Chromatographic Fidelity and Matrix /Analyte Solubility in Complex Polymer Systems using HPLC-MALD/I TOF MS

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CHEM 395

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Instructor : Prof J.Rusling

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Overview

- Introduction to MALDI
- Objective:
  - To demonstrate compatibility of matrix/analyte mixtures and to also ascertain the Chromatographic fidelity in MALDI analysis of polymers.
- Approach:
  - Coupling of HPLC with MALD/I TOF MS……Hence the name “LC-MALDI”
  - LC-MALDI interfaced using LC-Transform Series 600 (LabConnections, Inc.).
  - Selective use of solvents’ gradient over varying temperature range.
- Results:
  - Retention time/scan reproducibility over various samples analyzed and the signal to noise ratio.
  - Capability of functional HPLC separation combined with automated MALDI analysis is demonstrated using K-GH Polyol (thermal dye-receiver polymer).
  - Demonstrated advanced capability for differentiating materials.

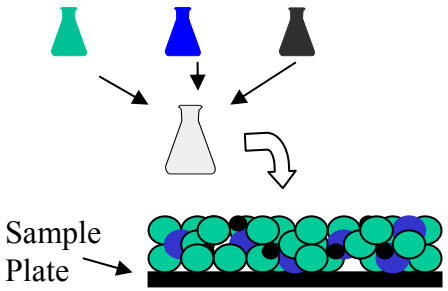
Introduction to MALDI

- Since its discovery in the late 1980s,Matrix Assisted Laser Desorption Ionization (MALDI) has emerge as an important soft-ionization MS technique.
- Applications in many biologically related research areas (e.g.,protein,peptides,DNA,carbohydrates) and materials-related fields (e.g., synthetic polymers)
- The fundamental mechanisms still remain unknown despite its routine usage

Introduction Cont'd

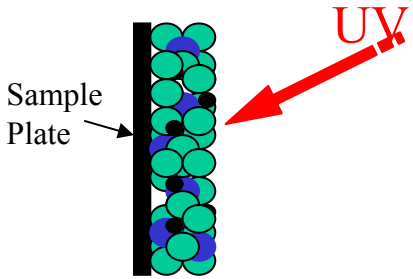
MALDI experiment can be reduce to four different steps:

- Sample Prep: Molar ratio of matrix to analyte is large (e.g. between 10^2 : 1 and 10^6 : 1)
- Desorption: Pulsed laser beam (typically a Nitrogen laser,337nm)
- Ionization: Gas phase ionization reactions (Cationized,protonated,de-protonated or radical species)
- Mass Analysis: Time-of-flight (TOF),FTICR ,QIT etc.

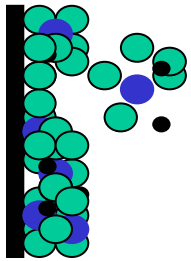


1) Sample Preparation

- Individual solutions of matrix material and analyte
- Solutions are mixed (high matrix-to-analyte ratio)
- Mixture is deposited onto the sample plate (solvent evaporated)
- Analyte is homogeneously embedded within the matrix crystal (solid solution)

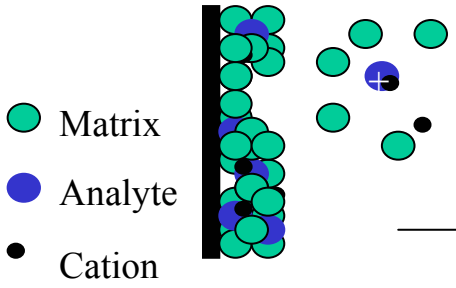


- Typically nitrogen laser (337 nm)
- Matrix resonantly absorbs the laser energy



2) Desorption

- Matrix is desorbed off the surface, transferring the analyte molecules into the gas phase.
- A supersonic expansion from the surface - the velocities of various components of the sample are found to be very similar



3) Ionization

- Reactions occur in the dense plume - Gas-phase ionization
- Formation of ions: cationized species, protonated species, de-protonated species, or radical cations

4) Mass Analysis - Time-of-Flight Mass Spectrometer…

TOF Cont'd

In linear TOF instrument, ions drift through the flight tube until they reach the detector.

Because ions of different species in a mixture have different masses, they have different velocities based on the following equation:

$$v = \sqrt{\frac{2KE}{m}}$$

ν=velocity, KE =kinetic energy, and m=mass

$$t = \sqrt{\frac{m}{2KE}}D$$

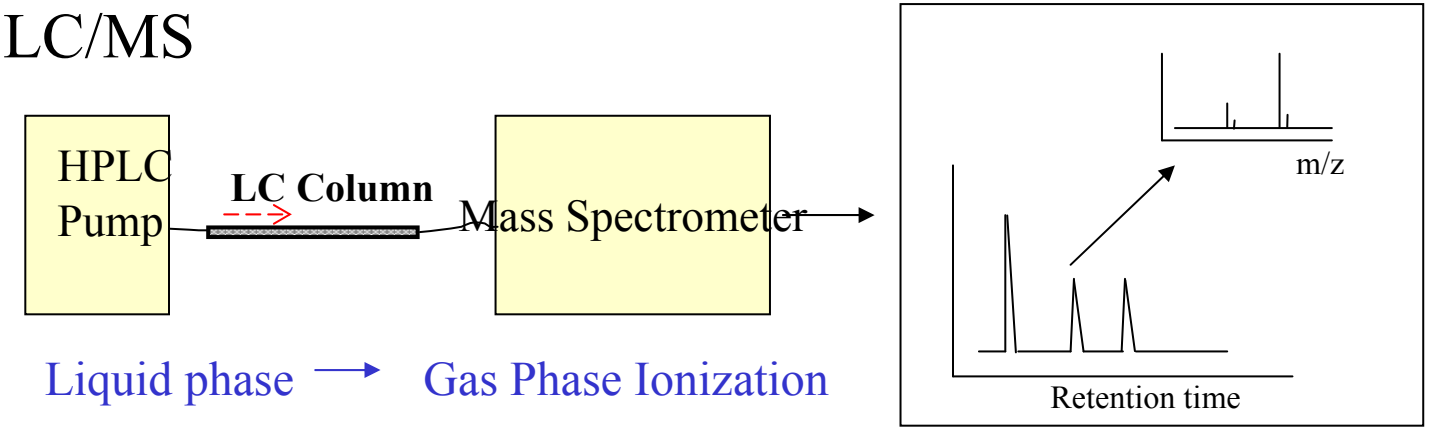
Where D is the flight distance

$$\frac{m}{z} = \frac{2Ut^2}{D^2}$$

z=number of charge on an ion, U=accelerating voltage
t=flight time

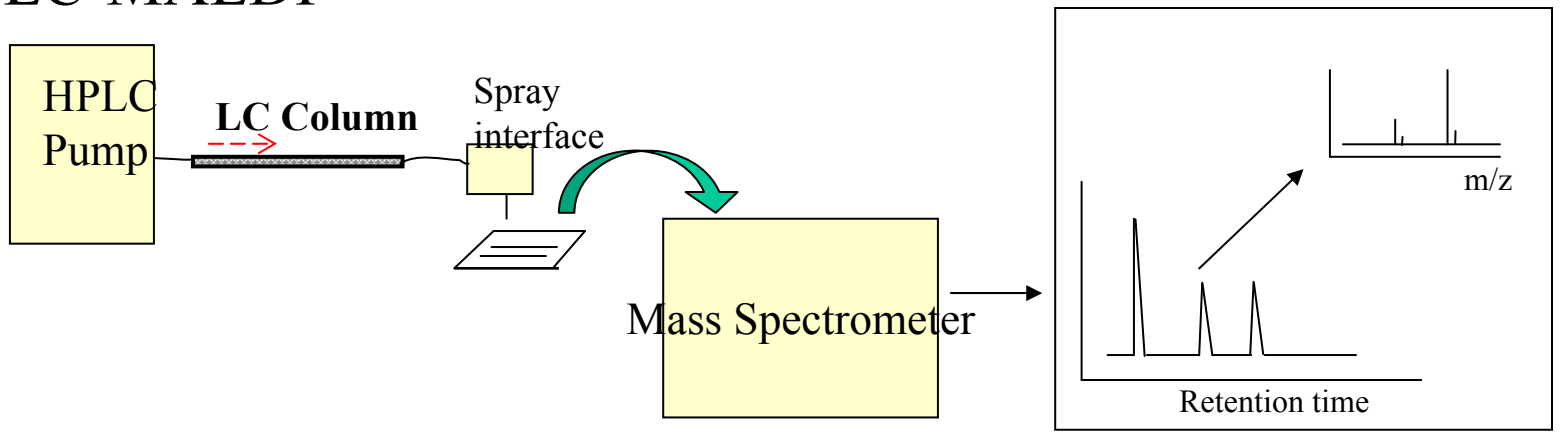
LCMS vs LC-MALDI

LC/MS



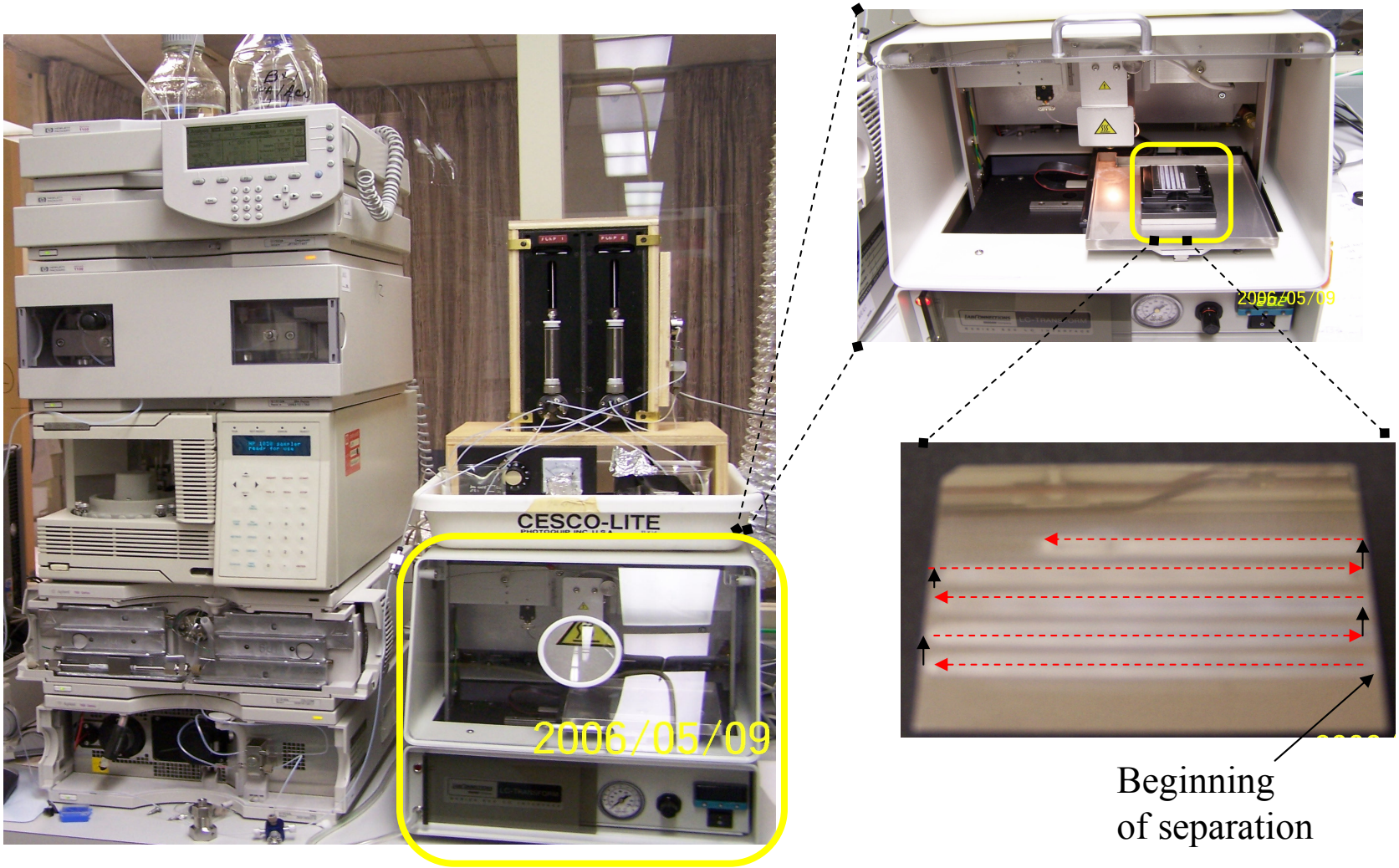
Liquid phase → Gas Phase Ionization

LC-MALDI



Liquid phase → Solid phase → Gas Phase Ionization

LC-MALDI Interface



Research & Development Laboratories, Eastman Kodak Company, Rochester, New York

LC-Interface Experimental Conditions

HPLC

Column: Zorbax Eclipse XDB-C8
2.1mm x 150mm; 5µm

A: Water

B: ACN/IPA (1:1)

Gradient:

- 50% B → 100%B in 20 min. at 250uL/min
- Hold 100% B for 5 min at 250uL/min
- 100% B → 50% B in 0.1 min at 350uL/min
- Hold 50% B for 5 min at 350uL/min

Inj Vol.: 2uL

Detection: DAD (monitor at 210 nm)

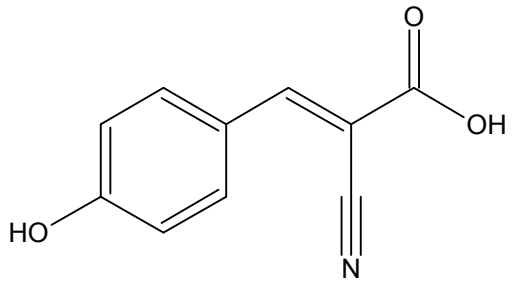
Matrix Pump

Pump 1: CHCA matrix 1 mg /mL in ACN:IPA

Pump 2: ACN:IPA

Isocratic pumping of 50:50 (pump 1 : pump 2)

Flow Rate: 100 uL/min



α-cyano-4-hydroxycinnamic acid (CHCA)

Sprayer

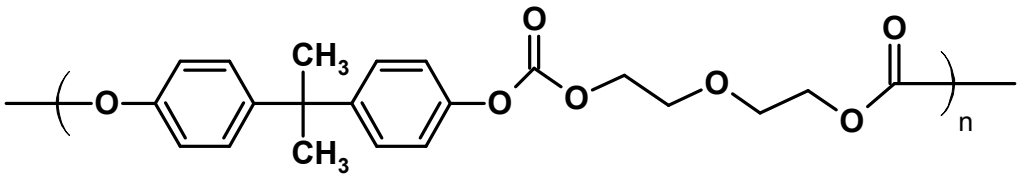
Temperature gradient: 172 °C at initial conditions to 133 °C at final conditions

N_2 Gas Nebulization: 30 psi

Application

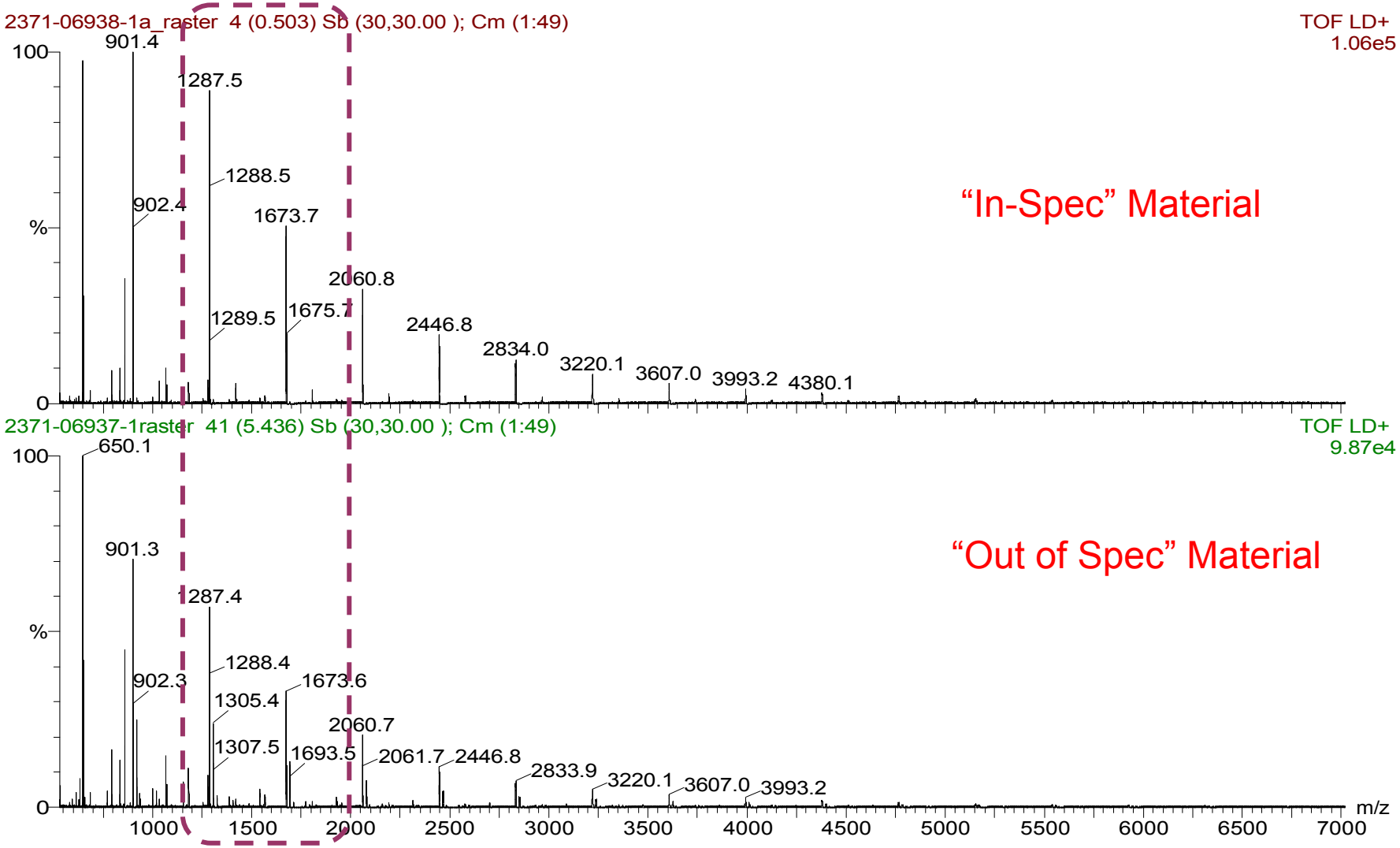
Example used for Demonstration of Capability

- K-GH Polyol (low MW polymer used in Thermal dye-receiver)



- Important to have correct end-group chemistry
- Multiple end-group combinations have been observed
- Interest in pursuing functional separation

Direct MALD/I TOF MS of K-GH Polyol (no separation)



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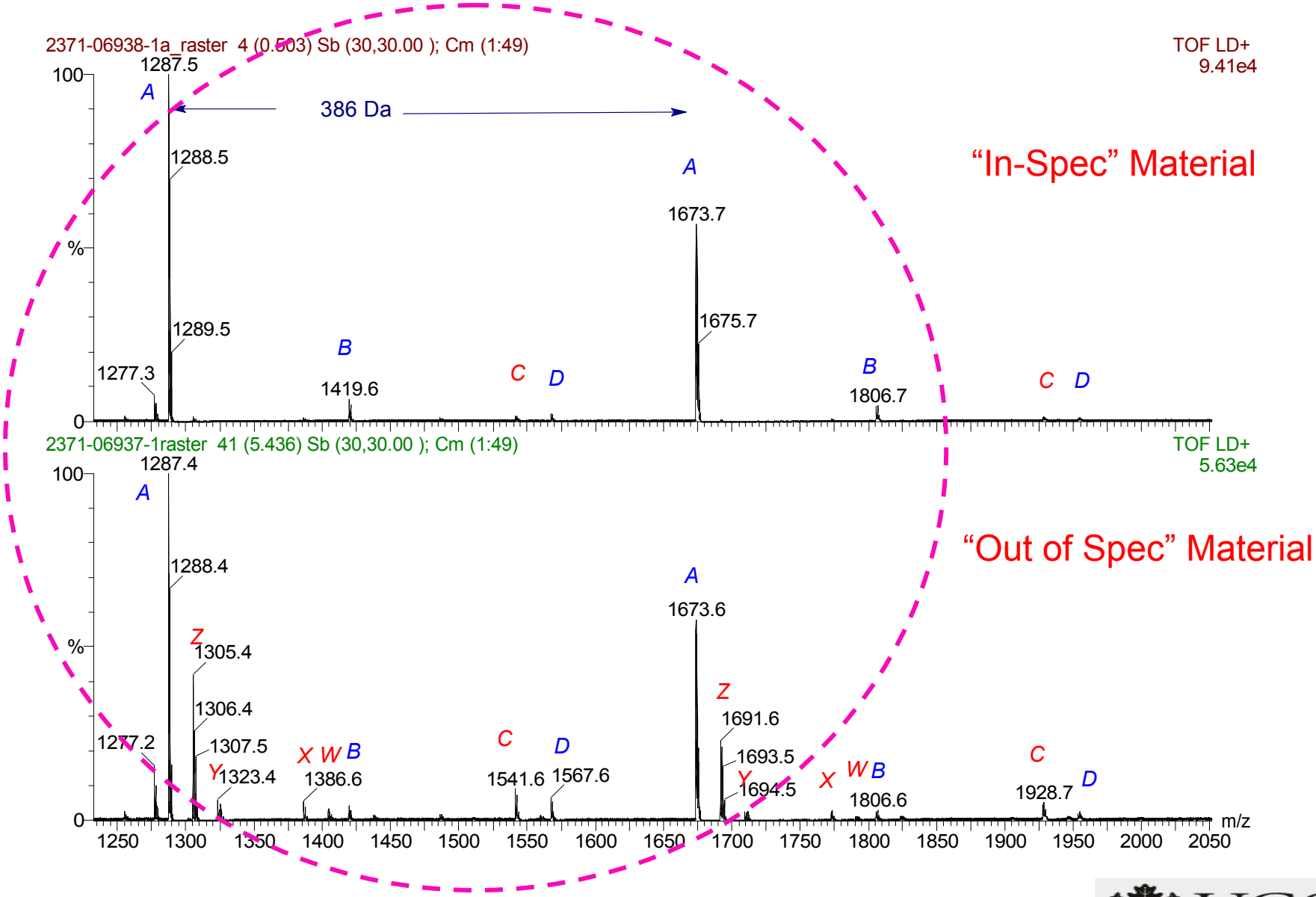
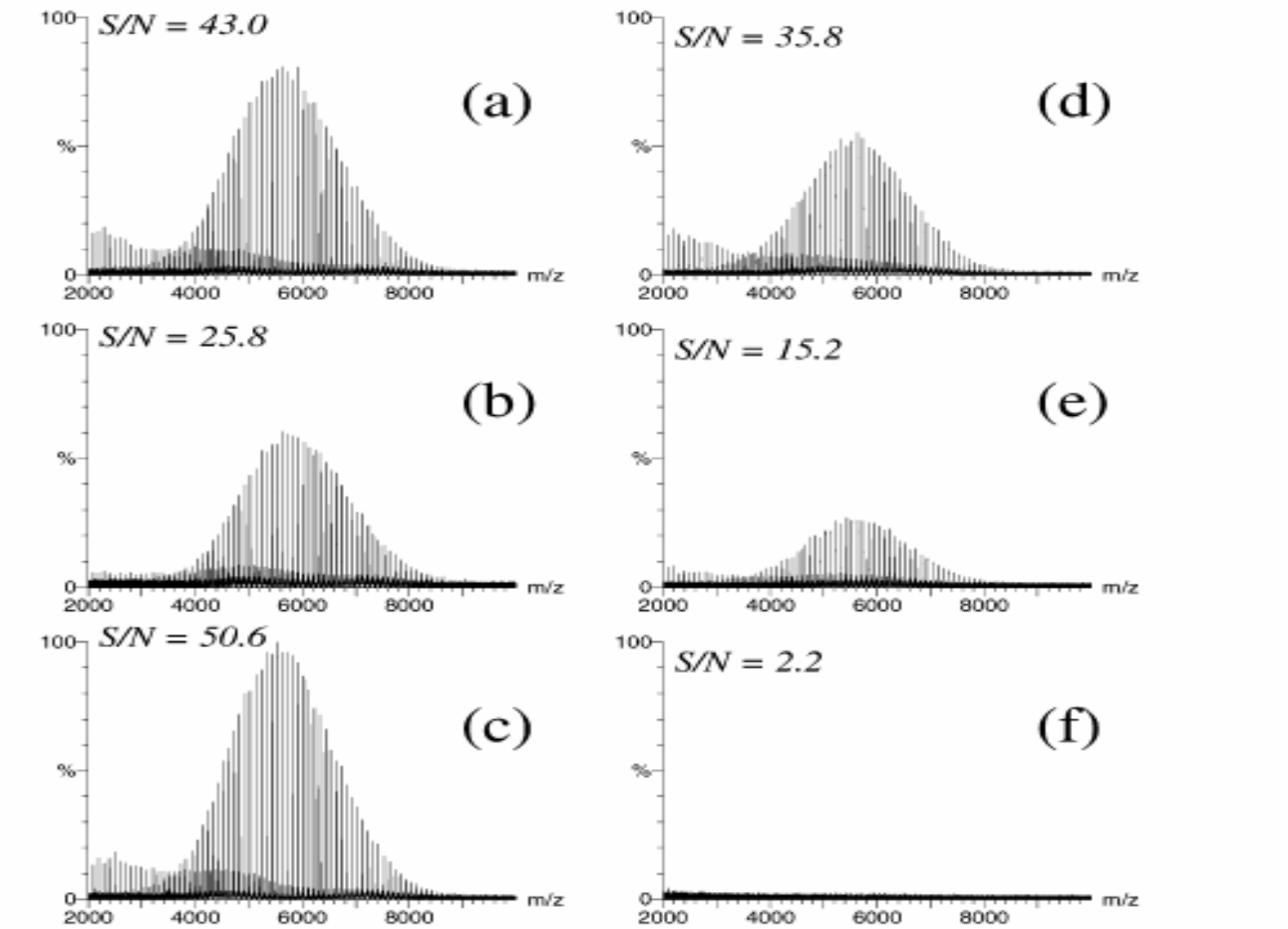
Direct MALD/I TOF MS of K-GH Polyol (no separation)

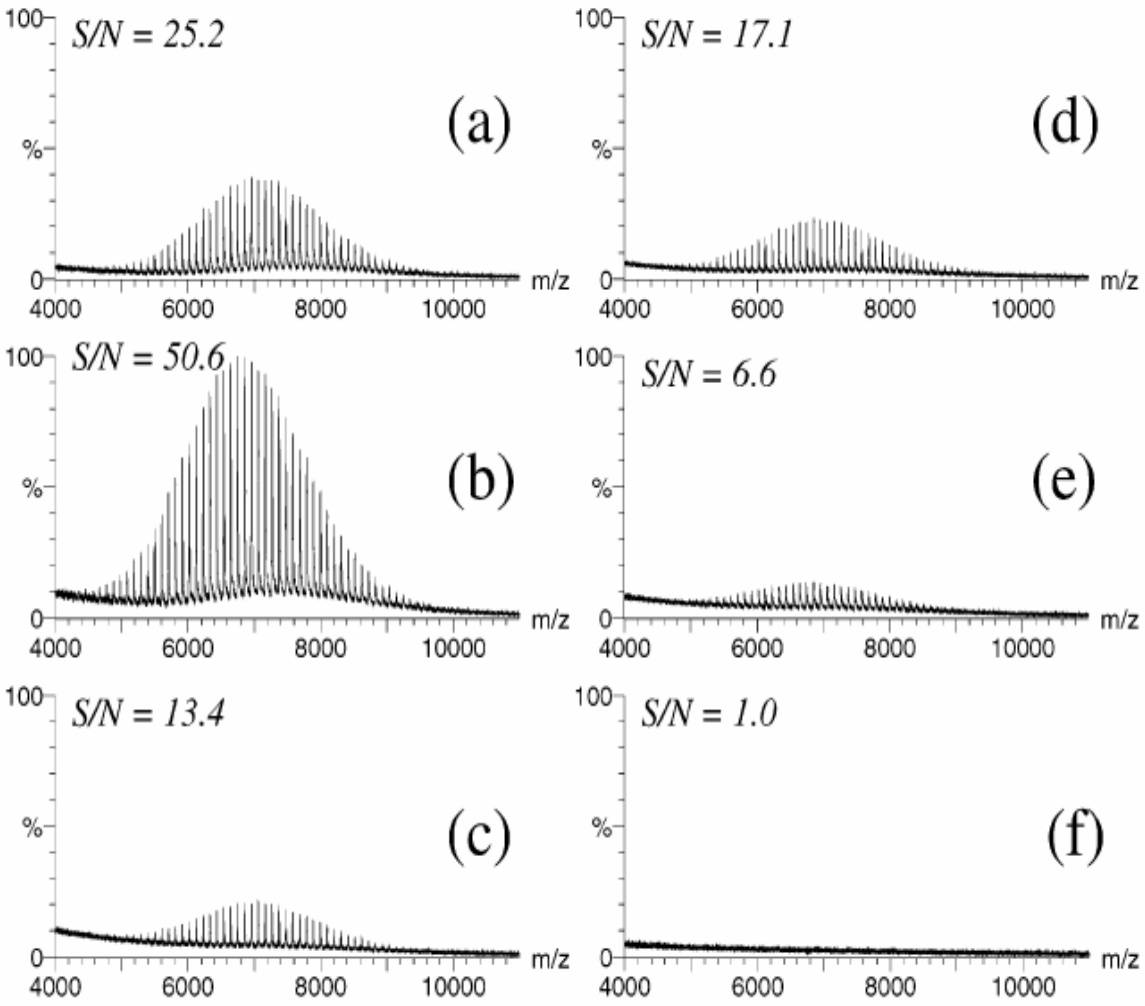




Spectral of PMMA 6300 using NaTFA as the cationization reagent with six matrixes (a) RA (b) DCTB (c) IAA (d) CHCA (e) DHB (f) DHBQ

Andrew, J.H.; William, J.E.; Robert, J.T.; Kelvin, G.O.; Anal.Chem. 2004, 76, 5157-5164

Results



DHBQ 2,5-dihydroxy-p-benzoquinone
DHB 2,5-dihydroxybenzoic acid
CHCA a-cyano-4-hydroxycinnamic acid
IAA trans- indoleacrylic acid
DCTB 2-[(2E)-3-(4-tert-butylphenyl)-2 methylpropenylidene] malanonitrile
RA all-trans-retinoic acid

Spectral of PS 7000 using AgTFA as the cationization reagent with six matrixes (a) RA (b) DCTB (c) IAA (d) CHCA (e) DHB (f) DHBQ

Andrew, J.H.; William, J.E.; Robert, J.T.; Kelvin, G.O.; Anal.Chem. 2004, 76, 5157-5164

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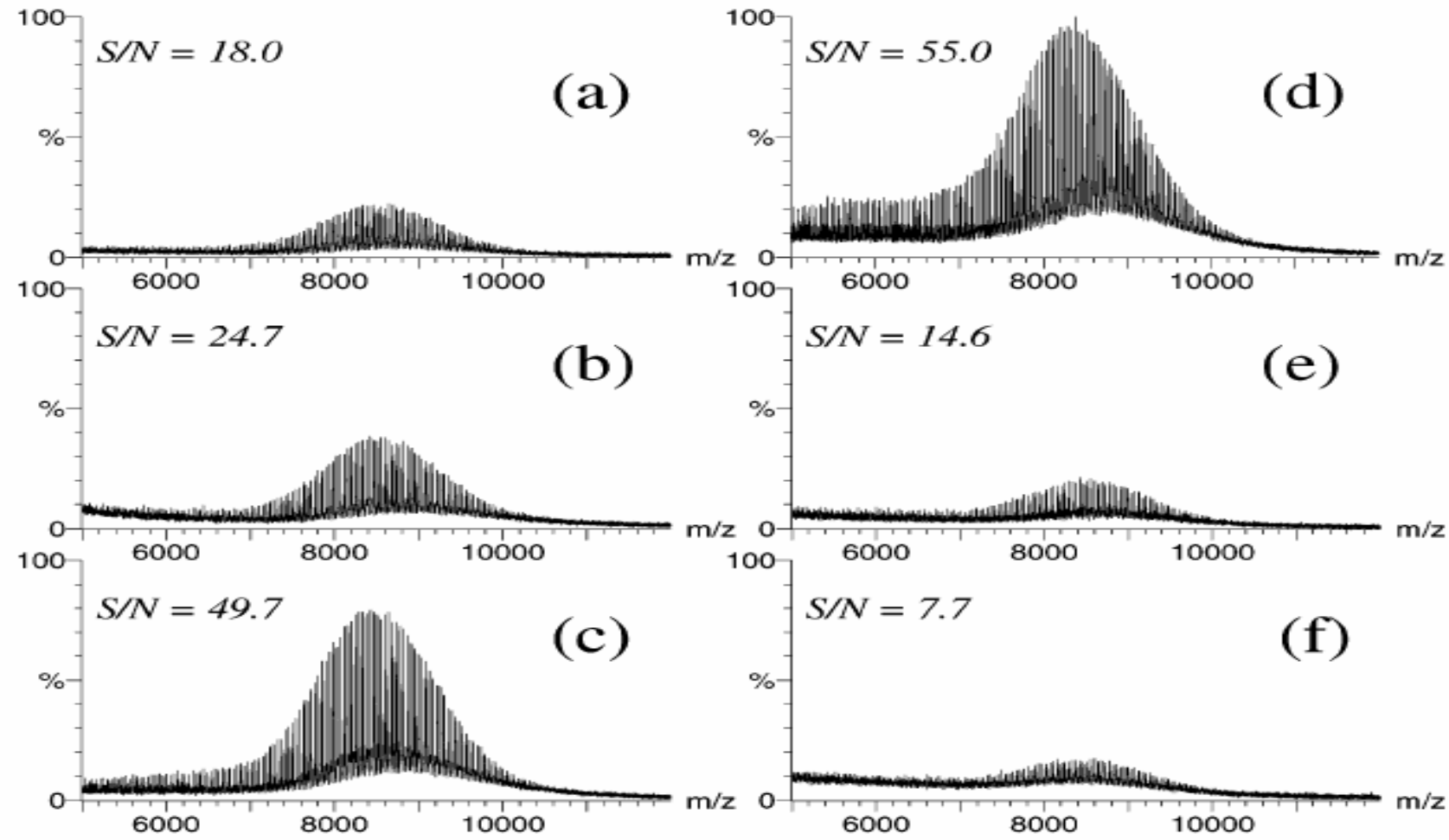
Results Cont'd



Spectral of PEG 10000 using NaTFA as the cationization reagent with six matrixes (a) RA (b) DCTB (c) IAA (d) CHCA (e) DHB (f) DHBQ

Andrew, J.H.; William, J.E.; Robert, J.T.; Kelvin, G.O.; Anal.Chem. 2004, 76, 5157-5164

Conclusion

- LC-MALDI retention time reproducibility is within a factor of 3, compared with conventional LC-DAD, despite the solid state transition. *(Within a factor of 2 compared with conventional LC/MS).*
- Demonstrated chromatographic fidelity for LC-MALDI compared to LC-DAD and LC-ELSD.
- Matrix-addition solvent effects were observed which compromised chromatographic fidelity. 3D MALDI Imaging being used to investigate the dynamics of this effect.
- Matching the RTs of the matrix and analyte consistently produced the best MALDI spectra with regard to S/N.
- The results presented here suggest that a good starting point for choosing the appropriate matrix for an “unknown” sample would be to match its relative polarity with that of the matrix.

Acknowledgements

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CHEM 395 Class of Spring ’07 !!

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Andrew J.H. PhD Thesis 2003