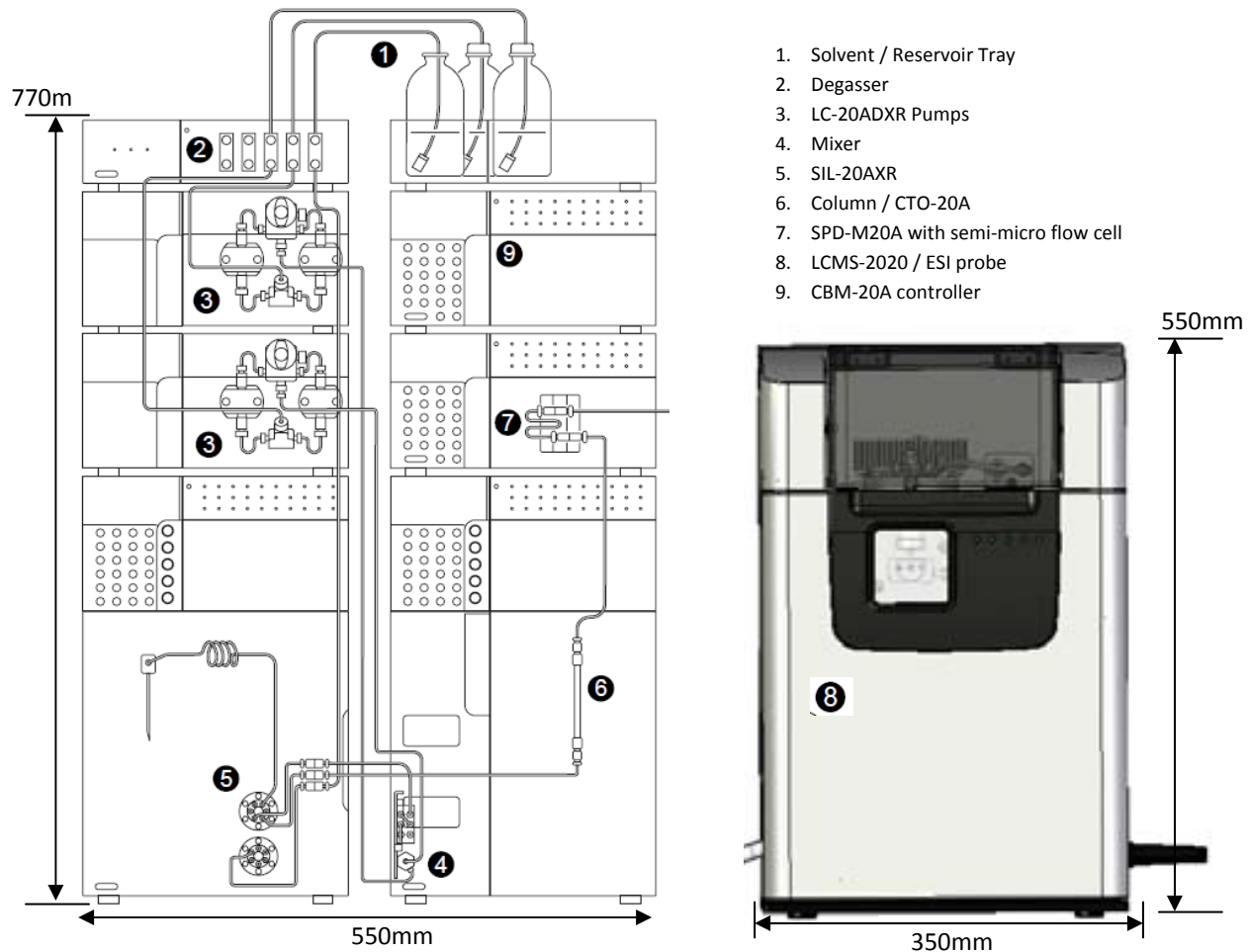


Figure 1: LCMS System Hardware Configuration

Method Settings: General method settings used for data acquisition:

LCMS 2020:	Scan 140-1000Da / Rate = 10000 Da sec ⁻¹
Data Acquire:	2 minutes / PDA = 40Hz / 200-300nm
Column Oven:	40°C
Flow Inlet to MS:	Approx 50% passive split after PDA
System Mode:	Pumps = XR mode / CBM = FastLC mode
Initial Pump:	1.00mL min ⁻¹ / Pmax = 9000psi / 5%B
LC Time Program:	Refer to FIGURE 2

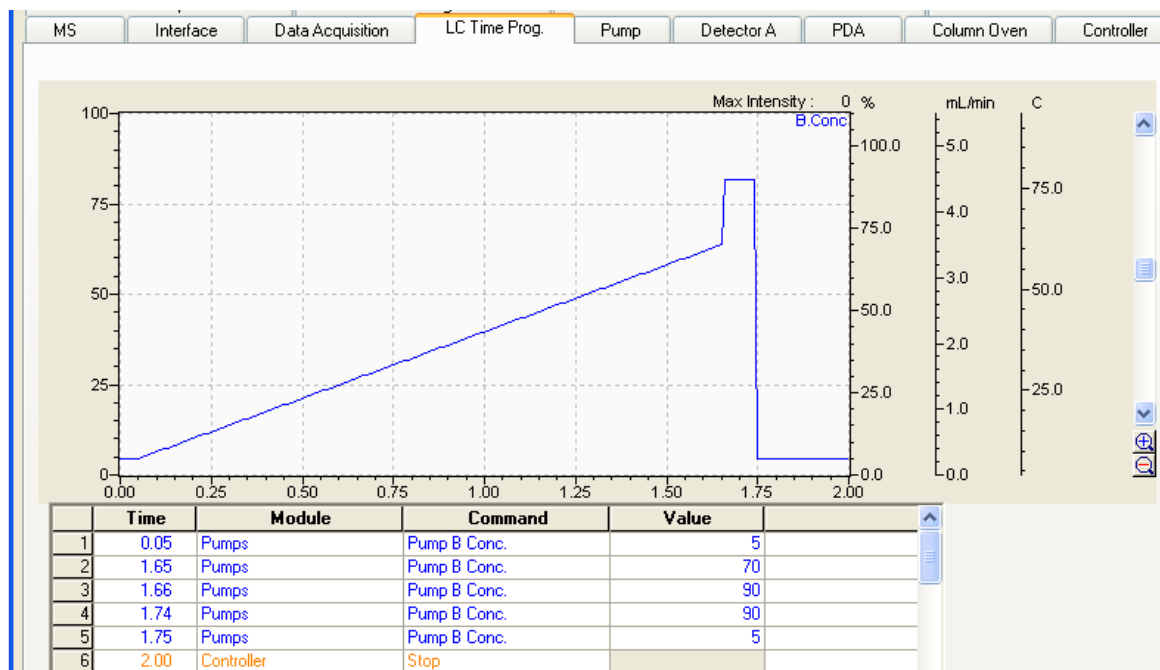
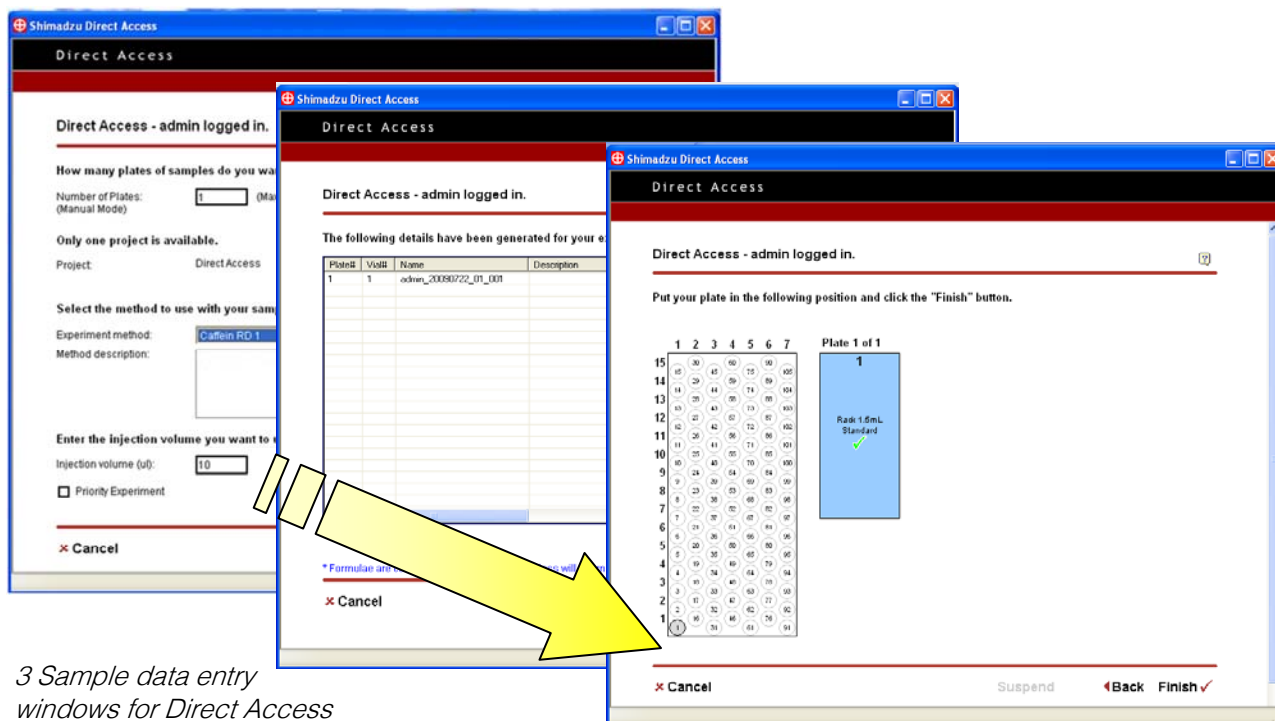


Figure 2: LC Time Program

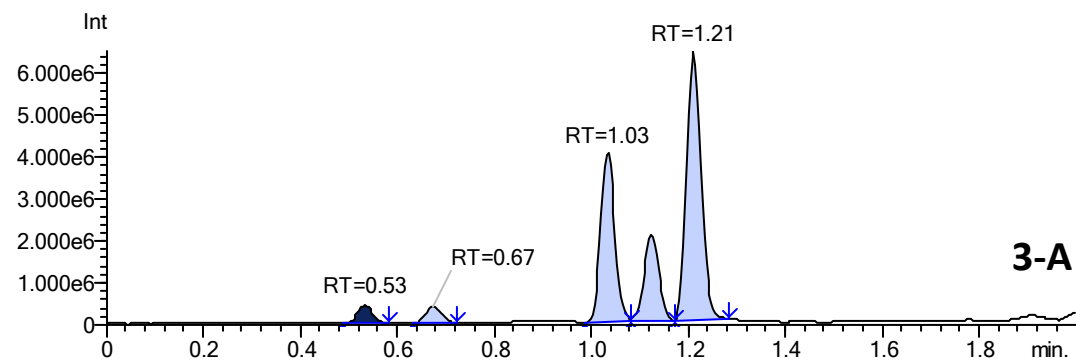
Results

- 1) CONFIDENTIAL Test mix was provided, scanned and analyzed in both (+) and (-) scan mode.
- 2) 1.0 uL of sample was injected per each analysis.
- 3) LCMS detector gain was set to 1.0kV to accommodate response of largest MS peak.
- 4) Using the 50mm 2.5 micron column, **all 5 peaks eluted at retention times representing the expected masses of 152, 195, 295, 311, and 472.4 m/z in under 1.3 minutes with a total run time of under 2 minutes.** TIC chromatograms for simultaneous POS/NEG ionization acquisition and spectra are presented in Figures 3-6. Results for CONFIDENTIAL sample #2 are shown in Figure 7.
- 5) Using the 75 mm, 2.2 micron column, **all 5 peaks eluted at retention times in less than 3 minutes under high-pressure conditions** with increased resolution for some peaks on the longer column (Figure 8 and Figure 9).



3 Sample data entry windows for Direct Access

Group#1 Scan(+) EI : TIC



Group#2 Scan(-) EI : TIC

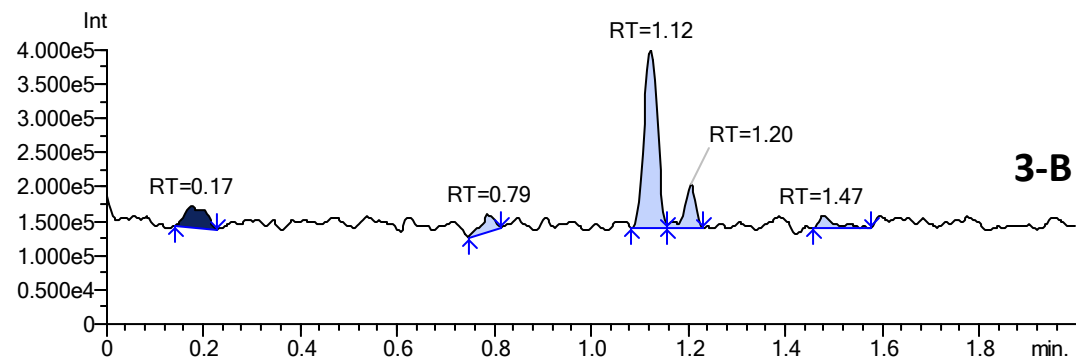
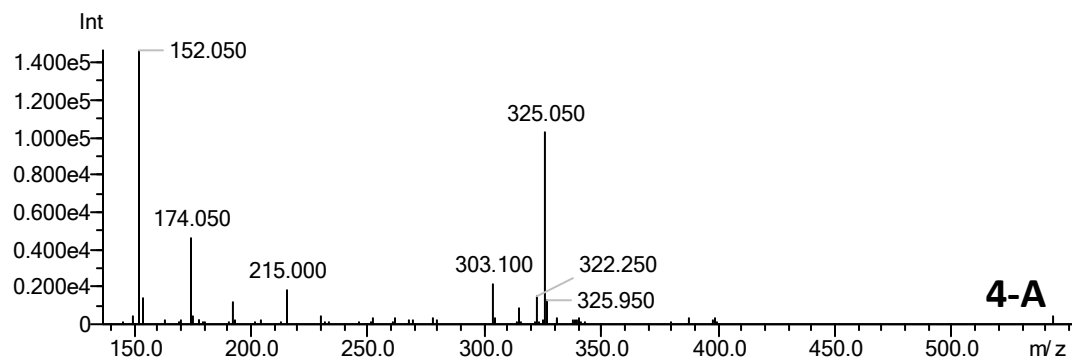


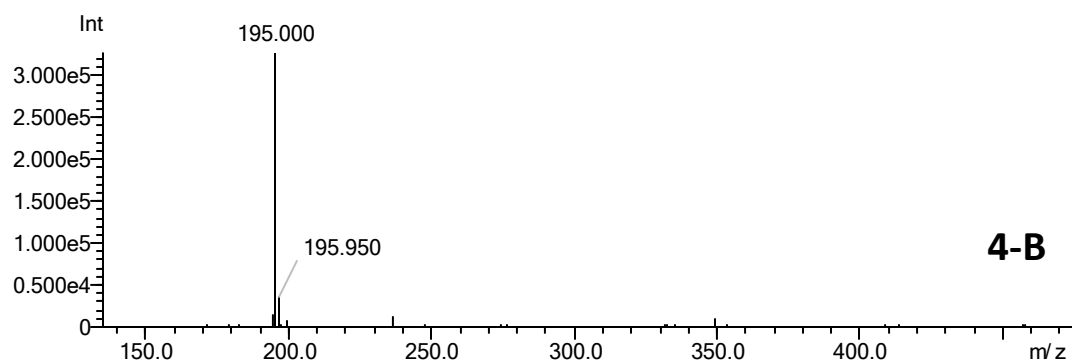
Figure 3: TIC Chromatograms CONFIDENTIAL test Mix sample

Group# 1 - MS Peak: 1, RT: 0.49 to 0.58 min



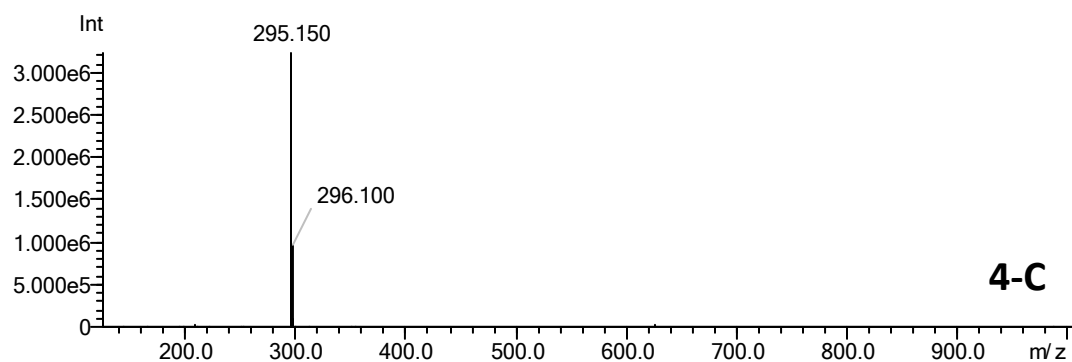
4-A

Group# 1 - MS Peak: 2, RT: 0.63 to 0.72 min



4-B

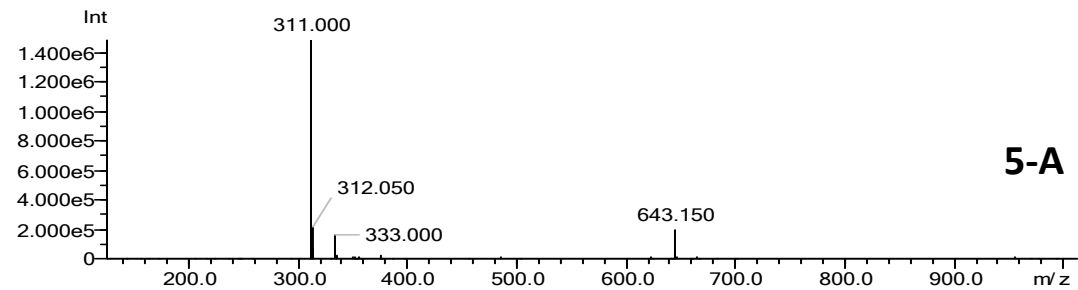
Group# 1 - MS Peak: 3, RT: 0.99 to 1.08 min



4-C

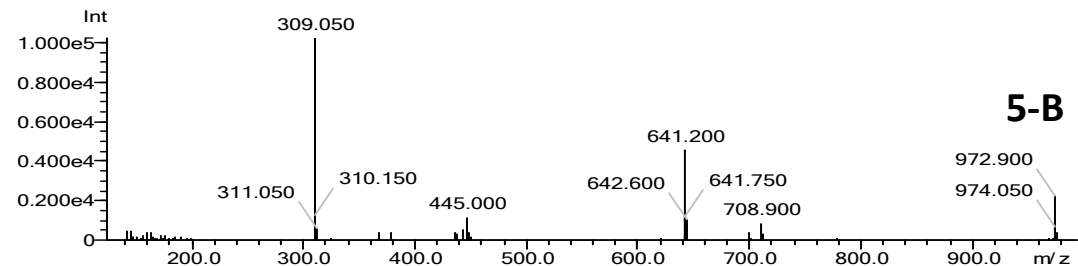
Figure 4: POSITIVE ESI spectra from peaks 1,2,3 TIC 3-A CONFIDENTIAL test Mix sample (no significant negative ion spectra)

Group#1 - MS Peak: 4, RT: 1.08 to 1.17 min



5-A

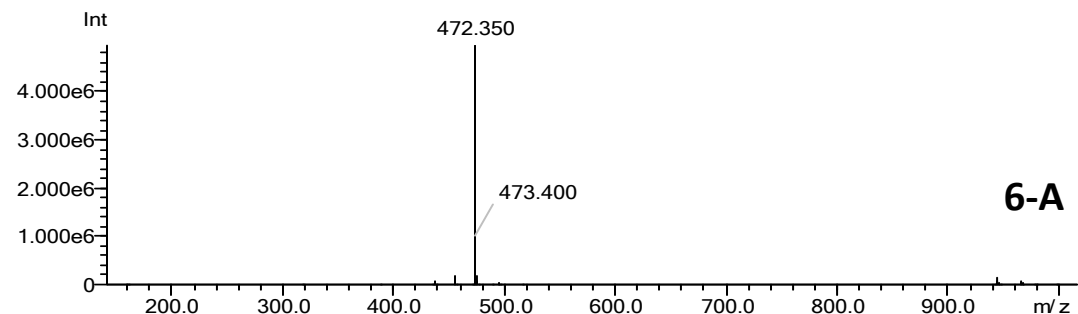
Group#2 - MS Peak: 8, RT: 1.08 to 1.16 min



5-B

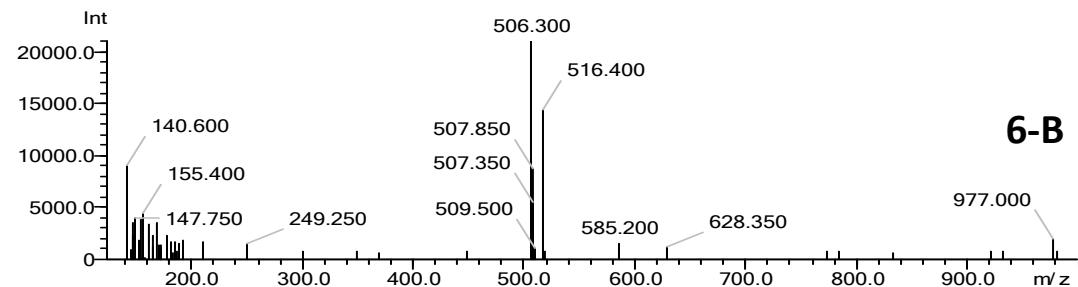
Figure 5: POSITIVE and NEGATIVE ESI spectra, peak 1.12 min TIC 3-A & 3-B CONFIDENTIAL test Mix.

Group#1 - MS Peak: 5, RT: 1.17 to 1.28 min



6-A

Group#2 - MS Peak: 9, RT: 1.16 to 1.23 min



6-B

Figure 6: POSITIVE and NEGATIVE ESI spectra, peak 1.21min TIC 3-A & 3-B CONFIDENTIAL test Mix.

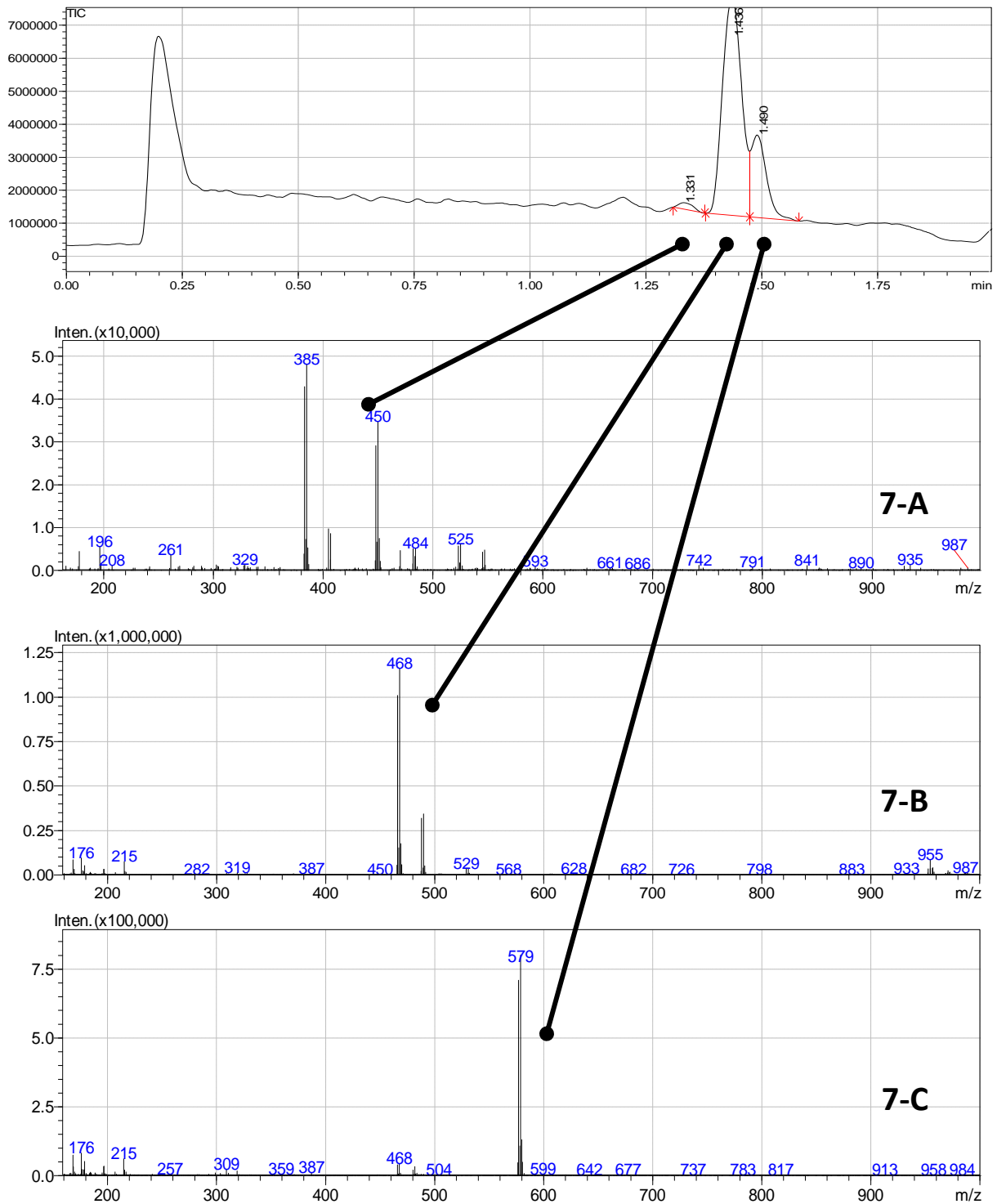


Figure 7: TIC and SPECTRA (7A, 7B, 7C) from CONFIDENTIAL sample 167015

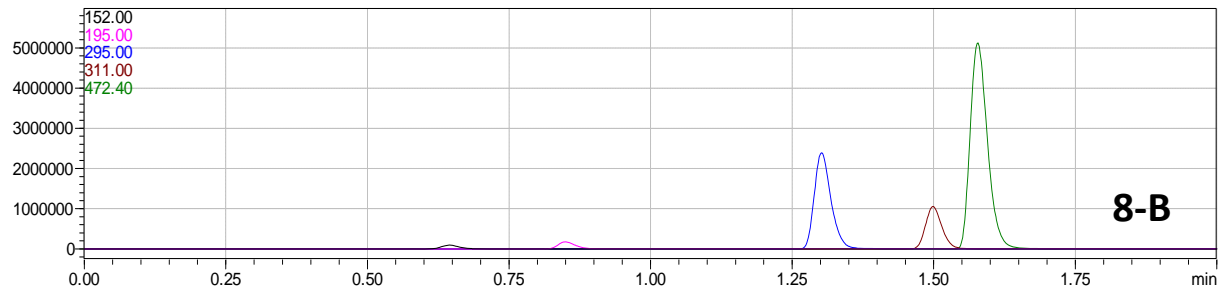
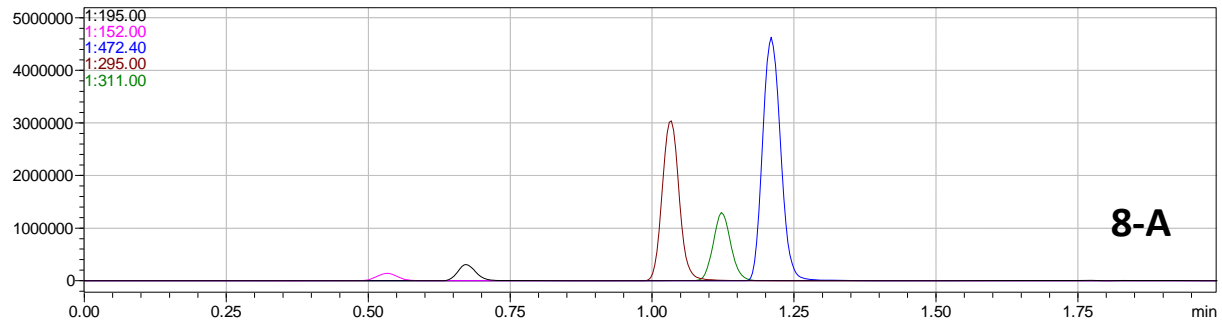


Figure 8: 50mm column peak separation(8A) vs. 75mm column (8B), Test mix sample.

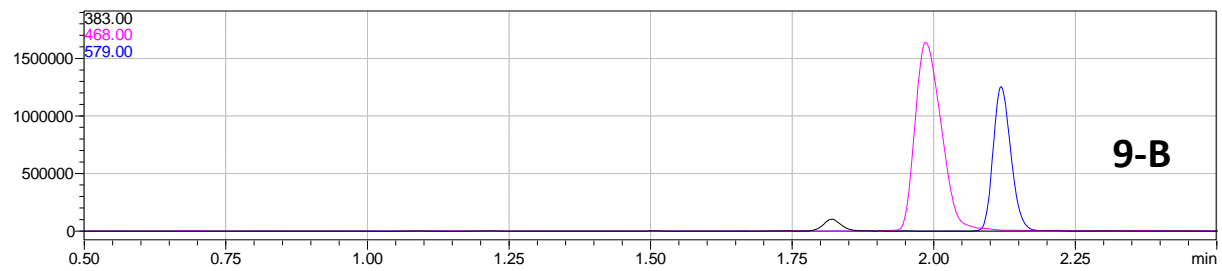
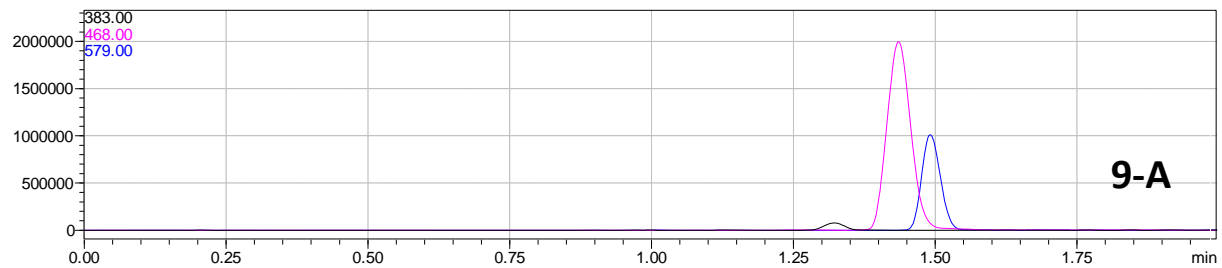


Figure 9: 50mm column peak separation(9A) vs. 75mm column (9B), Test sample 167015.

Discussion

Open Solution: The Open Solution software utility for LCMSsolution greatly simplifies complete system operation:

- automation – “cold” startup and equilibration automatic
- simplified sample login, in as few as 5 mouse clicks
- sample login and management by assigned sample location in autosampler for multi-user environment
- provides e-mail notification and results link when sample results are completed for an experiment (sample group)
- simplified retrieval and review of complete LCMS chromatogram and spectra, PDA chromatogram and spectra from any computer running Internet Explorer without “node” fees
- summary reports can be printed from local user computer through Internet Explorer

Fast LCMS Data Acquisition: The LCMS-2020 with fast data acquisition up to 15000Da sec⁻¹ and positive/negative polarity switching time of 15msec is best suited for fast gradient, high-resolution columns.

As demonstrated, a complex test mix containing 5 different compounds can be applied to the Shimadzu UFLC system with a gradient program, including column wash and re-equilibration at 2 minutes. The last eluting peak produced a retention time of 1.21 minutes, with all peaks having complete baseline resolution.

Simplified LCMS Calibration: The tuning of the Shimadzu LCMS-2020 is a completely automated process with a prepared tune solution. The LCMS-2020 tune program also includes a negative tune component for actual negative ion tuning - **NOT** simply tuning in positive mode and switching voltages.

All data produced in this report were achieved from a standard tune file. No sample-specific adjustments were needed to enhance or provide system performance (exception = detector sensitivity adjustment, probe position, drying gas flow). The LCMS was not re-tuned or adjusted for any compound specific optimized response.

UFLC-XR High Pressure LC: For extra separation, smaller particle size columns for even faster LC method development will require allowable pressure limits up to 9500+ psi.

For the samples analyzed in this study, the 50mm X 2.0mm ID 2.2 and 2.5 um particle size columns produced system pressures of 2000-5000 psi at 1 ml min⁻¹ and water/acetonitrile.

The longer 75mm X 2.0mm ID 2.2um particle size column produced system pressures up to 7800psi. The improvement in resolution is shown in the following table. The gradient was adjusted by a factor of 1.5 to maintain the same relative gradient velocity across the column bed.

Separation factors resulting from the analyzed samples were:

		Retention Time			Separation	
		50mm	75mm		50mm	75mm
Sample 1	Peak 1	0.53	0.64			
	Peak 2	0.67	0.85	Peak 1-2	1.264	1.328
	Peak 3	1.03	1.3	2-3	1.537	1.529
	Peak 4	1.12	1.5	3-4	1.087	1.154
	Peak 5	1.21	1.59	4-5	1.080	1.060
Sample 2	Peak 1	1.32	1.82			
	Peak 2	1.43	1.99	Peak 1-2	1.083	1.093
	Peak 3	1.49	2.12	2-3	1.042	1.065

LC Time Program
50mm X 2.0mm ID column

	Time	Module	Command	Value
1	0.05	Pumps	Pump B Conc.	5
2	2.45	Pumps	Pump B Conc.	70
3	2.50	Pumps	Pump B Conc.	90
4	2.65	Pumps	Pump B Conc.	90
5	2.70	Pumps	Pump B Conc.	5
6	3.00	Controller	Stop	

LC Time Program
75mm X 2.0mm ID column

	Time	Module	Command	Value
1	0.05	Pumps	Pump B Conc.	5
2	1.65	Pumps	Pump B Conc.	70
3	1.66	Pumps	Pump B Conc.	90
4	1.74	Pumps	Pump B Conc.	90
5	1.75	Pumps	Pump B Conc.	5
6	2.00	Controller	Stop	

Conclusion

The LCMS-2020 / UFLC-XR system combines simplified walk-up multi user capabilities and fast reliable data acquisition. Excellent baseline separations were made using 2mm X 50mm 2.2 or 2.5 um particle size columns in a total runtime of less than 2 minutes under standard pressure conditions. However, should the need arise for extra resolution, the Prominence UFLCXR pressure capabilities drive system performance with the use of a higher generation of column back pressure when using a longer column or a faster method cycle time using smaller particle size. The Prominence UFLCXR system also achieves higher linear velocity through the column for faster gradients and peak elution.

In order to fully utilize the benefits of any high-pressure HPLC, an LCMS system must be capable of sufficiently rapid scans across very narrow peaks. Shimadzu's LCMS-2020 is the only single quadrupole mass spectrometry system capable of scan speeds up to 15000 Da/sec, 15 msec positive/negative polarity switching, and extreme sensitivity all within its standard operating mode.