Disruptive Technology for Measuring Nutrients and Nourishment

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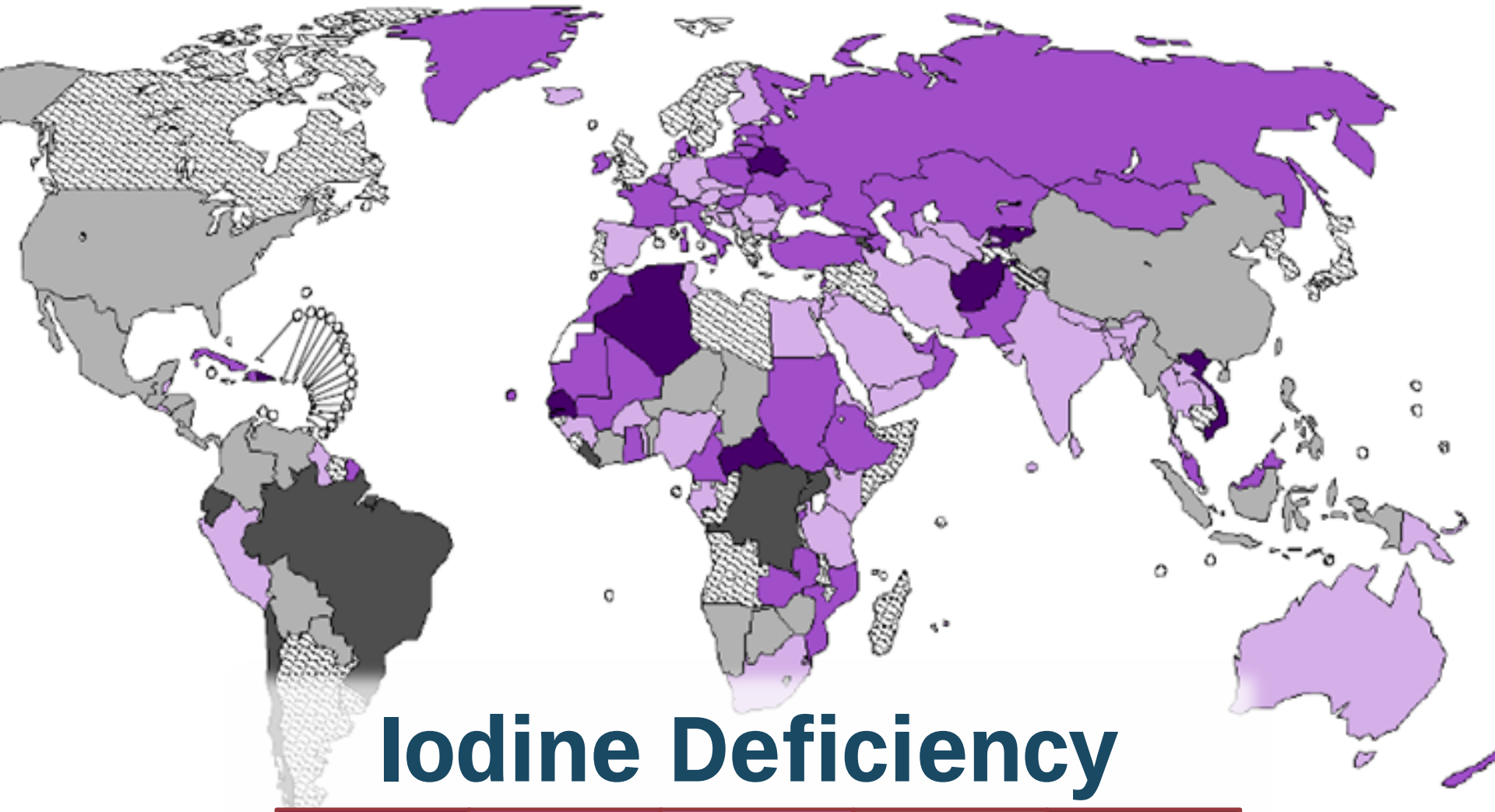
BILL & MELINDA GATES foundation

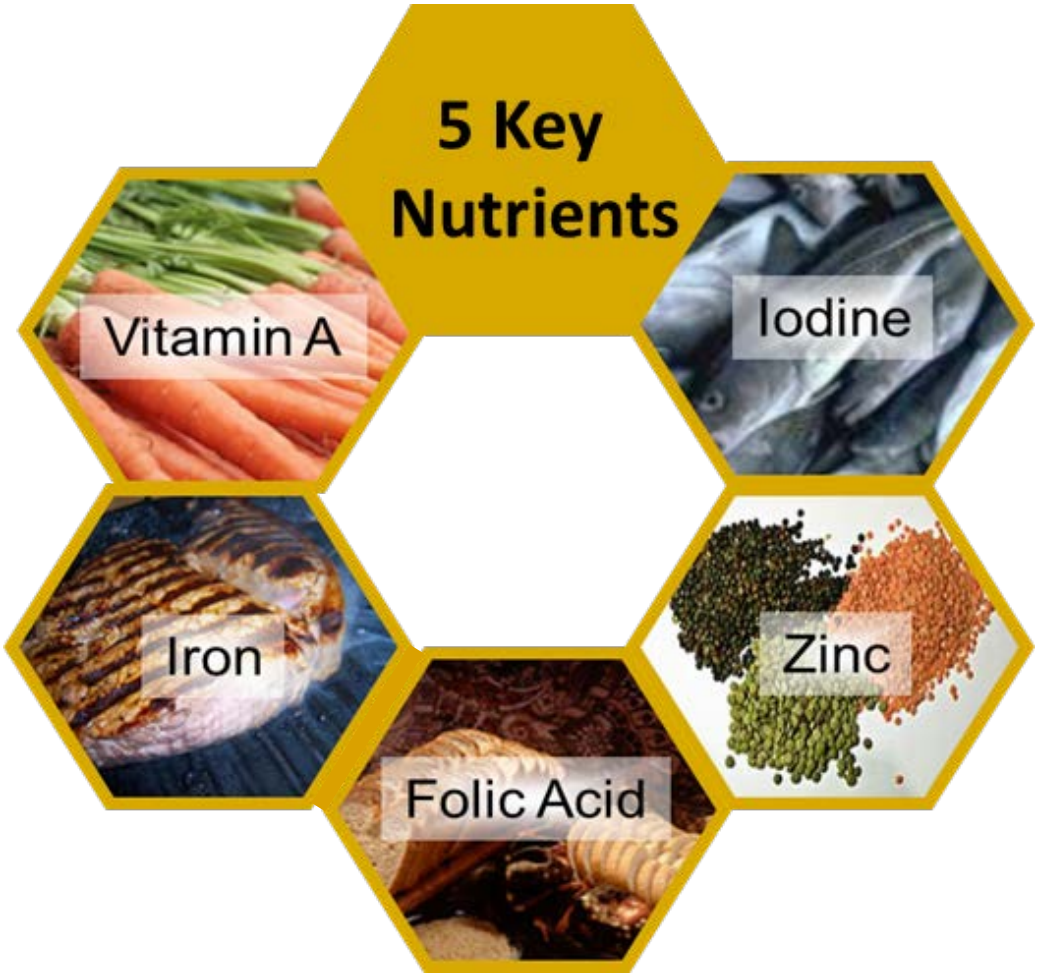


Iodine Deficiency

Moderate Deficiency	Mild Deficiency	Ideal	At Risk (hyperthyroidism)	Adverse Health Risks

Food and Nutrition Bulletin, **29** (3): 195-202 (2008)

Developing a Tool to Detect Nutrient Deficiencies



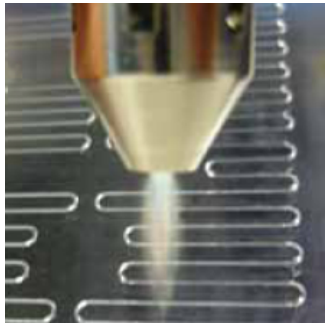
Goals:

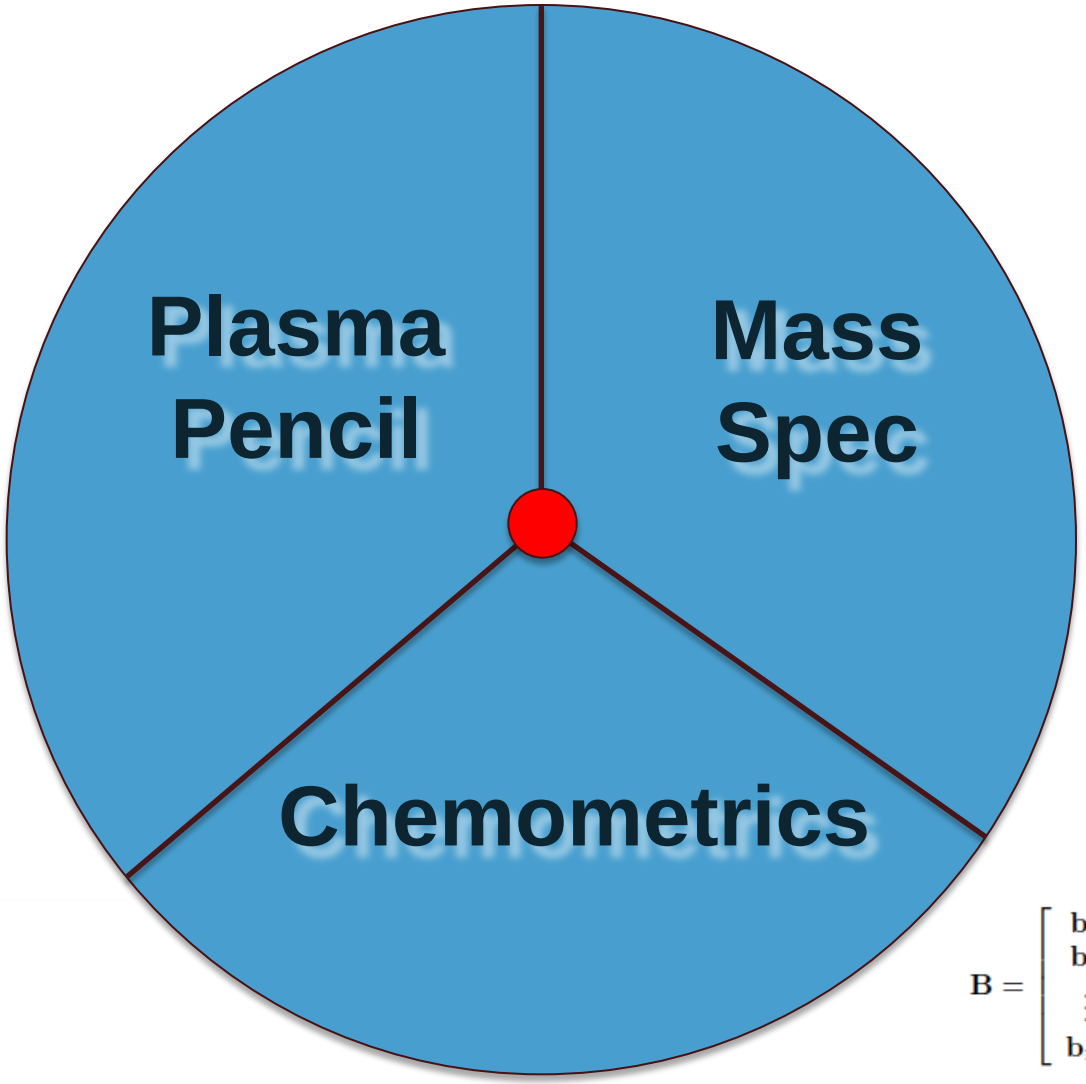
- Rapid
- Simultaneous
- Low Cost
- Durable
- Accurate

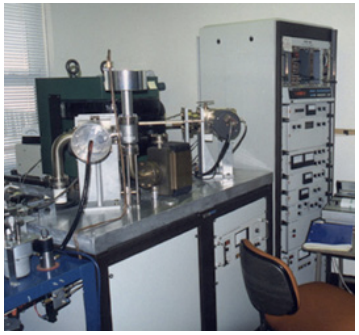
Can we develop one assay that can measure 5 micronutrients? YES!

Plasma Pencil Atmospheric Mass Spectrometry (PPAMS)

3 technologies intersect!

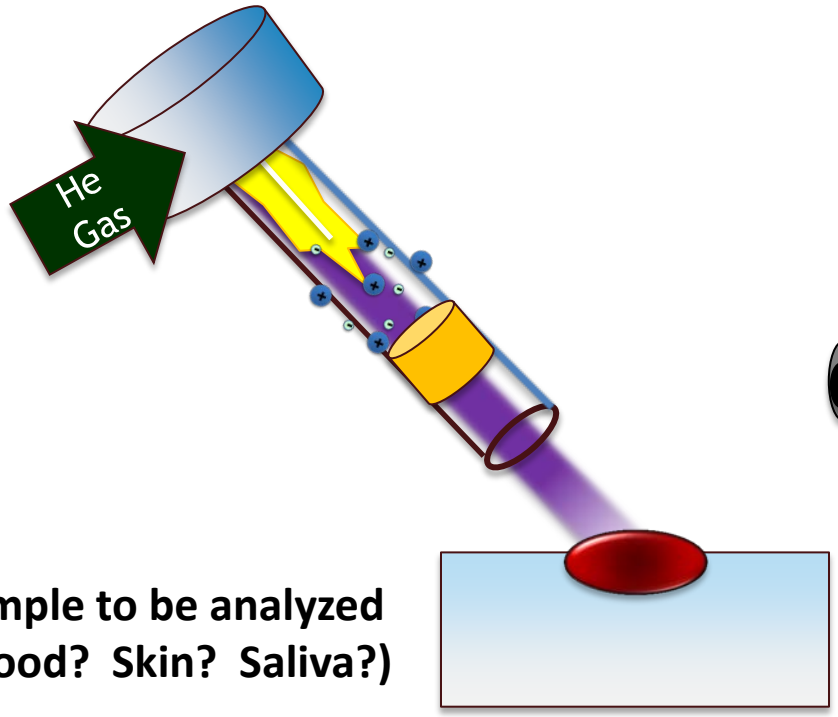






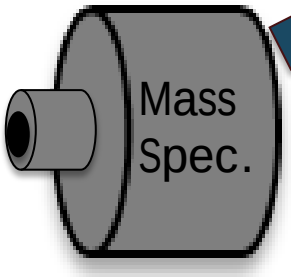
$$\mathbf{B} = \begin{bmatrix} \mathbf{b_1} \\ \mathbf{b_2} \\ \vdots \\ \mathbf{b_m} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \cdots & 0 \\ 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & 1 \end{bmatrix} = \mathbf{I}$$

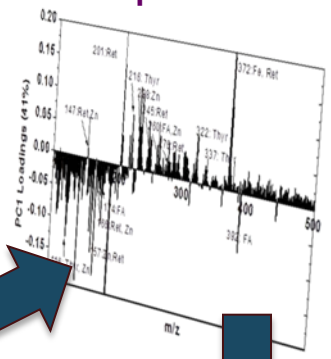
Plasma Pencil Atmospheric Mass Spectrometry (PPAMS) System



Sample to be analyzed (blood? Skin? Saliva?)

Mass Spectra





Nutrient Information:

Zn, Fe, I, Folic Acid, Vitamin A

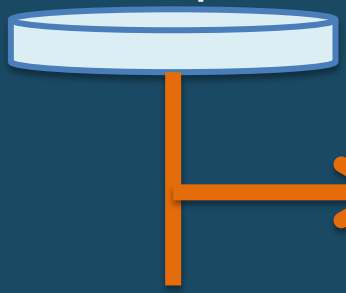
Working towards an easily portable system

Bench-top MS

Portable MS

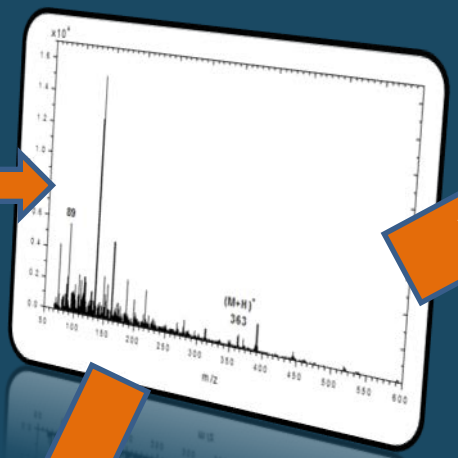
Data Analysis Workflow

1.5 mL Liquid in Petri Dish

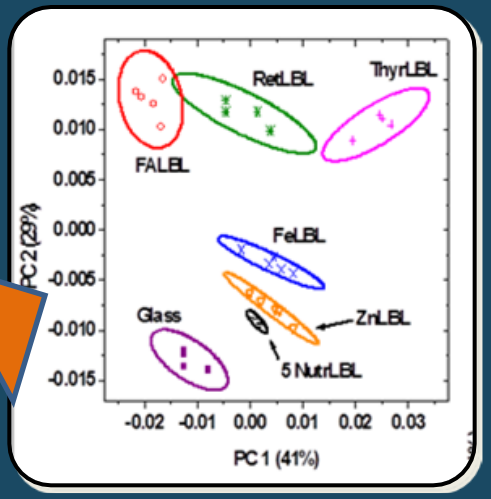


20µL Spot on Paper

PPAMS



PCA



Regression Software Program

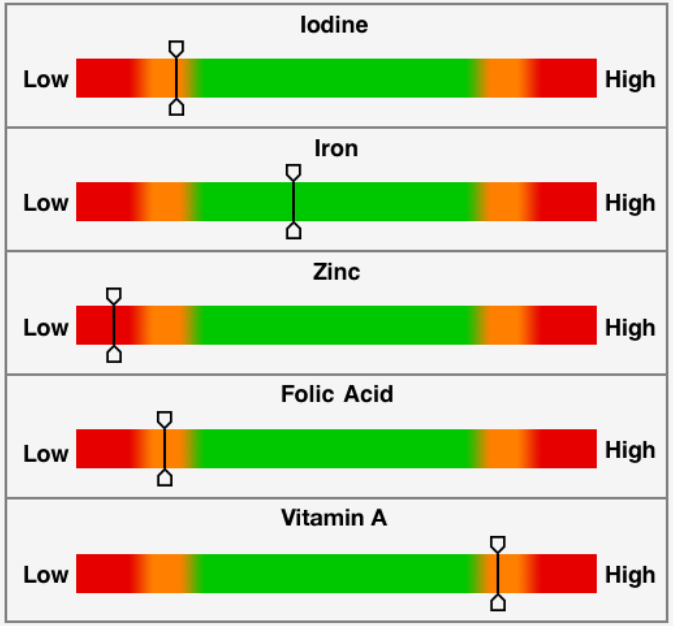
Folic Acid	%
Iodine	%
Iron	%
Vitamin A	%
Zinc	%

Results for Blood

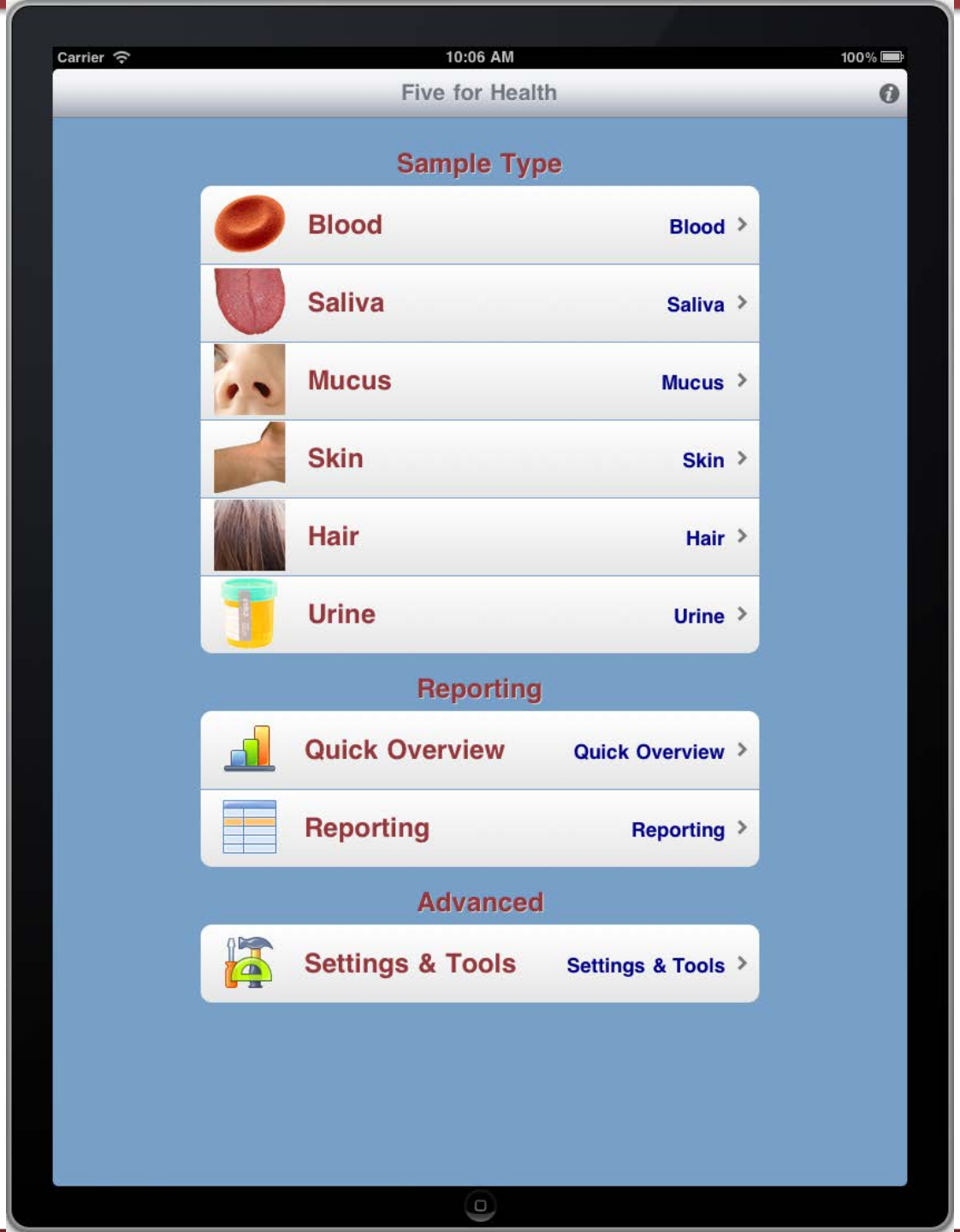
The sample may now be removed from the prep chamber

Mbomo Migunda, Male, 34yo

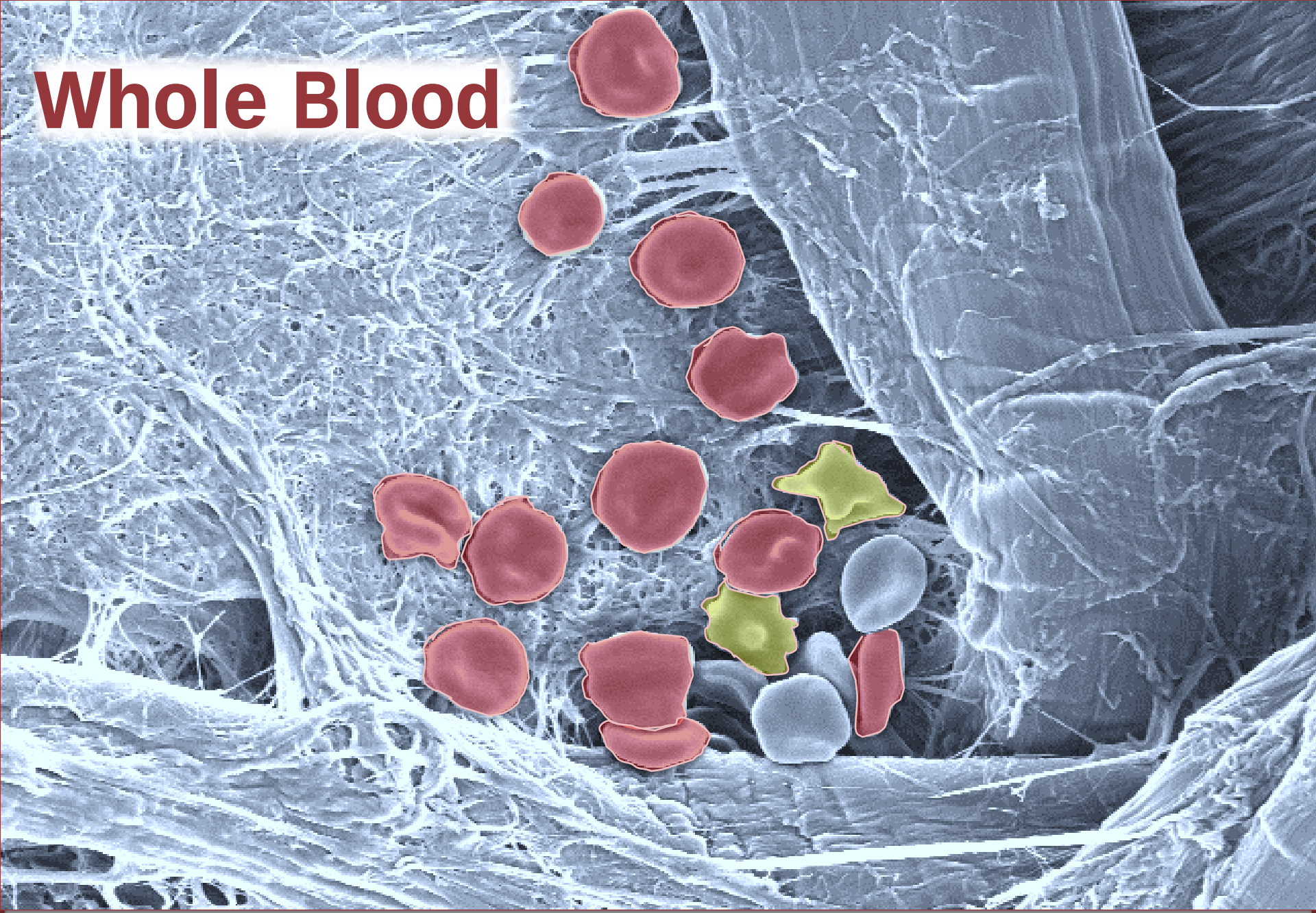
Iodine	**Date:** 3/16/2010
Iron	**Time:** 2:32PM
Zinc	**Location:** 0° 3' 36.40" S 36° 54' 57.18" E
Folic Acid	**Notes:** Nyadundo Village, Kenya
Vitamin A	



Graphical User Interface



Whole Blood



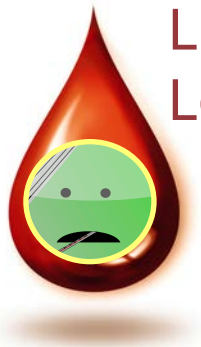
PPAMS was taken on raw nutrient powder and on dried blood samples

Adult human blood chosen as sample media to detect micronutrient levels

HBLC: High Blood Level Concentrations

For Each Nutrient Group:

- Iron: 2 ppm
- Zinc: 20 ppm
- Folic Acid: 50 ppb
- Retinol (Vitamin A): 625 ppb
- Thyroxine (Iodine): 105 ppb



LBLC: Low Blood Level Concentrations

For Each Nutrient Group:

- Iron: 0.5 ppm
- Zinc: 10 ppm
- Folic Acid: 5 ppb
- Retinol (Vitamin A): 288 ppb
- Thyroxine (Iodine): 46 ppb

PPAMS on Examples on Patient Models with Single Nutrient Variations

"Relatively Healthy"

For Each Nutrient Group:

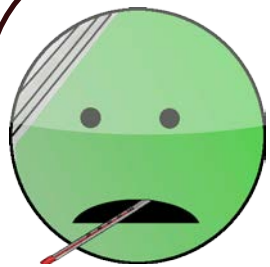
- 1 Nutrient at 1x LBLC
- 4 Nutrient at 1x HBLC

Control:

- All Nutrients at 1x LBLC

Media:

- 10% Porcine Plasma in citrate Phosphate Buffered Saline



"Relatively Unhealthy"

For Each Nutrient Group:

- 1 Nutrient at 1x HBLC
- 4 Nutrient at 1x LBLC

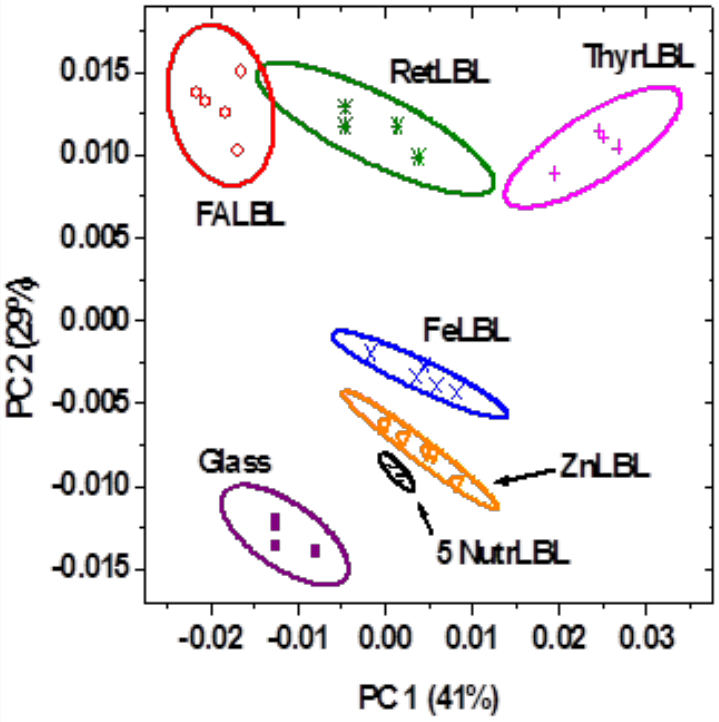
Control:

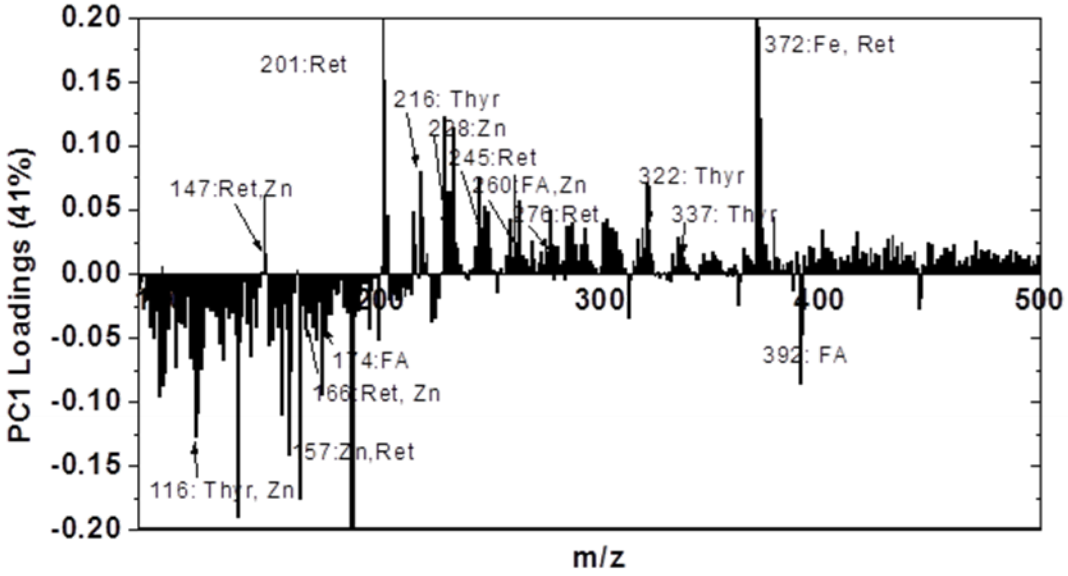
- All Nutrients at 1x HBLC

Media:

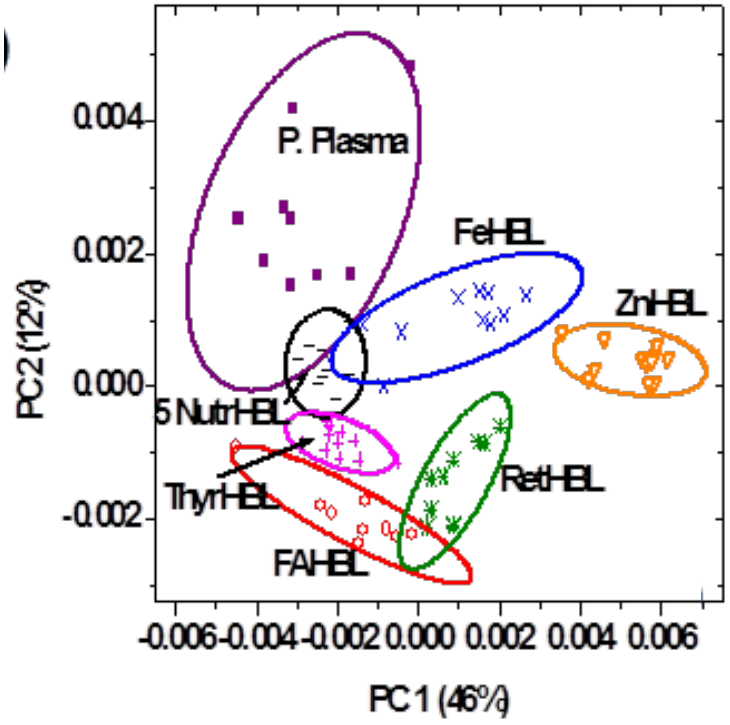
- 10% Porcine Plasma in citrate Phosphate Buffered Saline

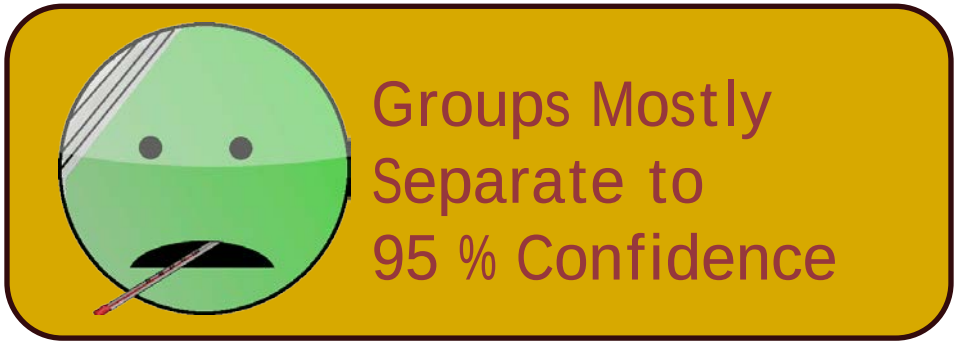
“Relatively Healthy” sample groups separate nicely

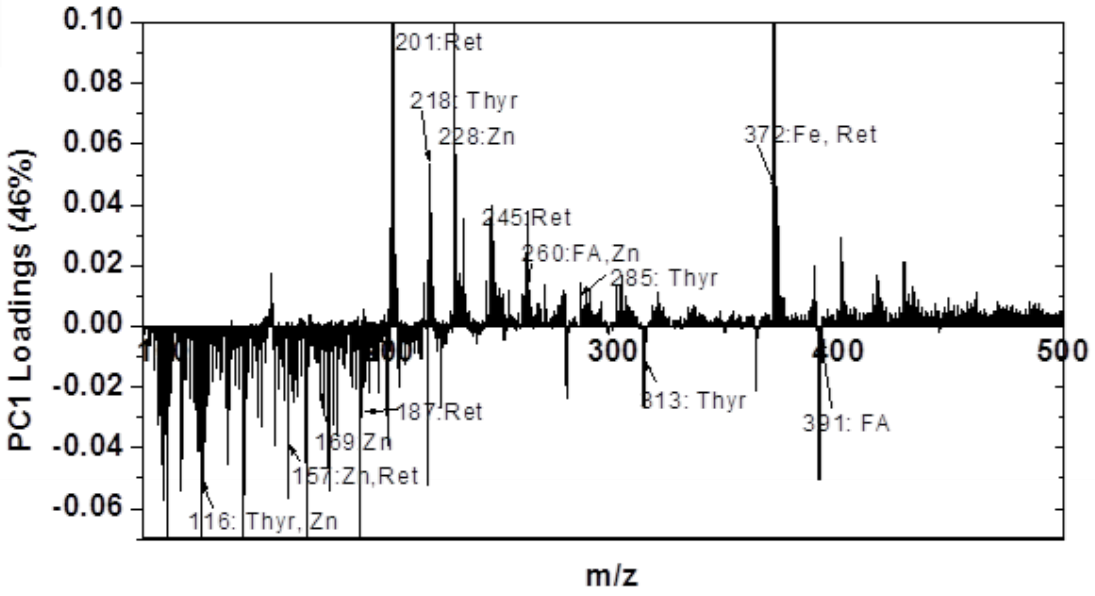




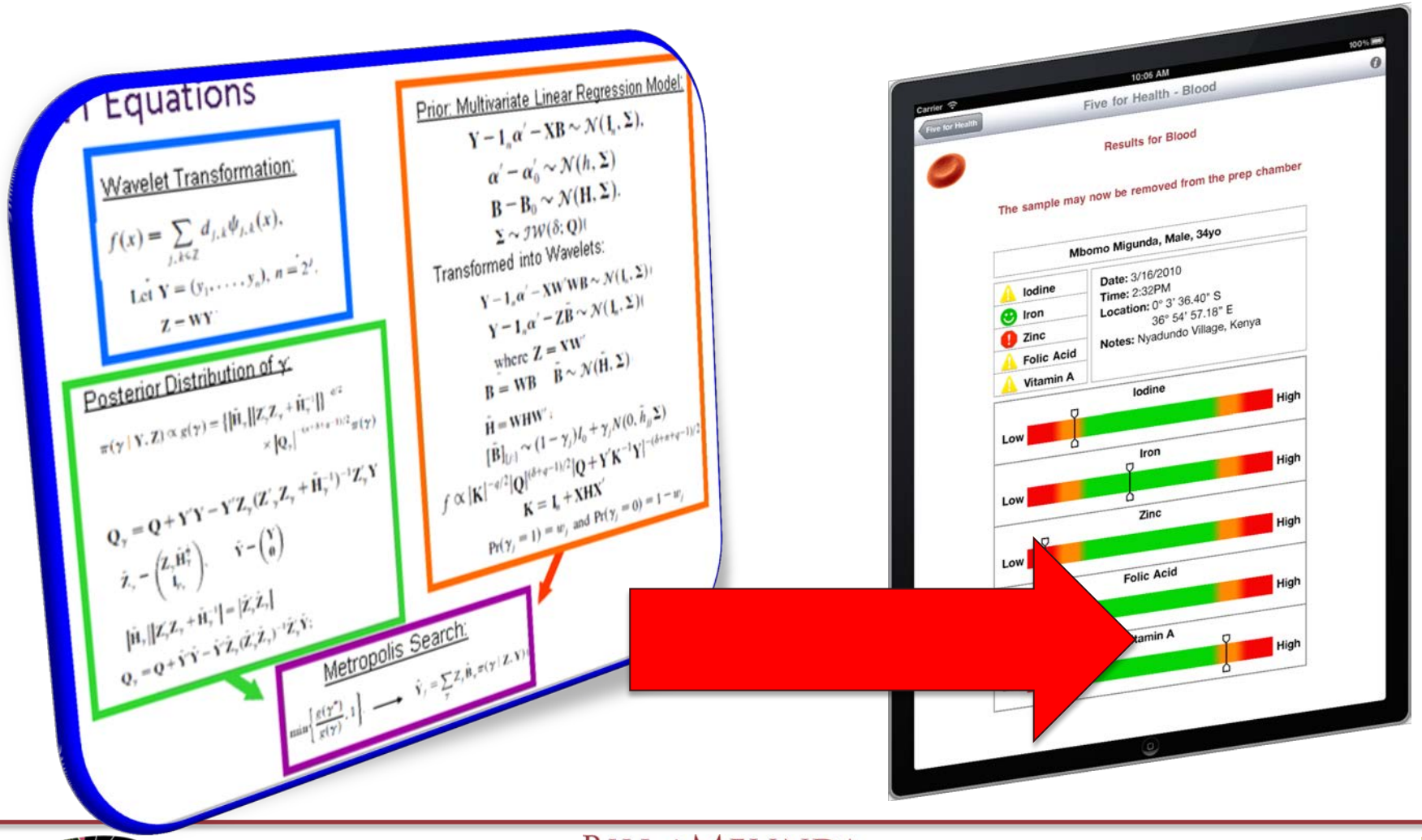
“Relatively Unhealthy” sample groups separate reasonably well



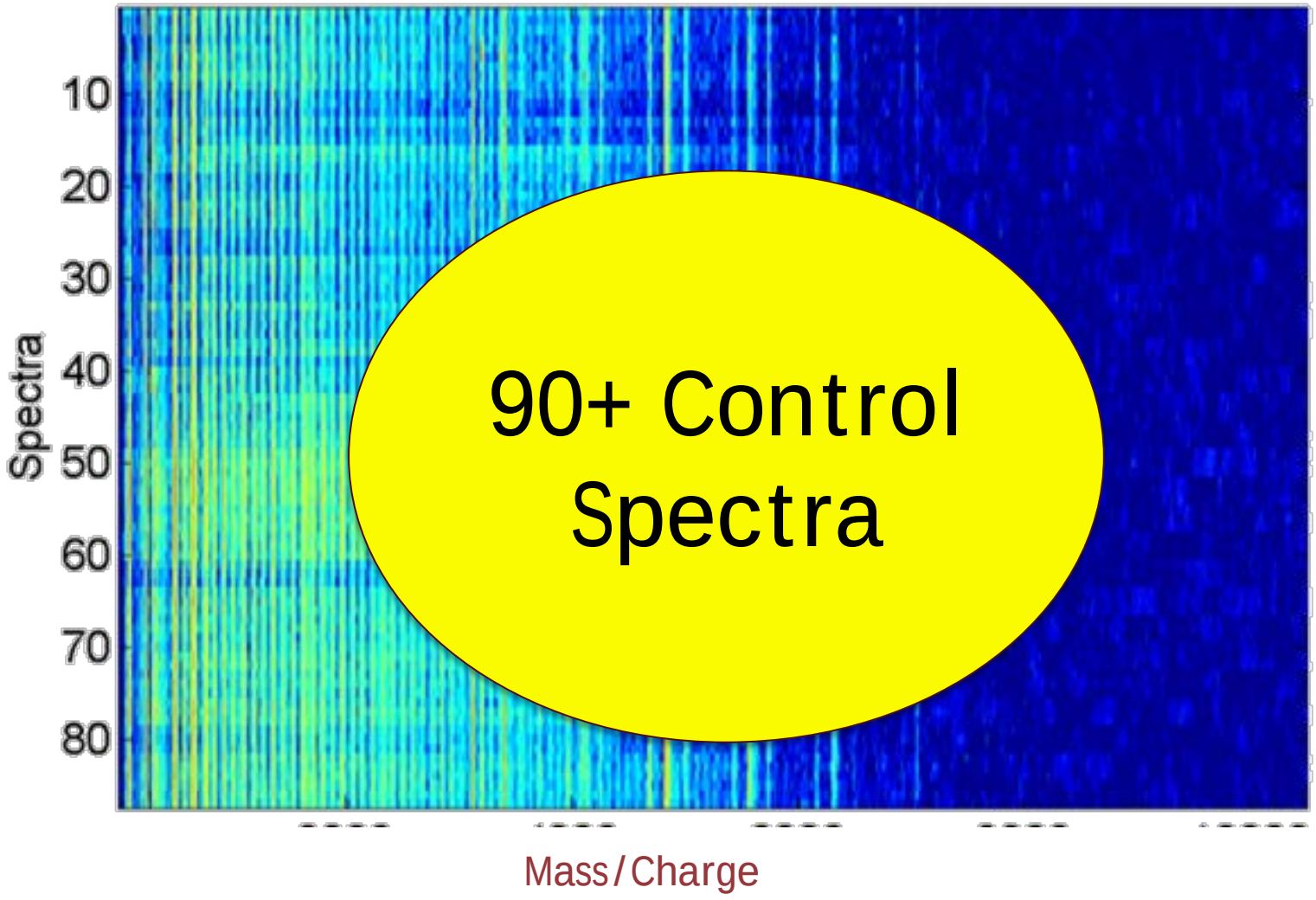




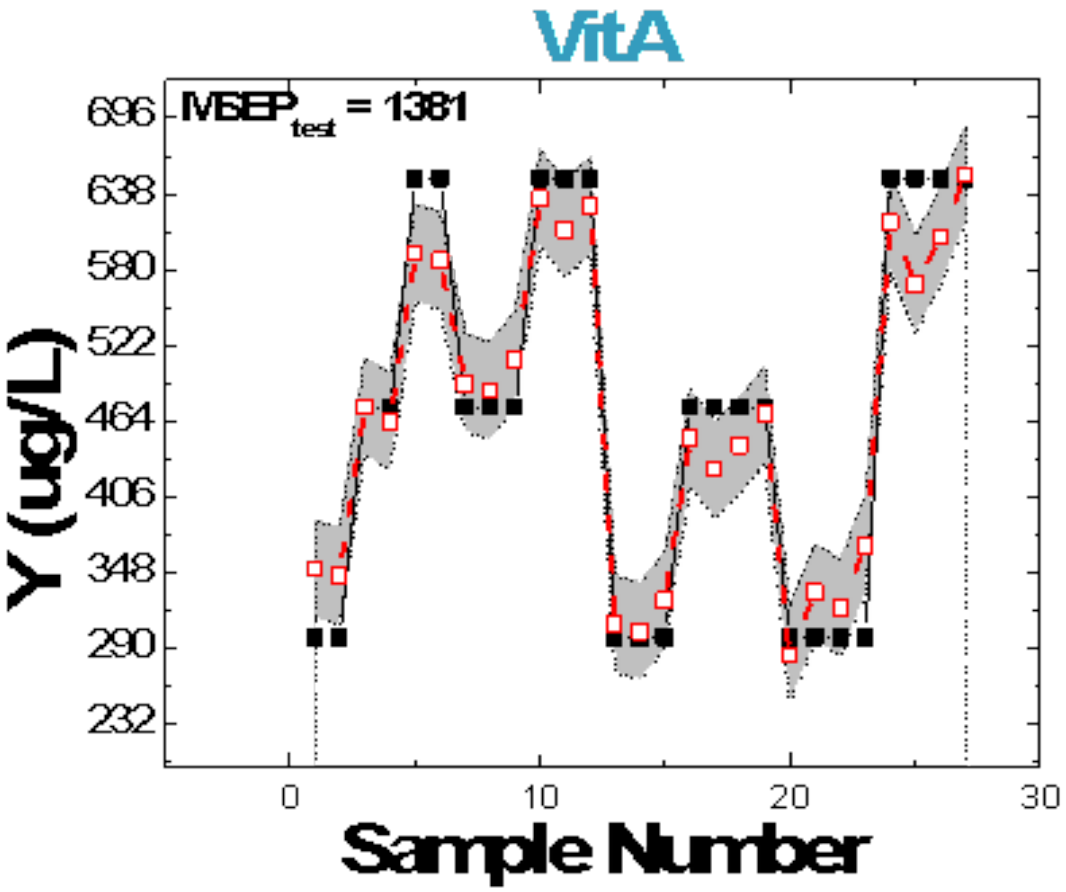
If you utilize a more complex mathematical model…



… And utilize a large number of spectra as controls…



… then one can begin to move from the qualitative to the quantitative

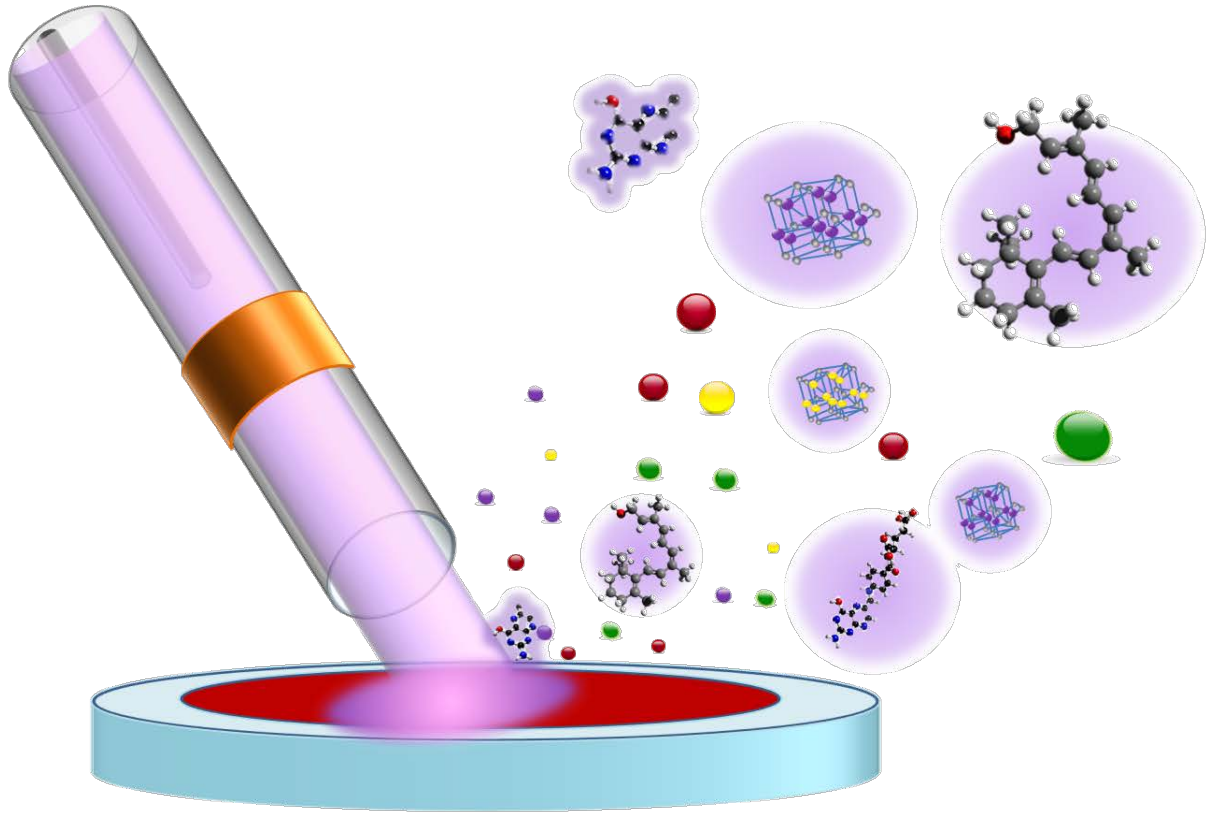


- Predicted Value
- Actual Value
- Matrix: Porcine Plasma

Strategic Advantages

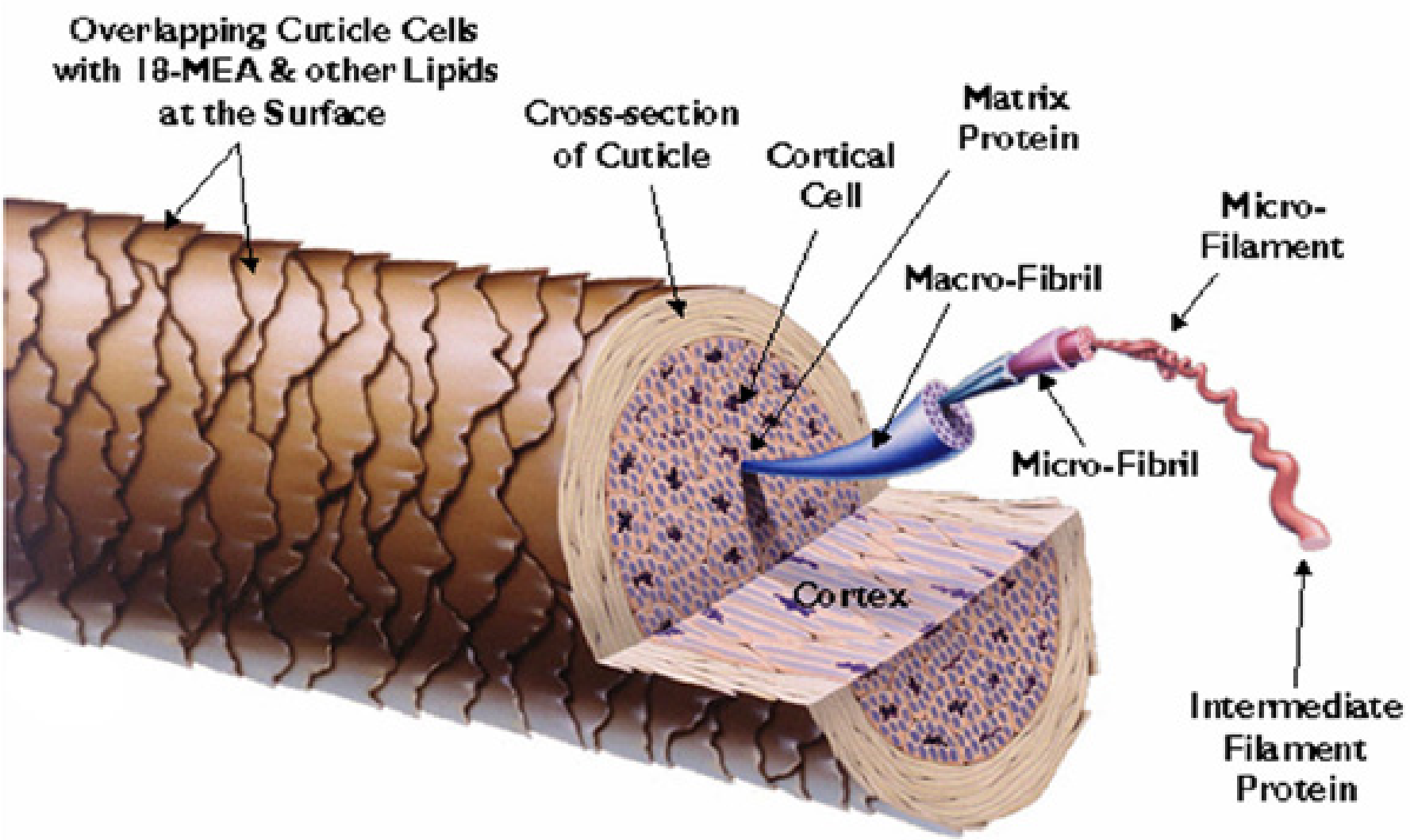
- PPAMS was rapid (~3-5 min), ~20 uL solution
- Physiological range of micronutrients in blood was within spectrometer detection range even in the presence of salt and proteins
- Multivariate PCA yielded simultaneous separation of the nutrients by type and quantity at both the lower ranges expected for a “malnourished” individual and at the higher ranges expected for a “well-nourished” individual.

Disruptive Technology for Measuring Nutrients and Nourishment



Venturing forth to measure new biological sample types

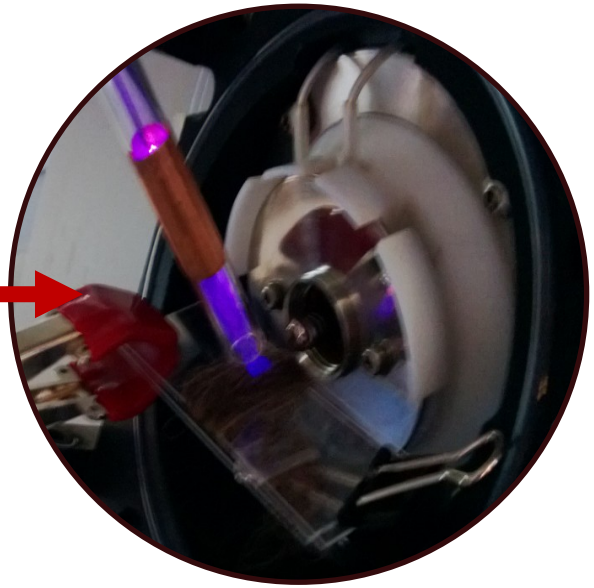
Physiology of Hair



PPAMS was taken on a fresh hair and cleaned hair samples

Hair obtained from donor

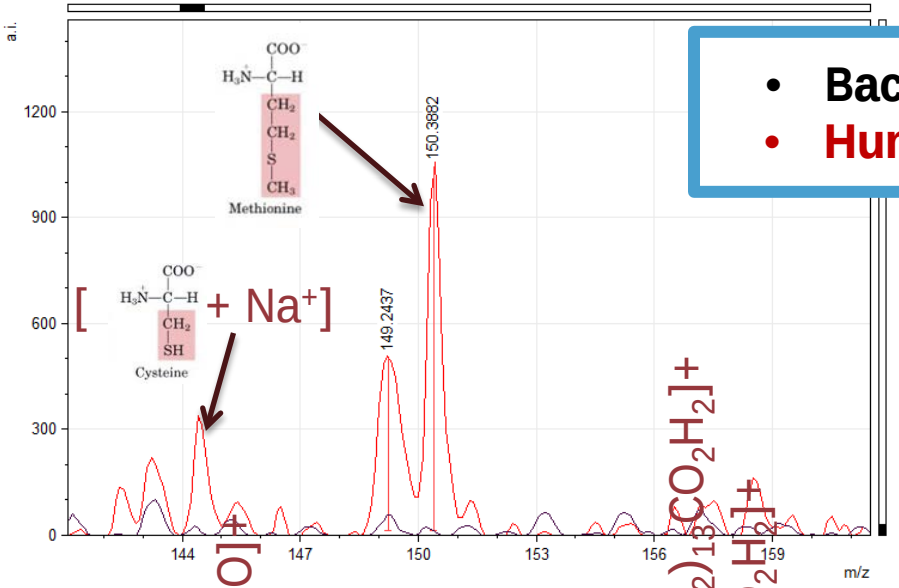
Analyzed “as-is”

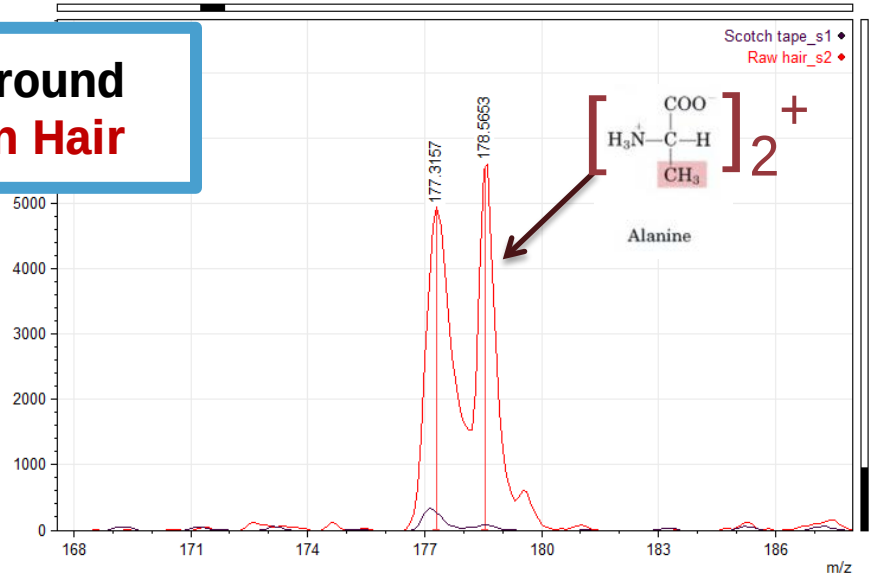


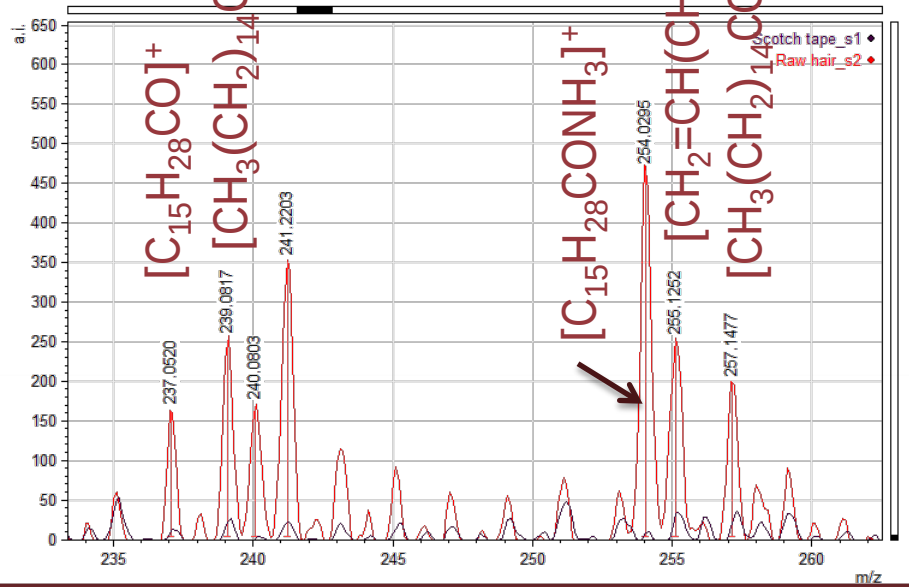
Analyzed via PPAMS

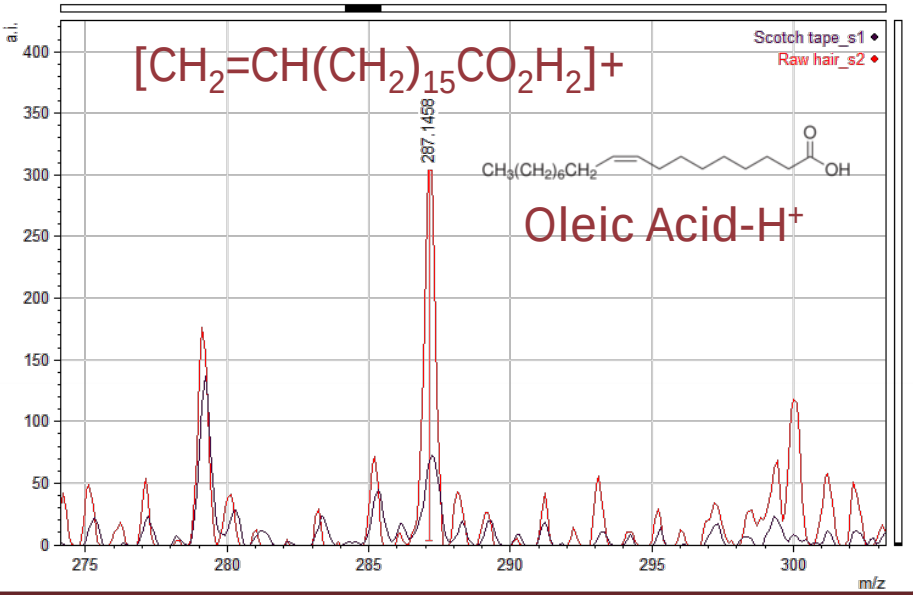
Several peaks were found to be present only in the sample of human hair

- **Background**
- **Human Hair**





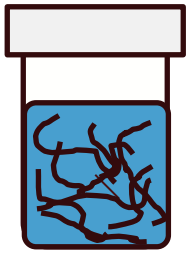




PPAMS was taken on a fresh fingernail clippings

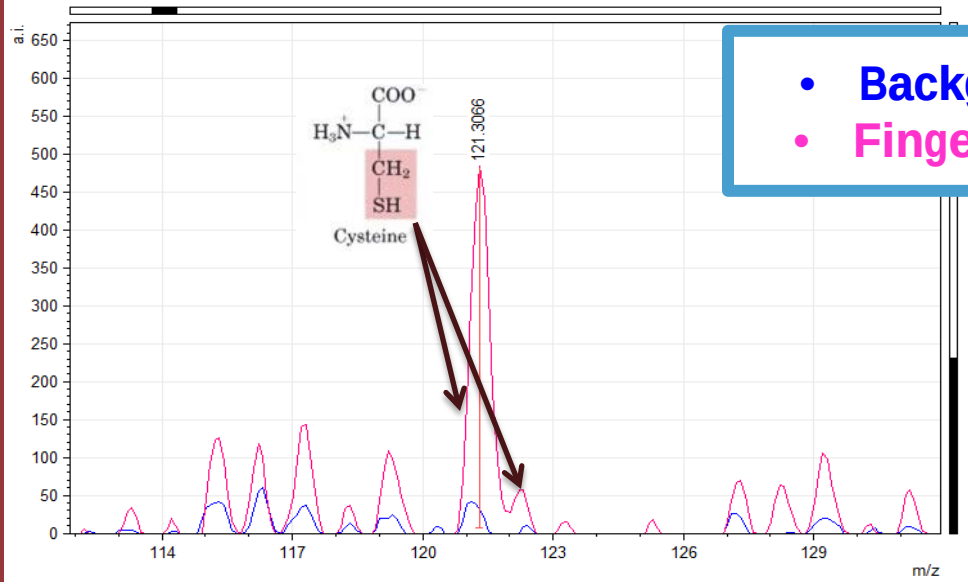
Fingernail clippings taken from healthy donor

Washed with 2% Triton X
Solution: Water , Acetone
Rinse

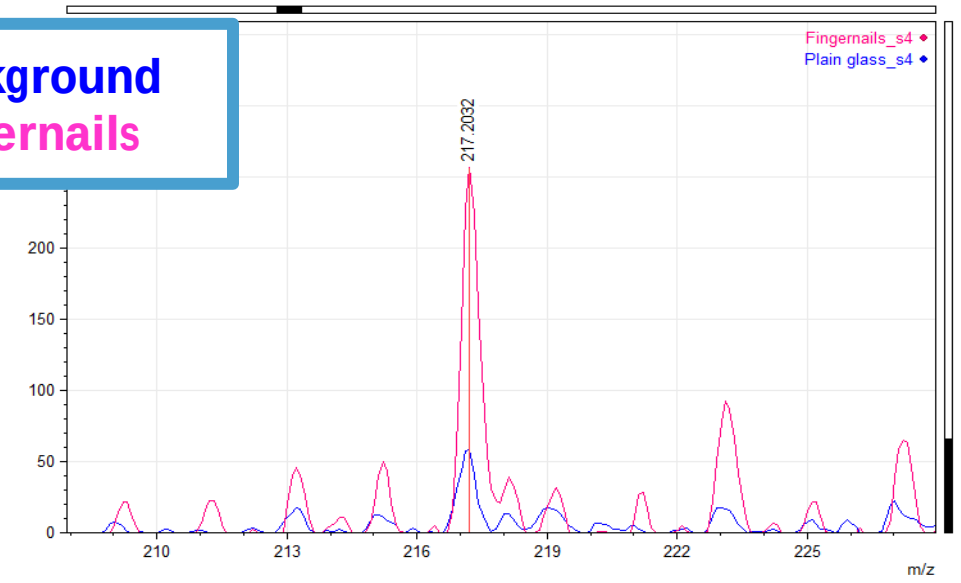


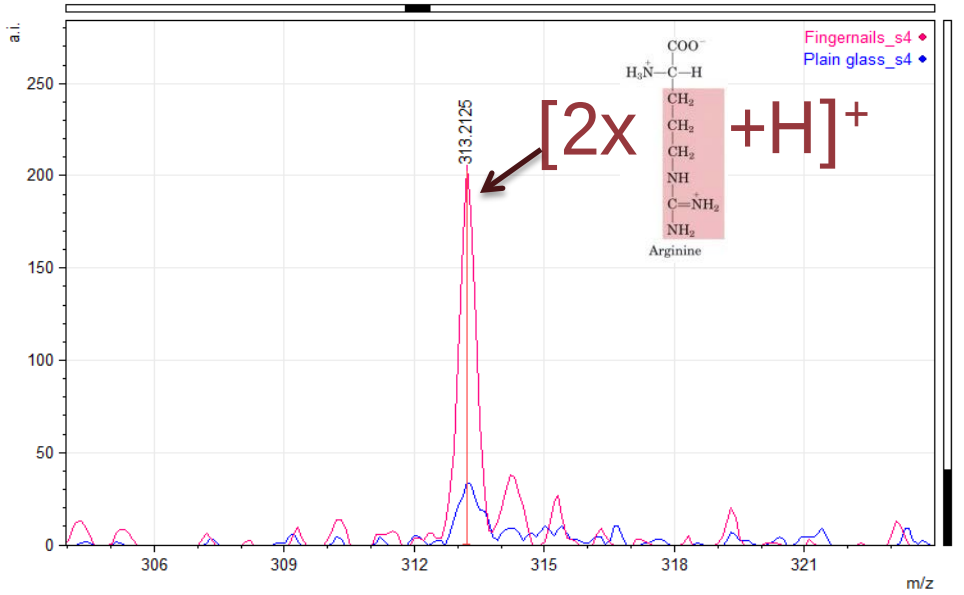
Analyzed via PPAMS

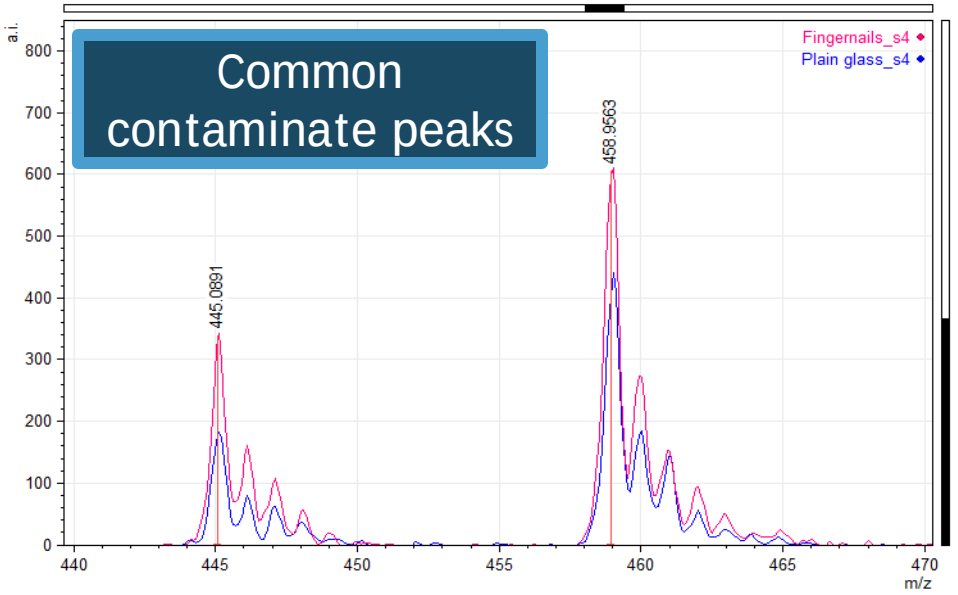
Several peaks were found to be present only in the sample of human fingernails



- **Background**
- **Fingernails**







PPAMS was taken on samples of raw, unpasteurized cow's milk with and without nutrient doping

Additional nutrients were added:

Zinc

Iron

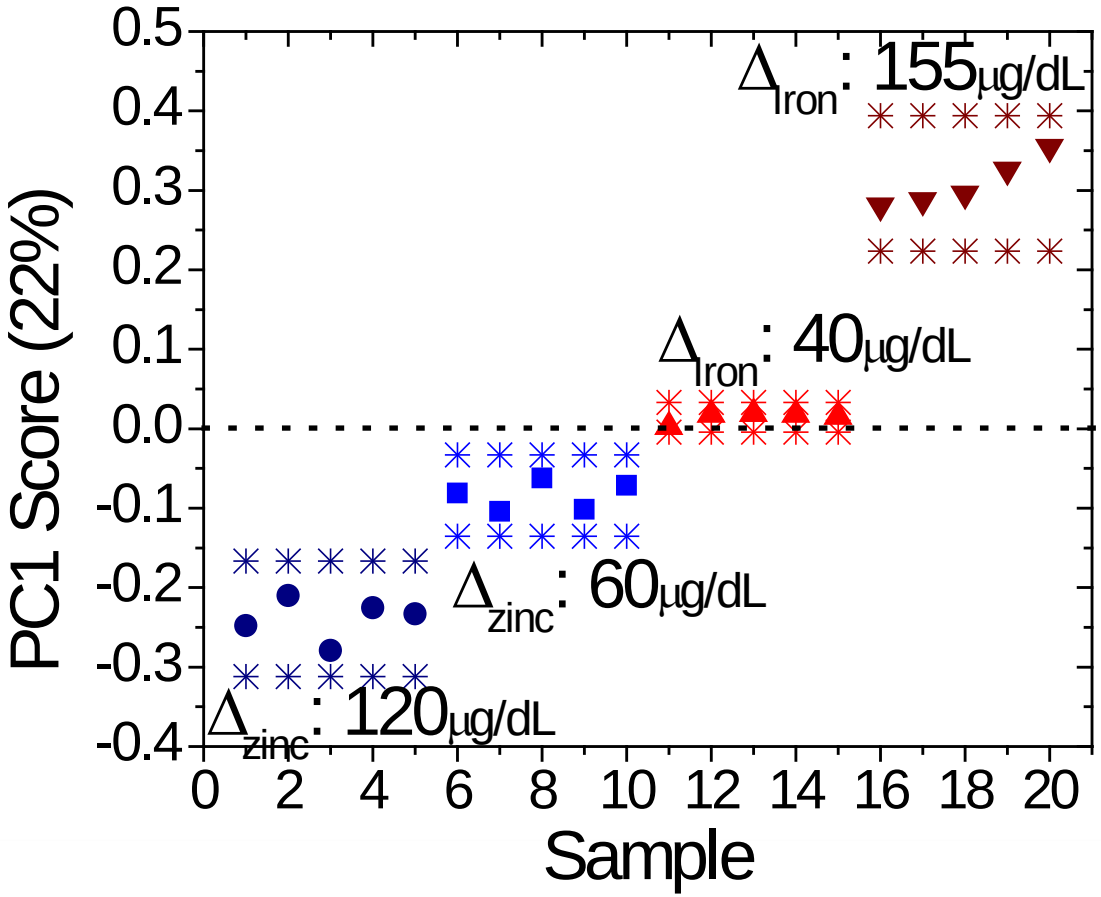
Vitamin A

Fingernail clippings taken from healthy adult

Deposited on a substrate and allowed to dry

Analyzed via PPAMS

PPAMS was able to distinguish changes in vitamin A and iron content in unpasteurized, raw cow's milk



Venturing forth to measure new biological sample types

What is the best measurement of nourishment in a population?

Acknowledgments

Project Advising:
- Buddy D. Ratner, PhD
- David G. Castner, PhD

Project Team:
- M. Jeanette Stein
- Edward Lo
- Joelle Roelfs
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- Lurdes Inoue, PhD
- Laura Sangare, PhD
- Collin Waterton

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- James Bollinger (Mass Spec)
- William Beaty (Plasma Pencil Construction)

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- Daniel J. Graham, PhD
- Bonnie Tyler, PhD
- Ratner lab
- Castner lab
- Chris Barnes, PhD (Project Alumni)
- Marvi A. Matos, PhD (Project Alumni)

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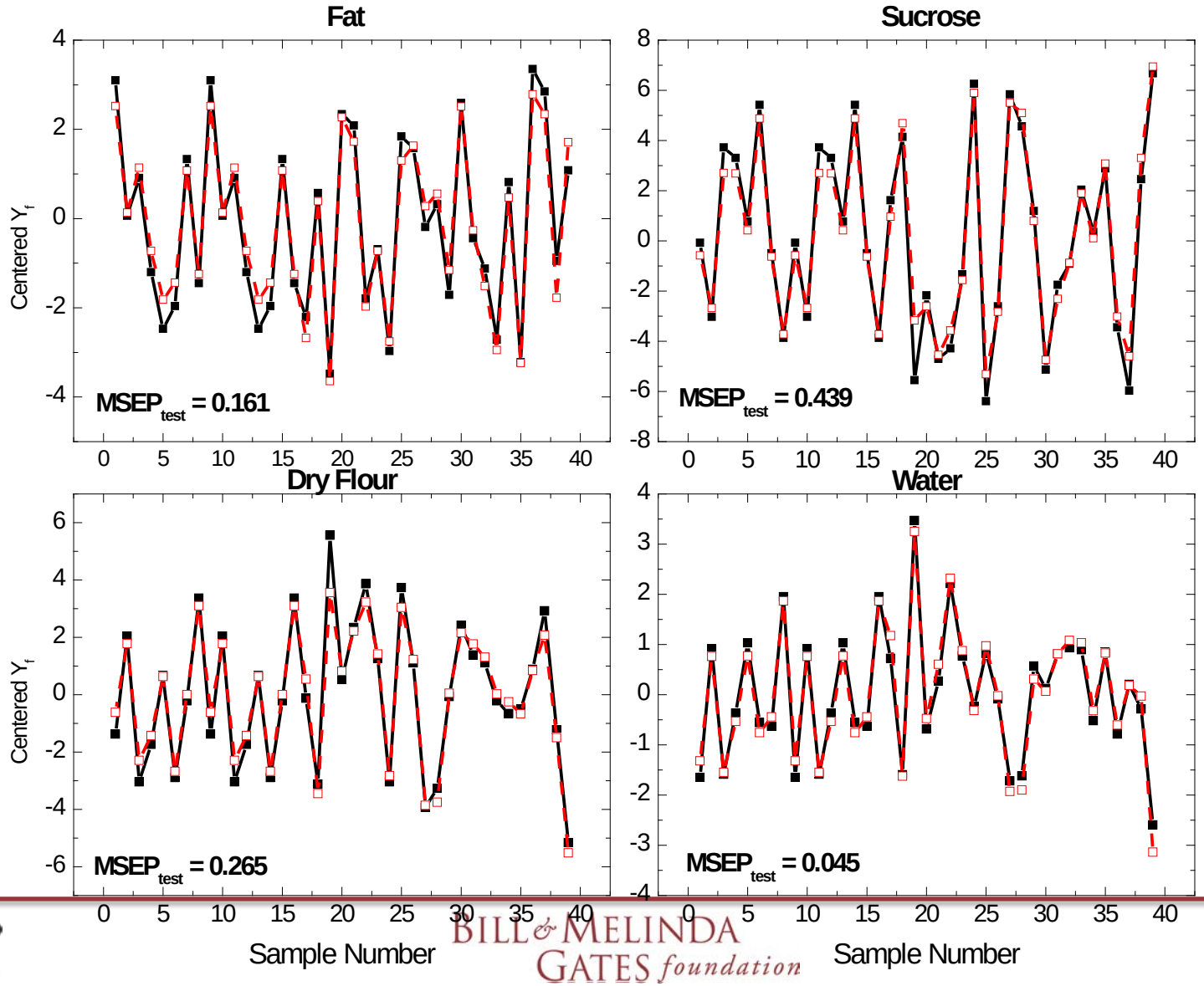
BILL & MELINDA GATES foundation

Biscuit Dough Composition Results



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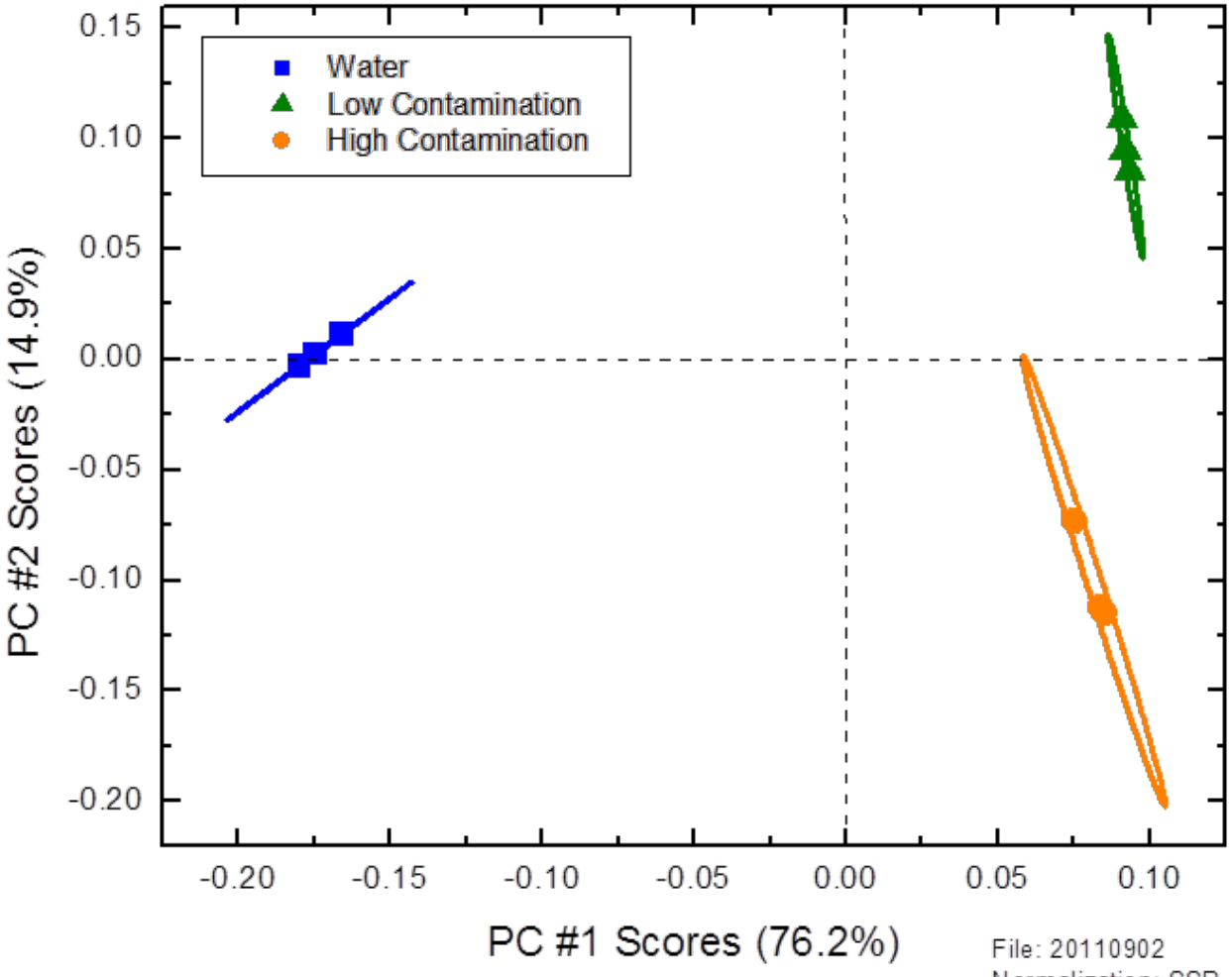
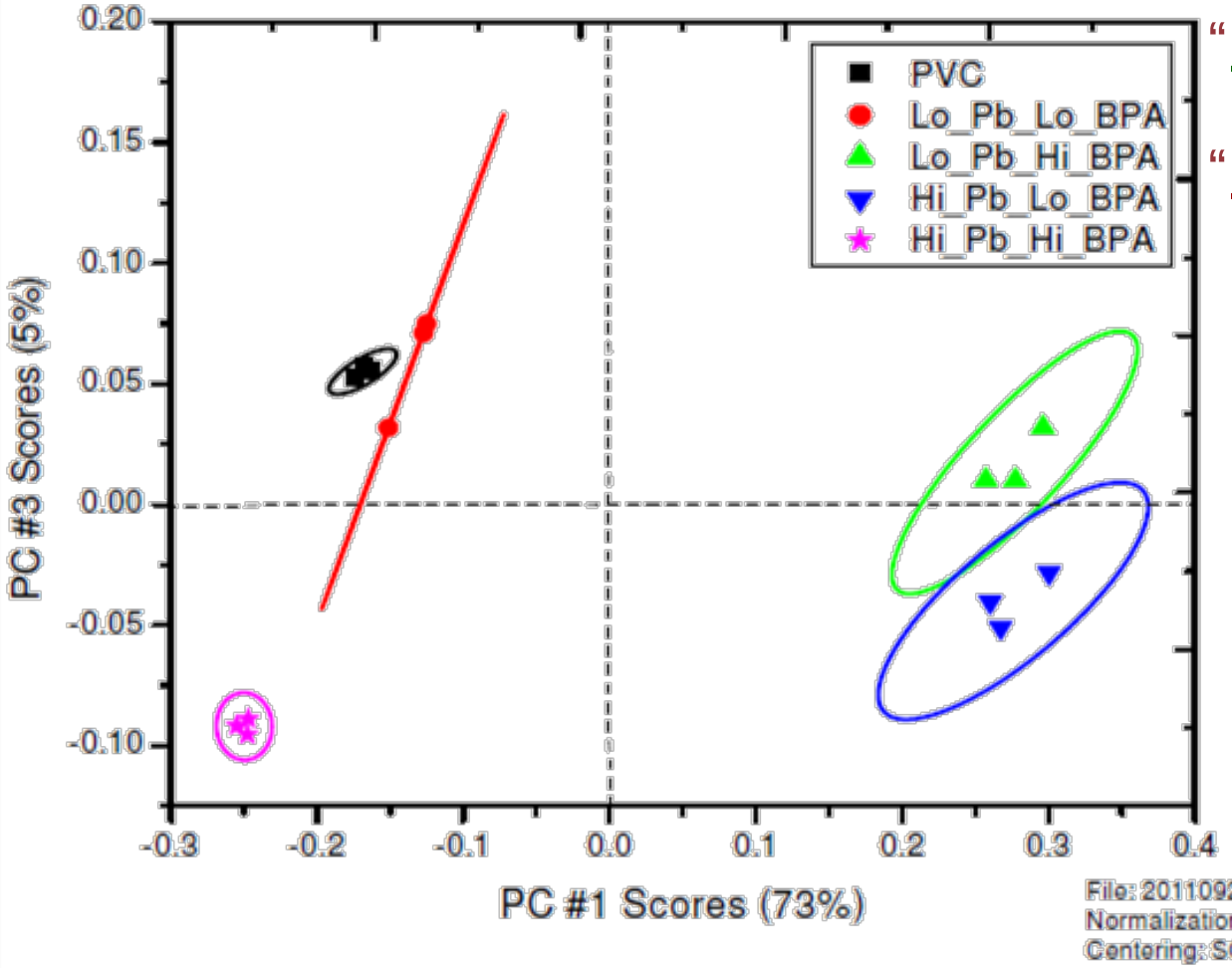
Water Safety: Lead, Copper & Zinc



"**Low**" Levels = Pass Safety

"**High**" Levels = Fail Safety

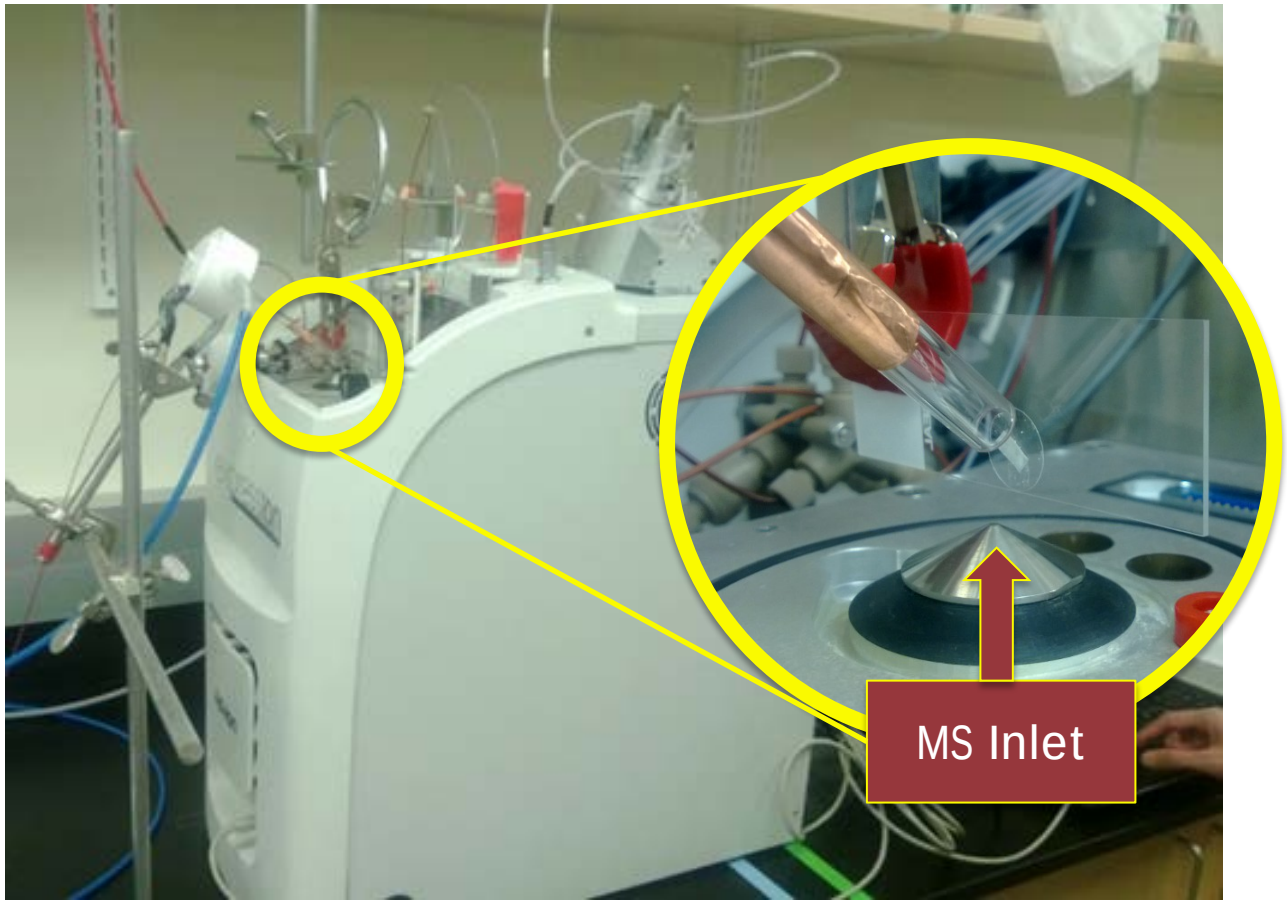
Toy Safety: BPA and Lead in PVC



"**Low**" Levels = Pass Safety

"**High**" Levels = Fail Safety

Plasma Pencil Atmospheric Mass Spec



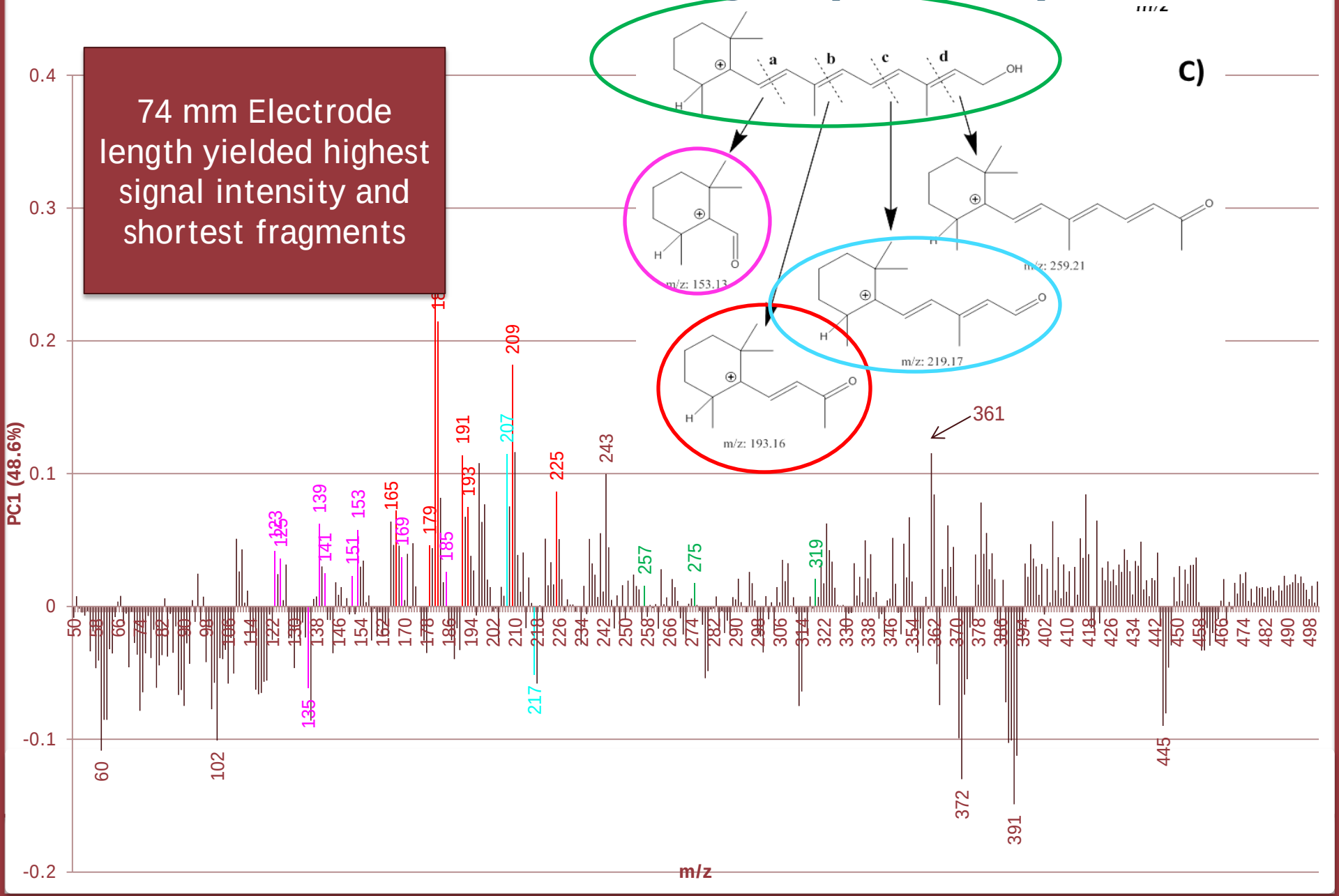
Other options:

Analysis via hair, fingernails, skin, saliva, urine

Melanoma detection
Esophageal cancer analysis
Disease diagnosis based on breath analysis
Food Analysis (spoilage, origin, authenticity)
Wine analysis (grape? Quality? Adulteration?)
Perfume analysis
Counterfeit detection (drugs, consumer goods)
Airport security, explosives detection
Industry quality control
Toys
Water supplies and water safety
Mineral identification

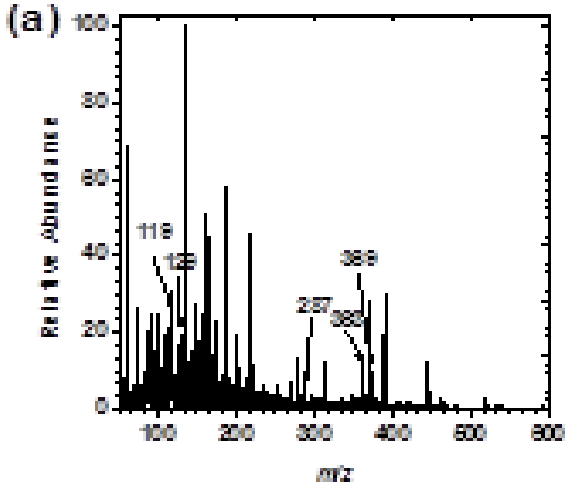
PCA- Loadings (zoom)

74 mm Electrode length yielded highest signal intensity and shortest fragments

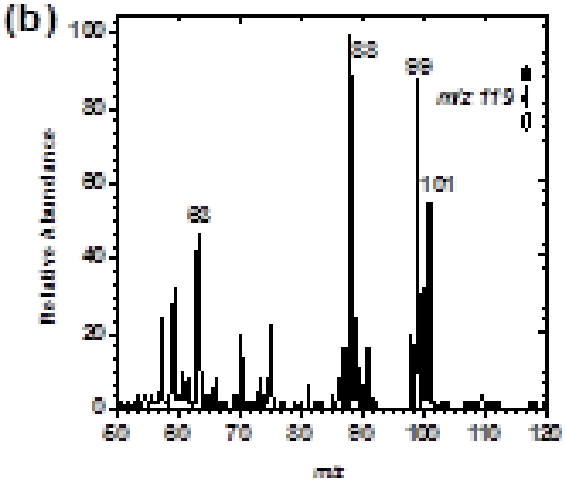


Positive ion PPAMS and PPAMS/MS spectra on a mixed HBLC nutrient sample in methanol

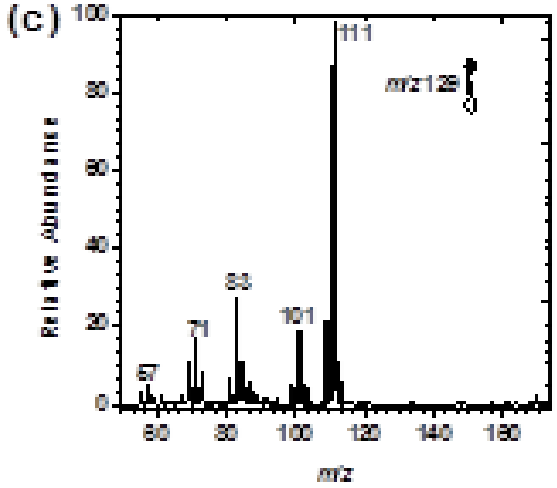
MS Spectrum



MS/MS for Zn



MS/MS for Fe



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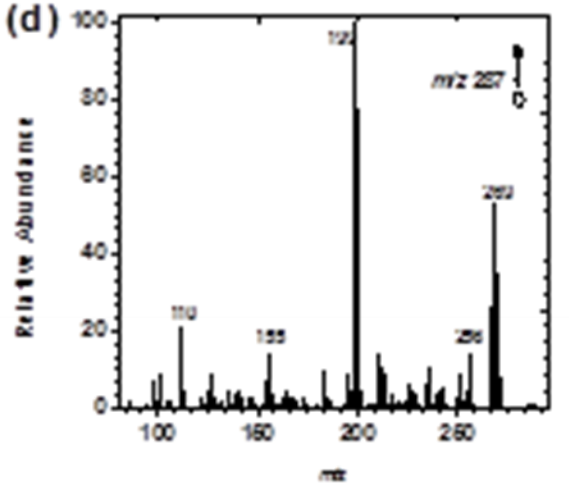
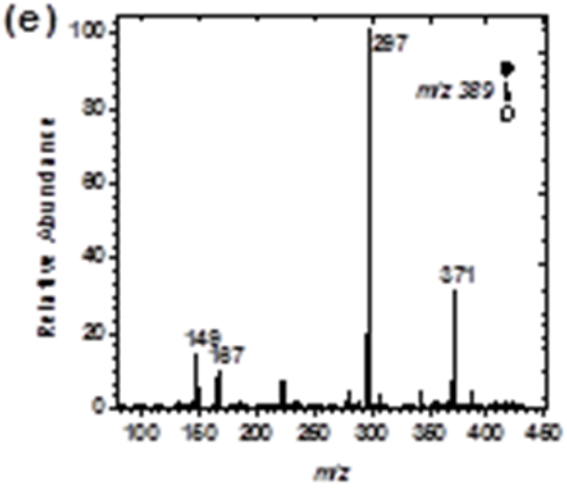
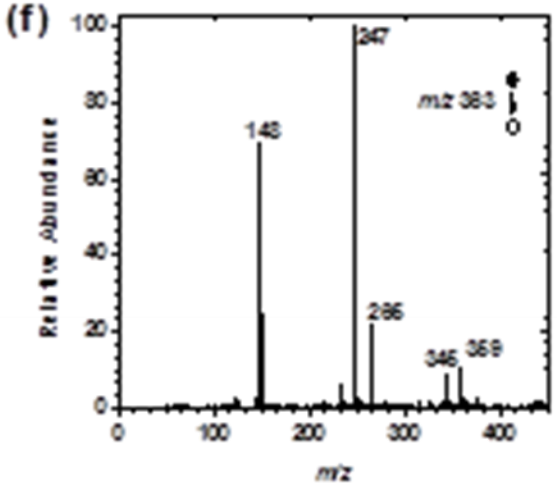


MS/MS for FA

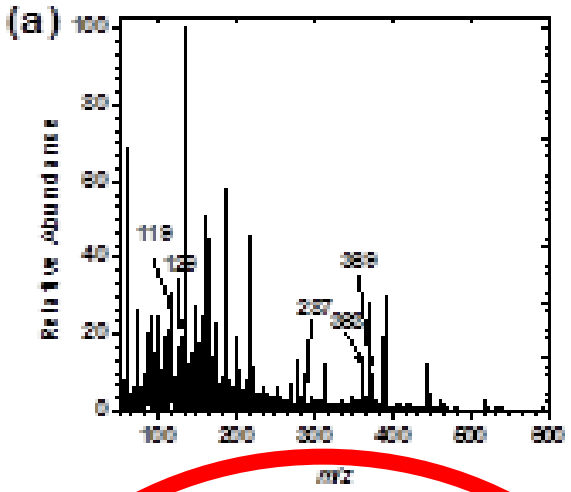


MS/MS for Thyr

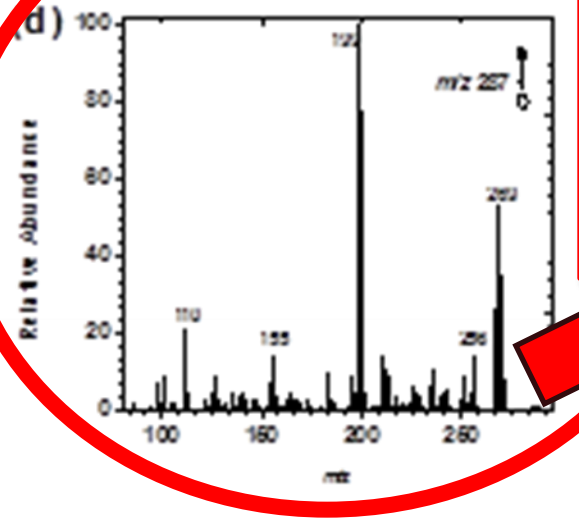


Positive ion PPAMS and PPAMS/MS spectra on a mixed HBLC

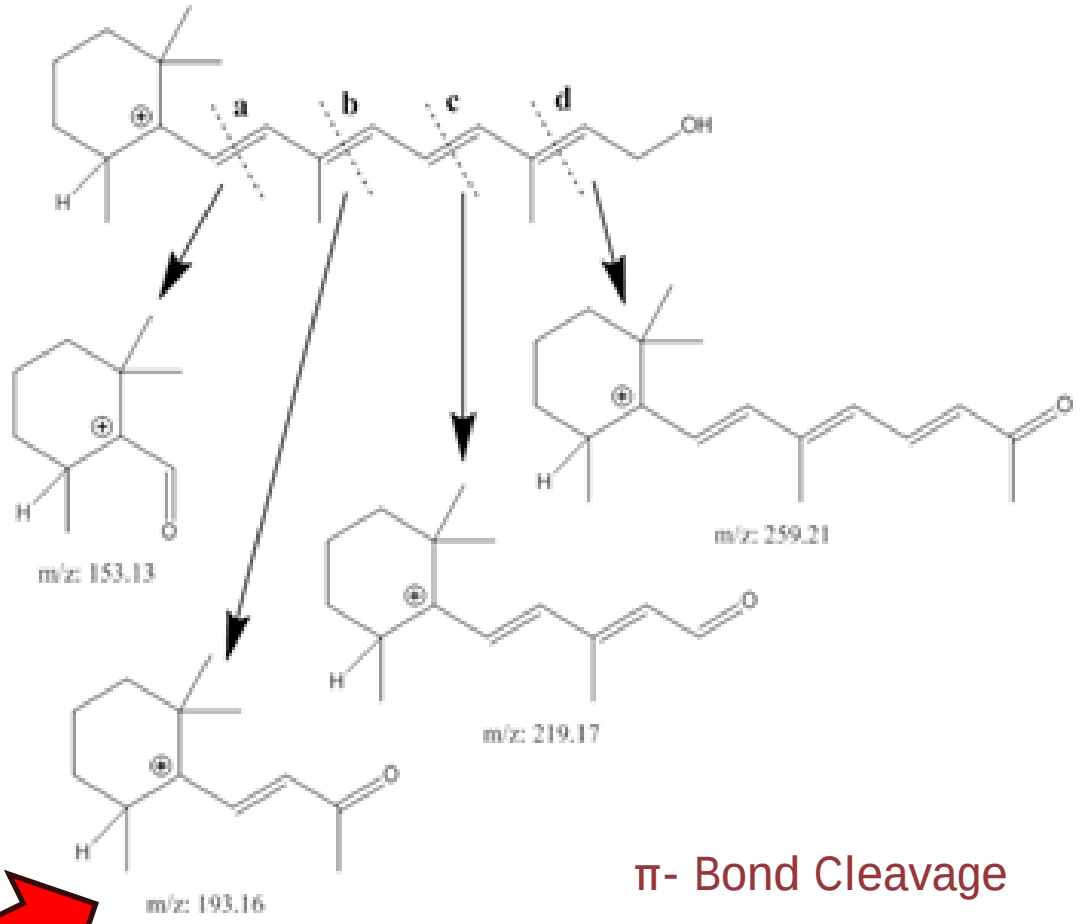
MS Spectrum



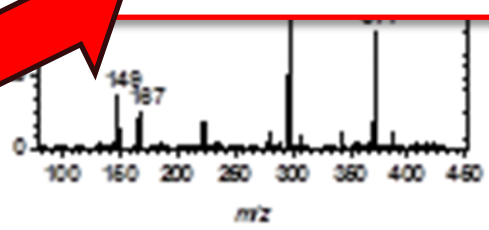
MS/MS for Ret

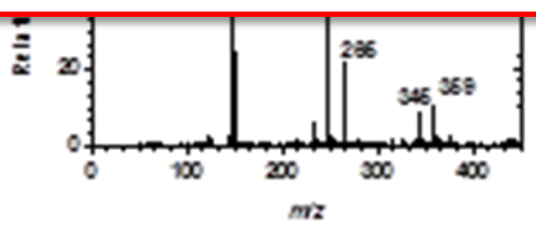


MS/MS 287: Retinol Molecule



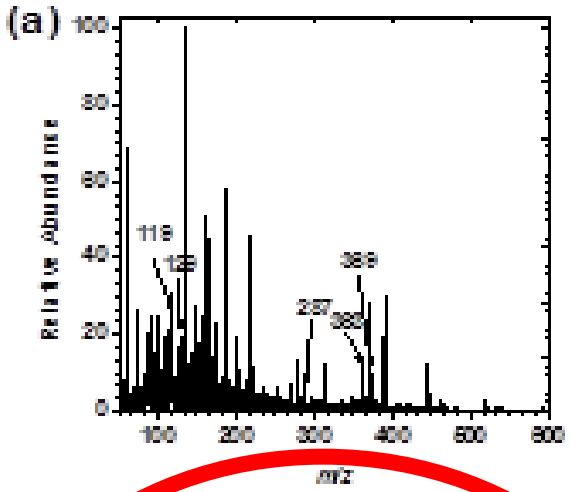
π- Bond Cleavage



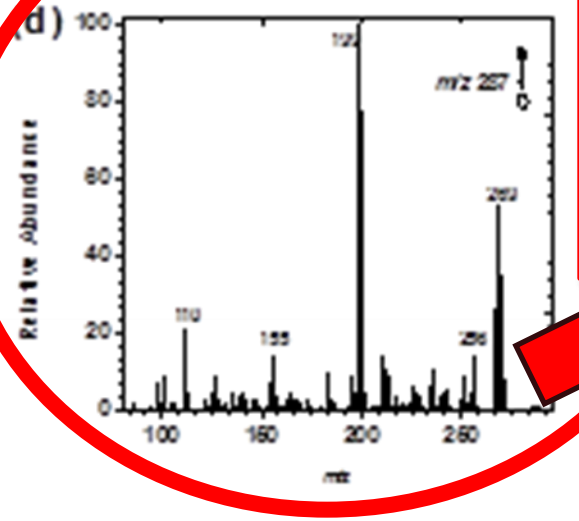


Positive ion PPAMS and PPAMS/MS spectra on a mixed HBLC

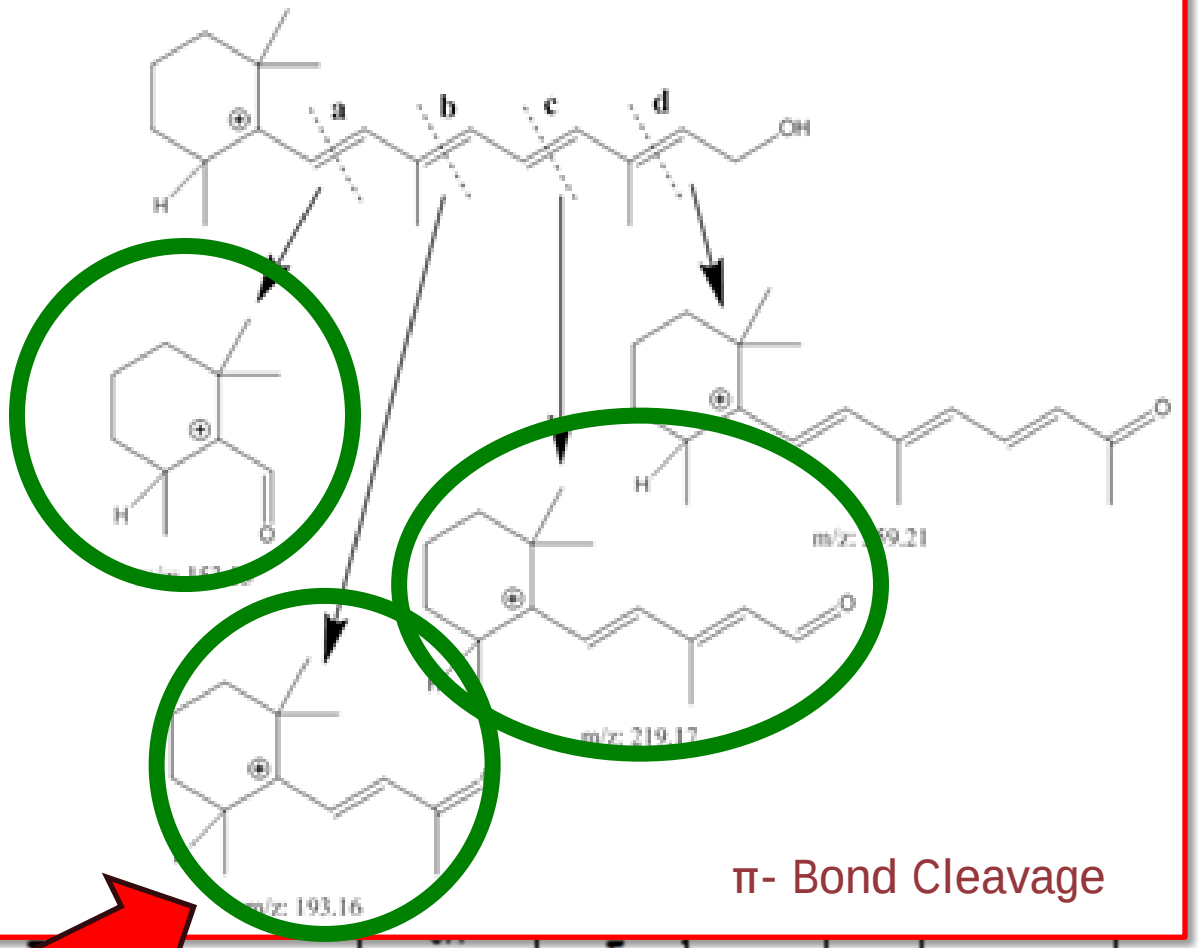
MS Spectrum



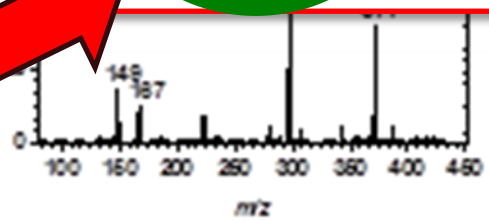
MS/MS for Ret

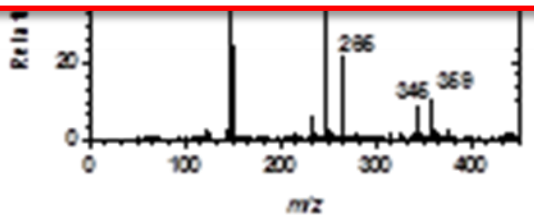


MS/MS 287: Retinol Molecule



π- Bond Cleavage



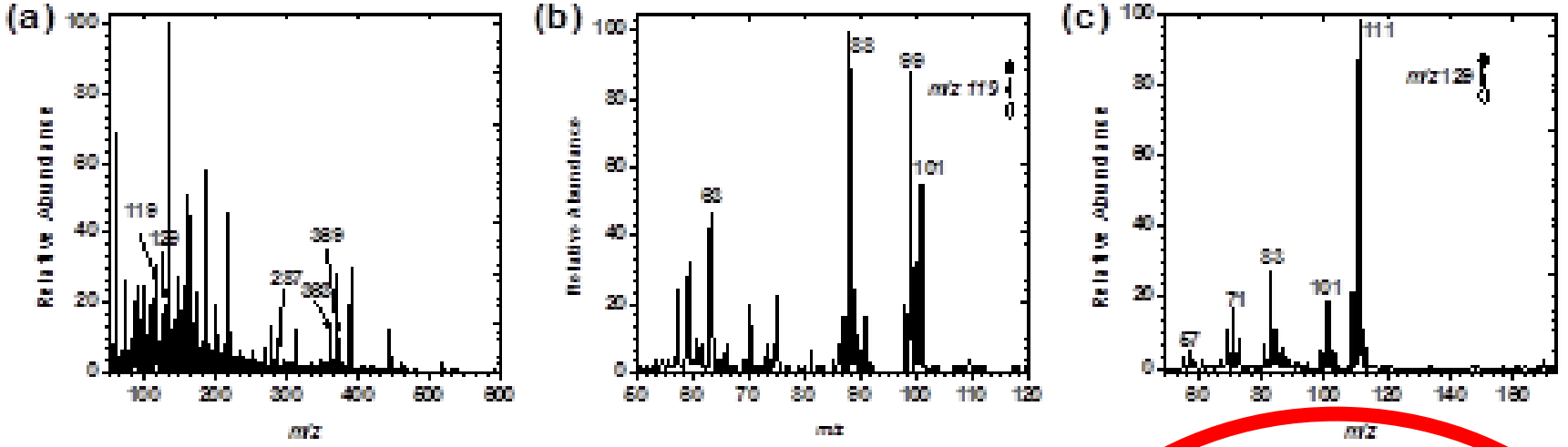


Positive ion PPAMS and PPAMS/MS spectra on a mixed HBLC nutrient sample in methanol

MS Spectrum

MS/MS for Zn

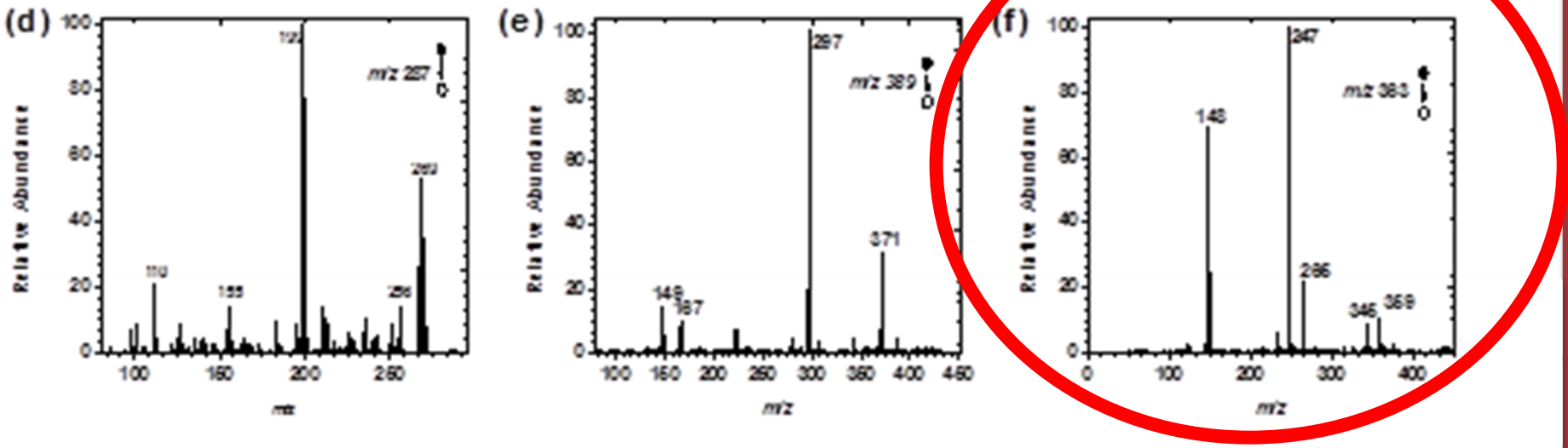
MS/MS for Fe



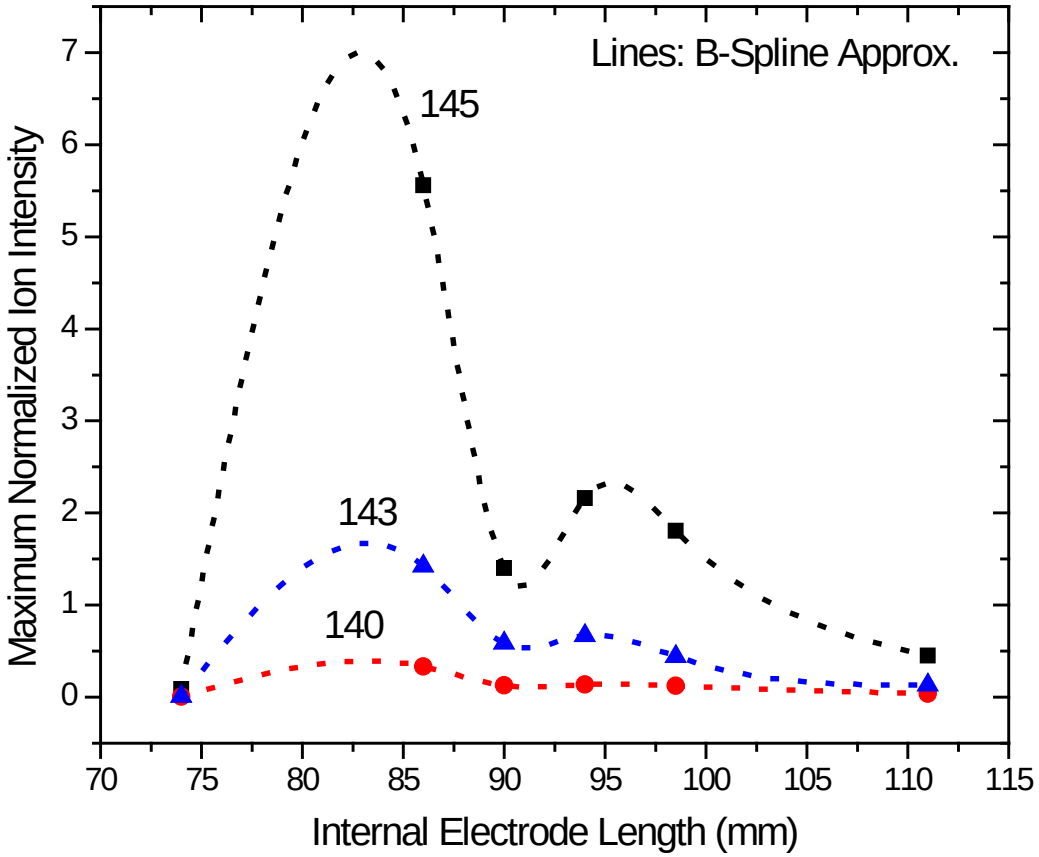
MS/MS for Ret

MS/MS for FA

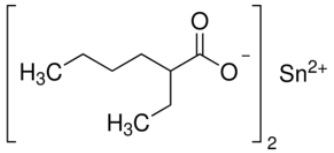
MS/MS for Thyr



Variation of normalized signal intensities for some ions observed for $Sn(Oct)_2$

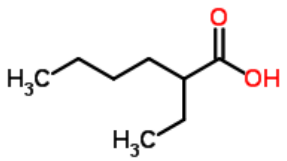


Stannous Octoate



145:
2-Ethylhexanoic acid

+



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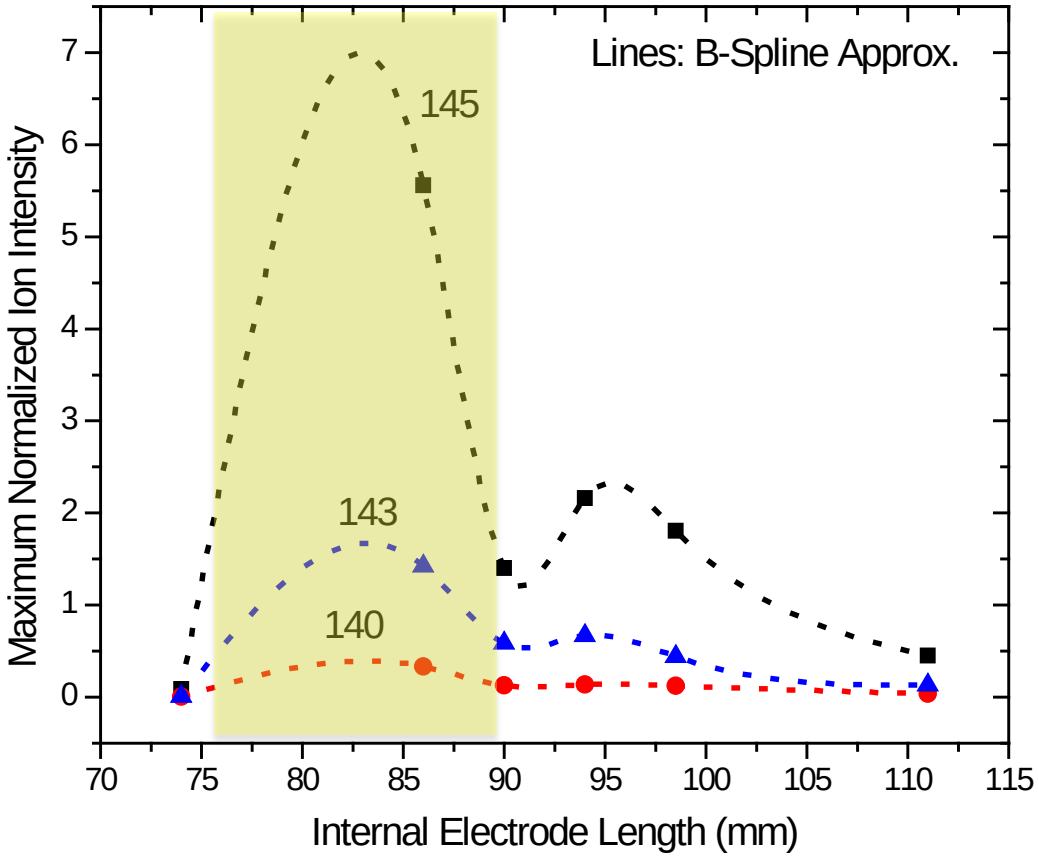

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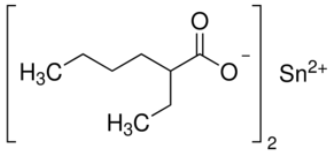

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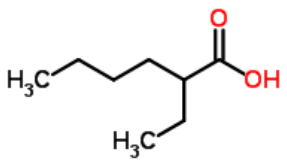
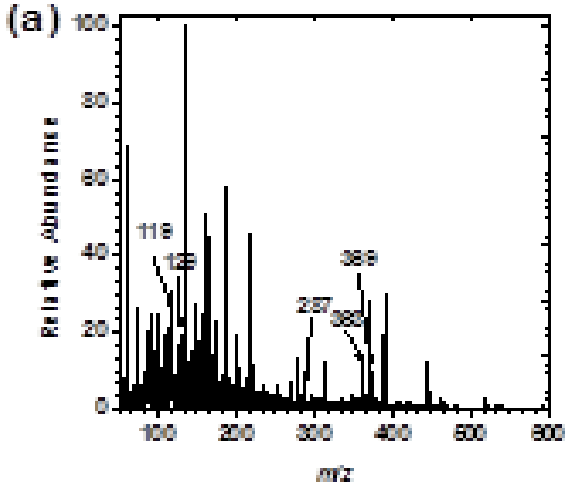


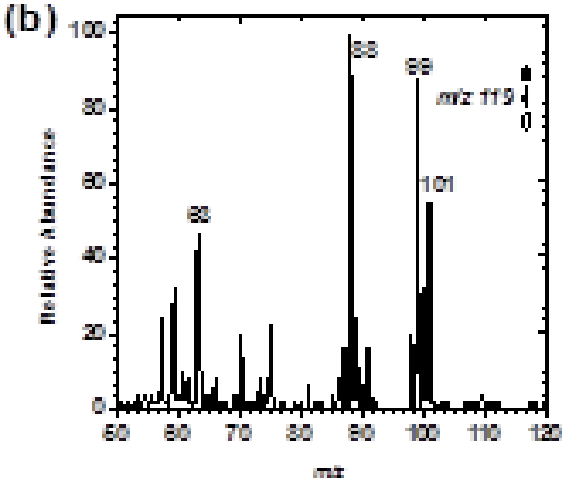
END

Positive ion PPAMS and PPAMS/MS spectra on a mixed HBLC nutrient sample in methanol

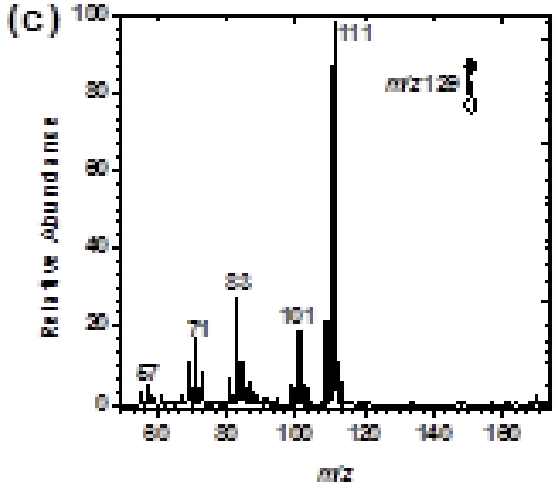
MS Spectrum



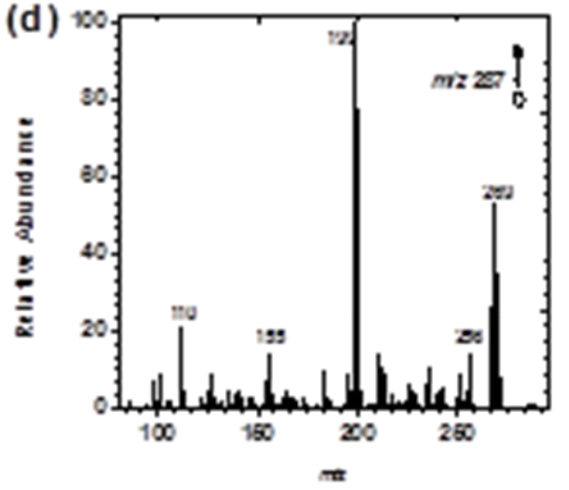
MS/MS for Zn



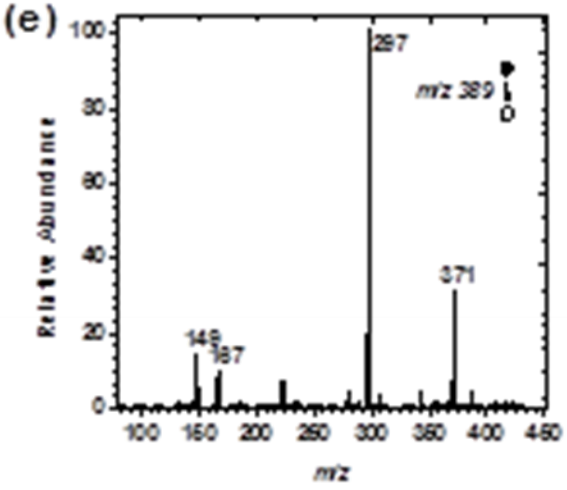
MS/MS for Fe



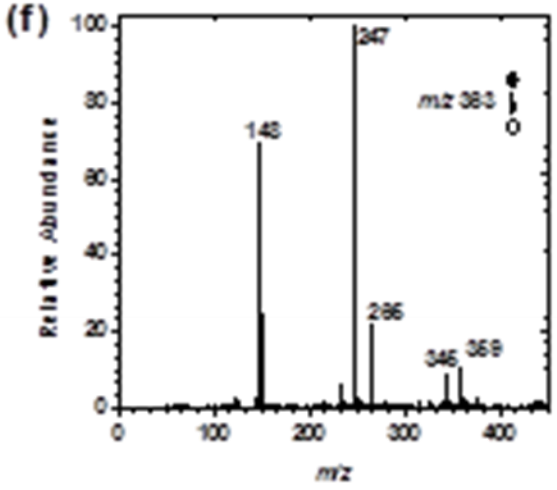
MS/MS for Ret



MS/MS for FA



MS/MS for Thyr



Positive ion PPAMS and PPAMS/MS spectra on a mixed HBLC

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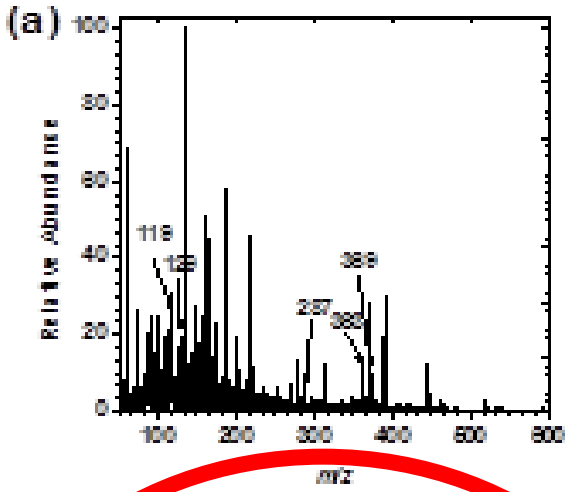
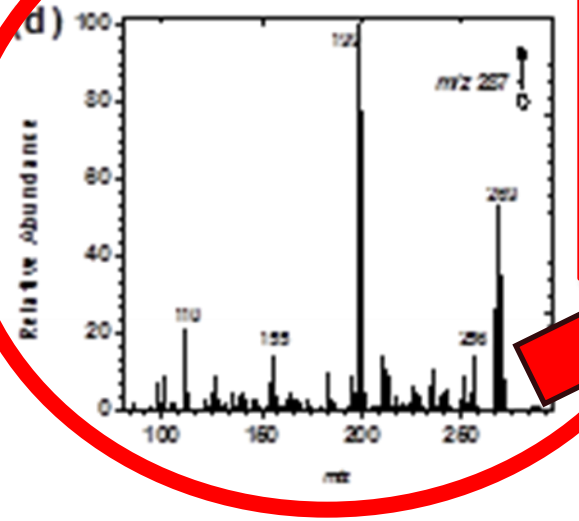
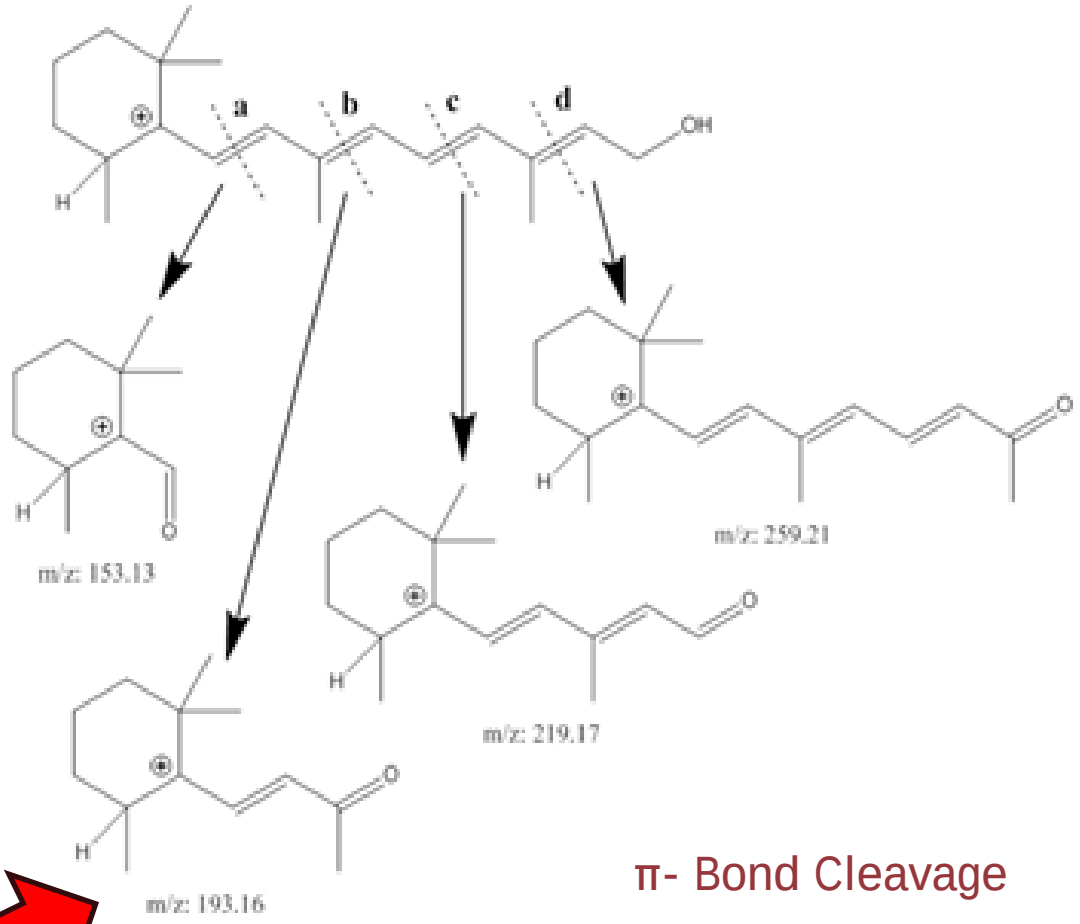


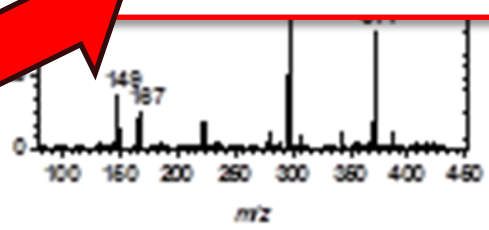
MS/MS for Ret

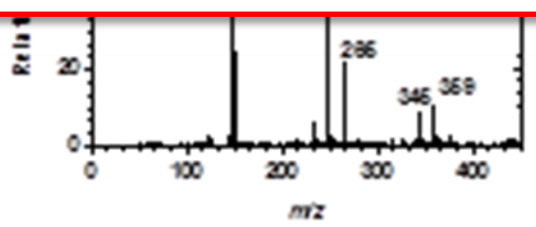


MS/MS 287: Retinol Molecule



π- Bond Cleavage





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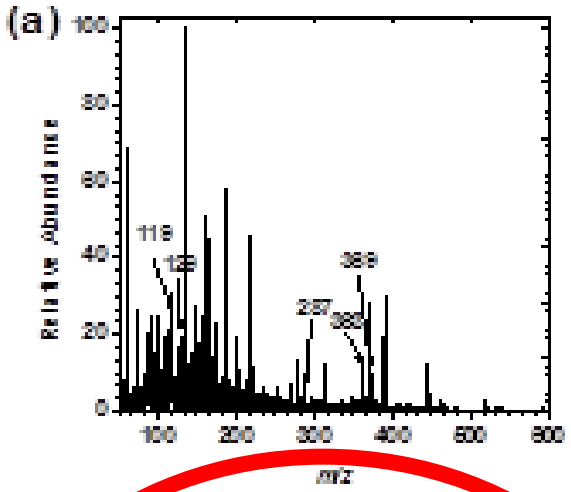

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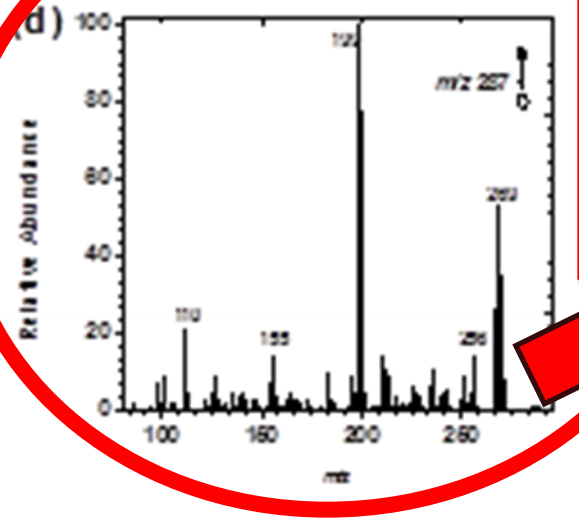

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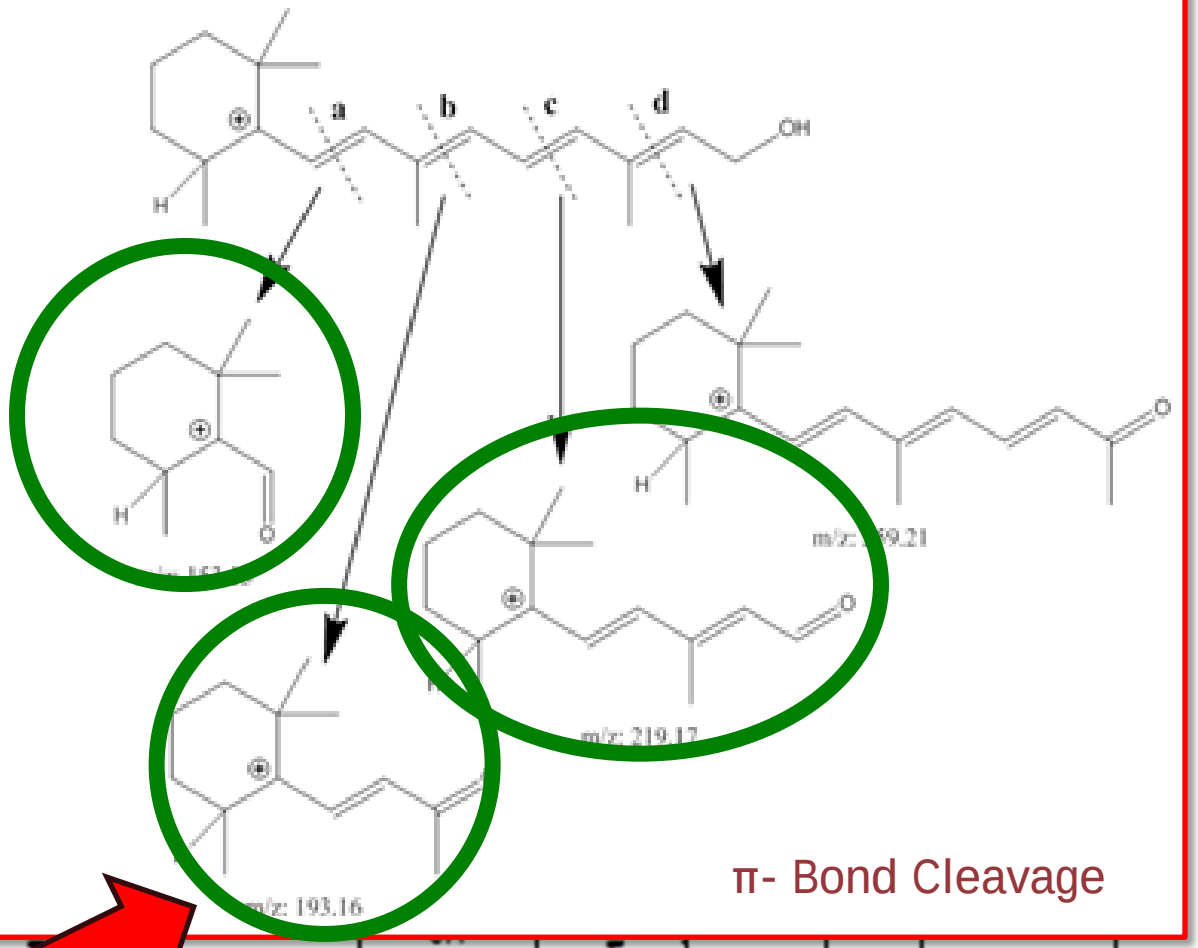

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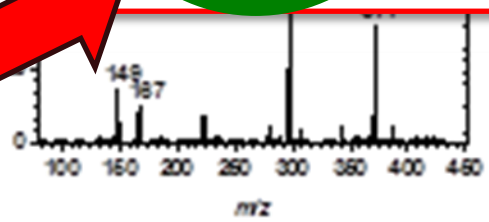
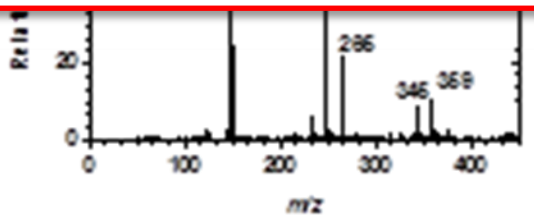
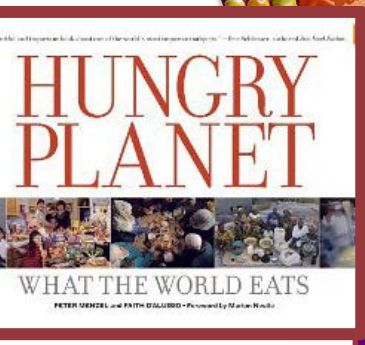




Magic wand



Iodine Deficiency

Moderate Deficiency	Mild Deficiency	Ideal	At Risk (hyperthyroidism)	Adverse Health Risks

Food and Nutrition Bulletin, **29** (3): 195-202 (2008)

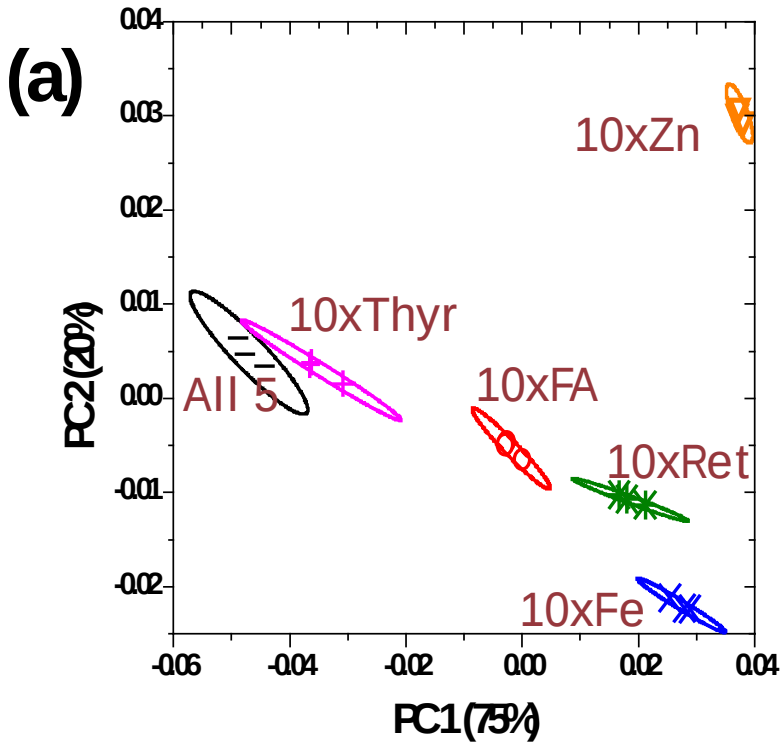
Multivariate Data Analysis

Analytical and clinical chemistry generally focuses on one test to measure each analyte?

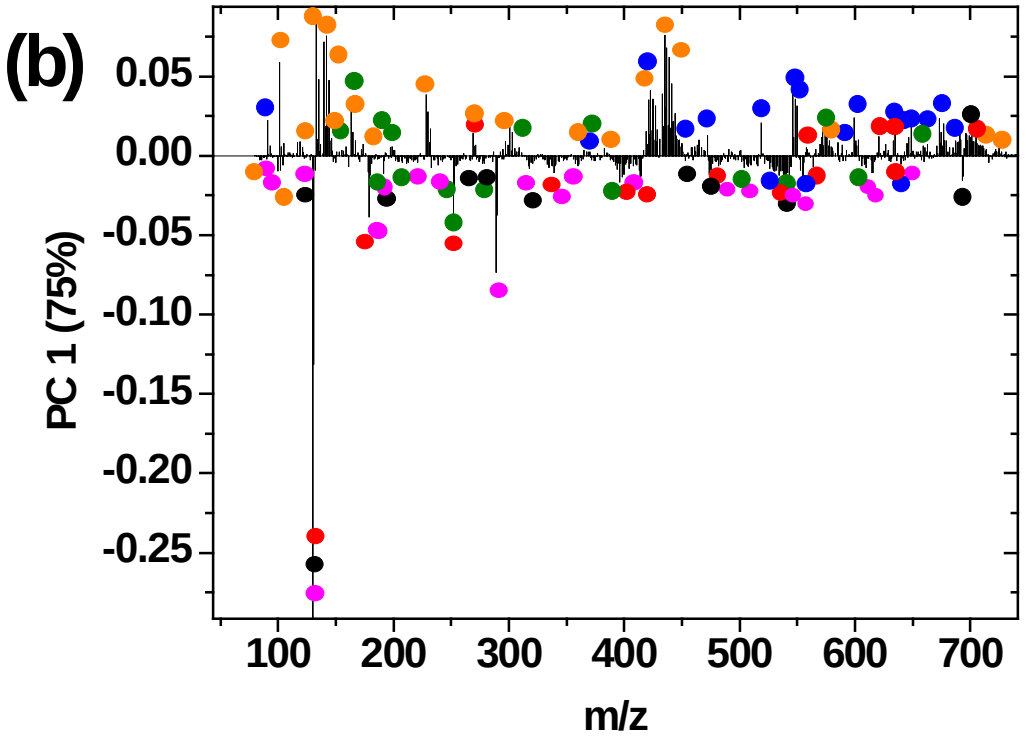
Can we measure many things simultaneously?

Micronutrient mass spectra successfully separated by PCA

Scores Plot



Loadings Plot



PCA data for mixed samples with four nutrients at 1xHBLC and one nutrient at elevated 10x HBLC

You can’t manage what you can’t measure

George Soros

To measure is to know

Lord Kelvin

How such analysis is done now:

Why Use Multivariate Data Analysis?

- **It is difficult to identify people based only on their heights, weights, eye color or hair color…**
- **…however, given all this information, identification becomes easier**

PCA - Principal Component Analysis

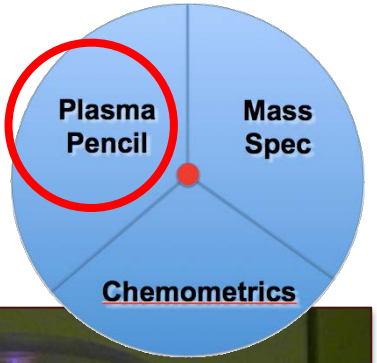
What are the important differences and key identifiers of different samples?

Separate the signal from the "dross"

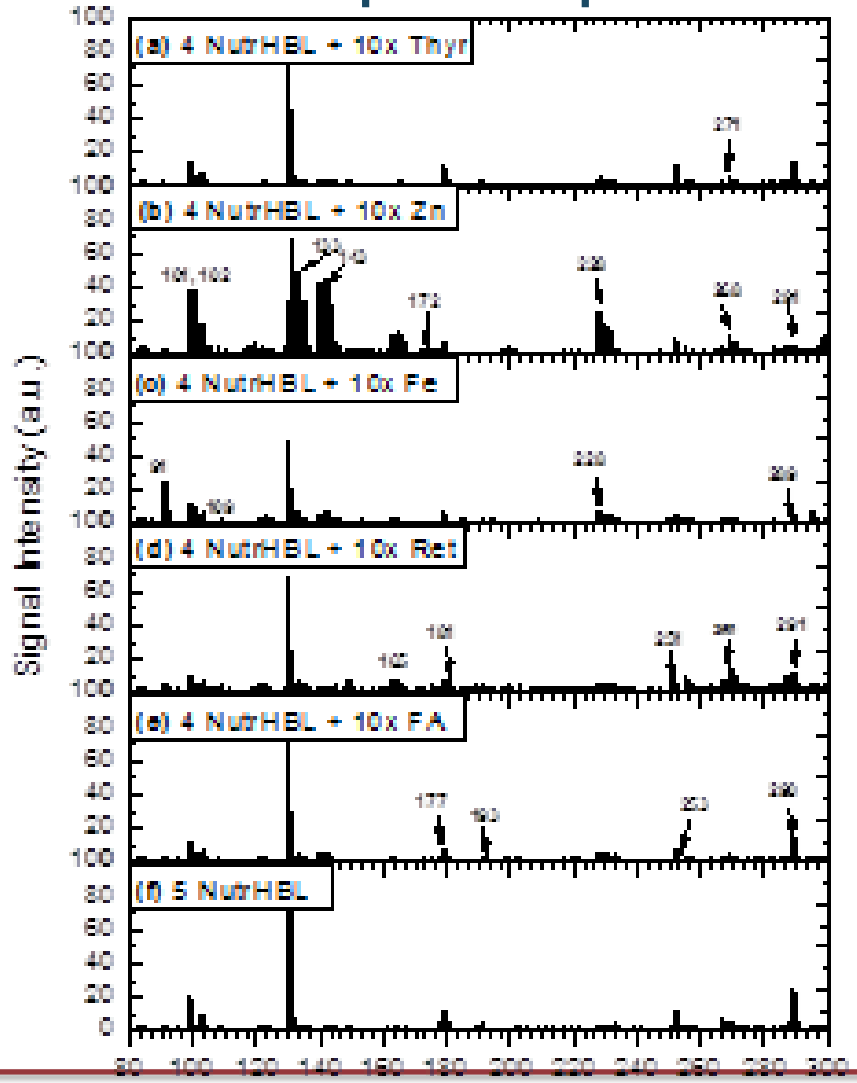
PLS – Partial Least Squares

Develop a quantitative calibration model

Plasmas (physics)



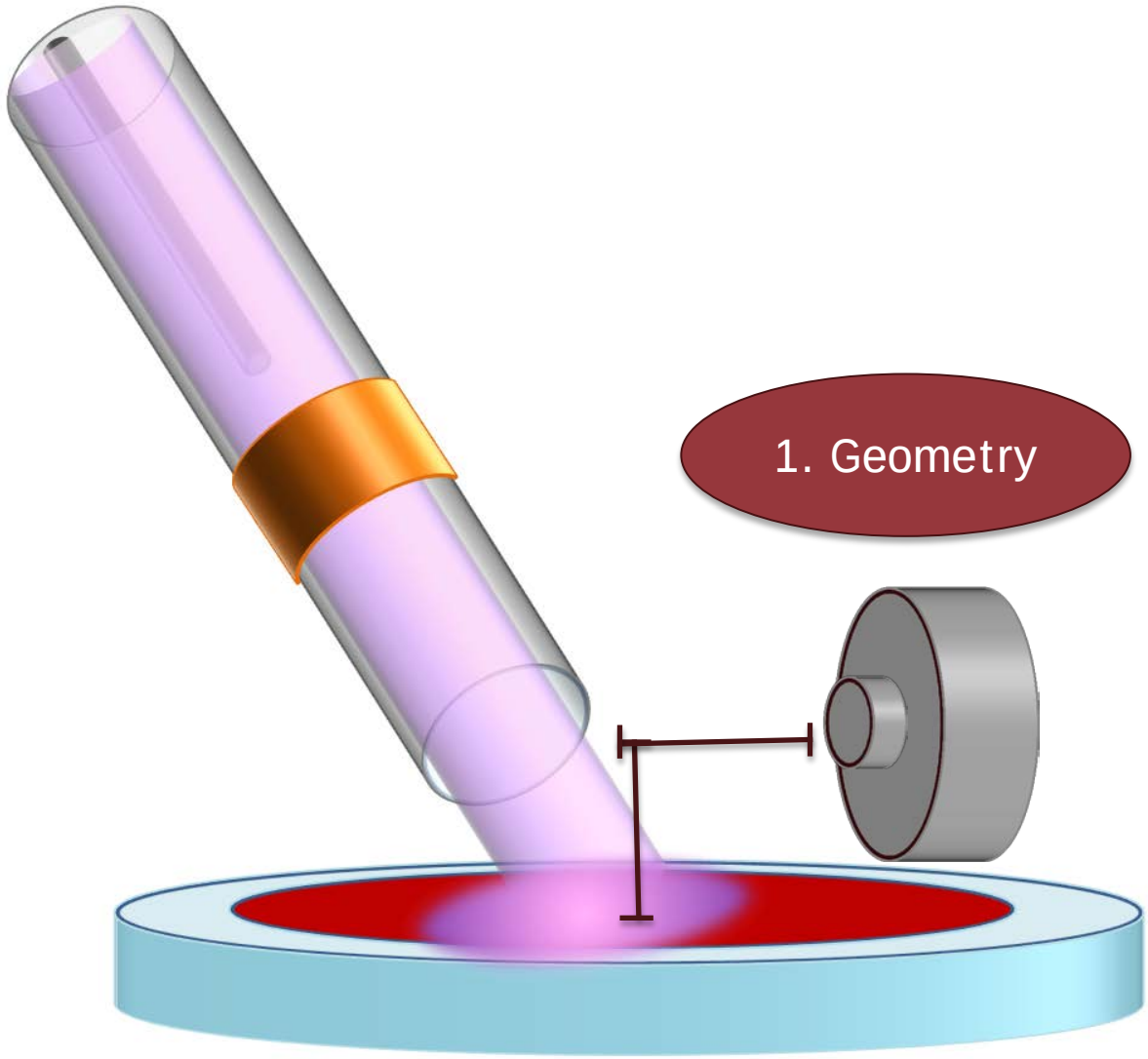
Positive ion ESI-MS spectra separation includes many of the peaks present in raw powder spectra



(multi-component mixtures consisting of one nutrient at a 10-fold concentration of its HBL concentration and the remaining four nutrients at a 1x HBL concentration)

- **Each spectrum had signature peaks for the respective nutrient as confirmed by raw nutrient spectra (data not shown).**
- **The presence of the other nutrients did impact some key nutrient peaks**
  - **Ex. Peak at 777 for Thyroxine by itself disappears in mixed solutions.**

What can we alter to affect LTP ionization?



Analytical Chemistry, Vol. 80, No. 23, December 1, 2008

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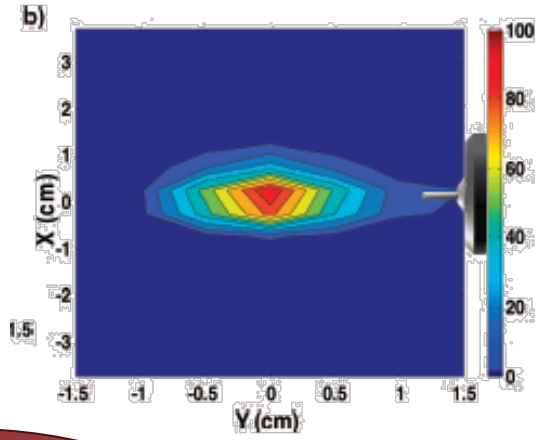

1. Geometry

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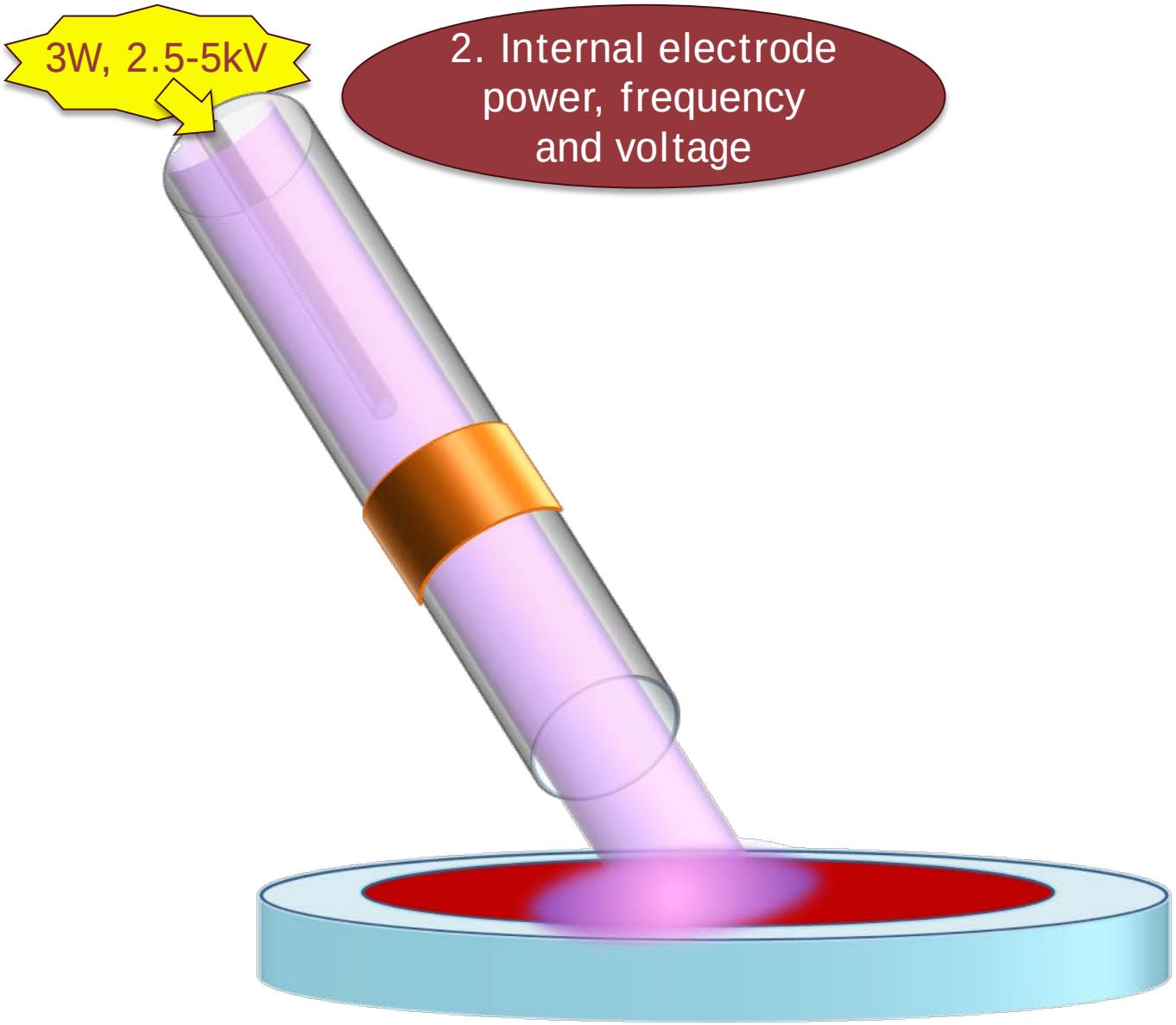
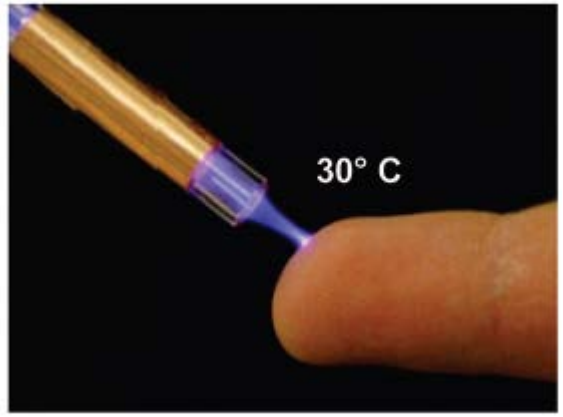


What can we alter to affect LTP ionization?

3W, 2.5-5kV

2. Internal electrode power, frequency and voltage



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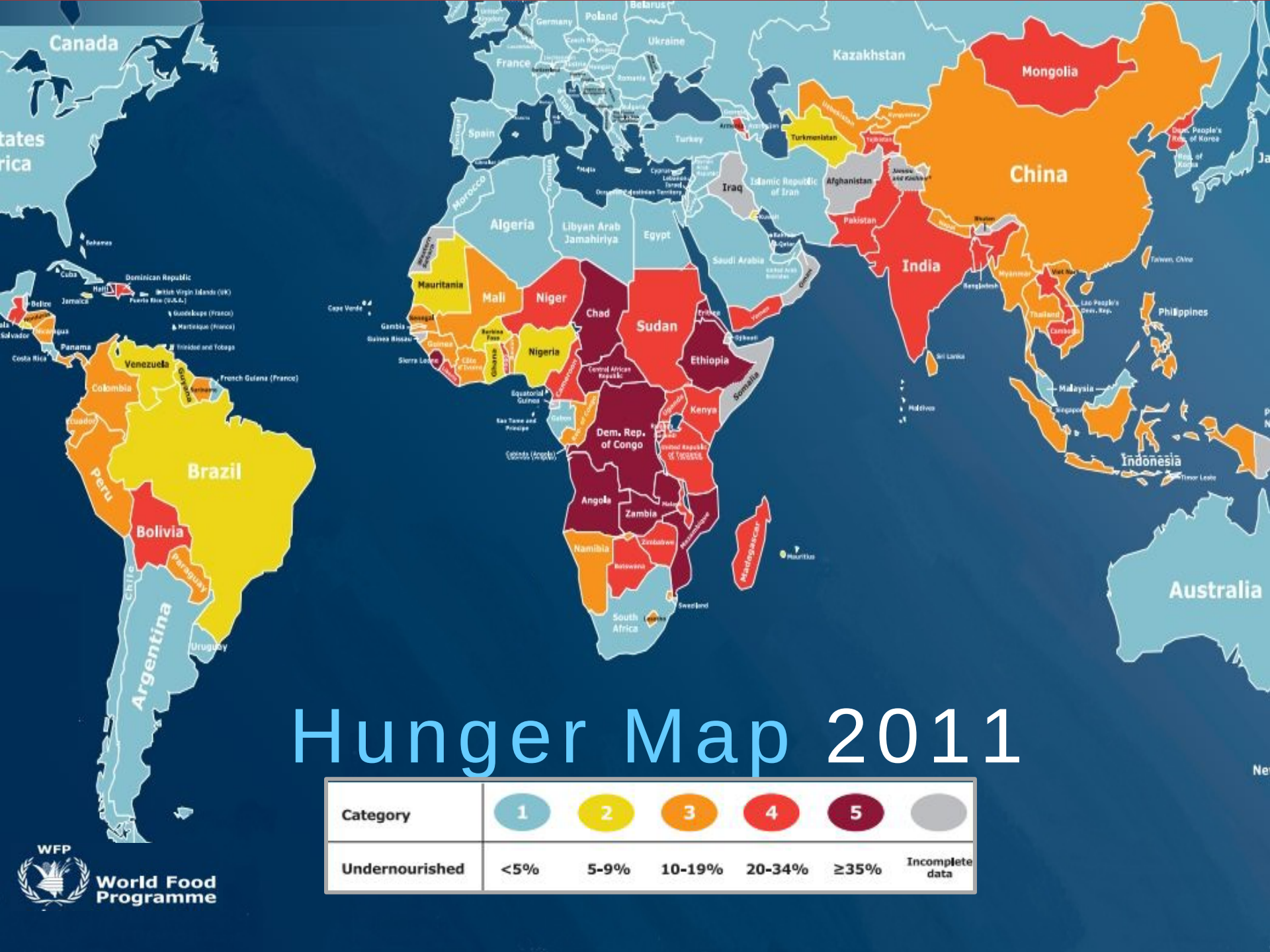
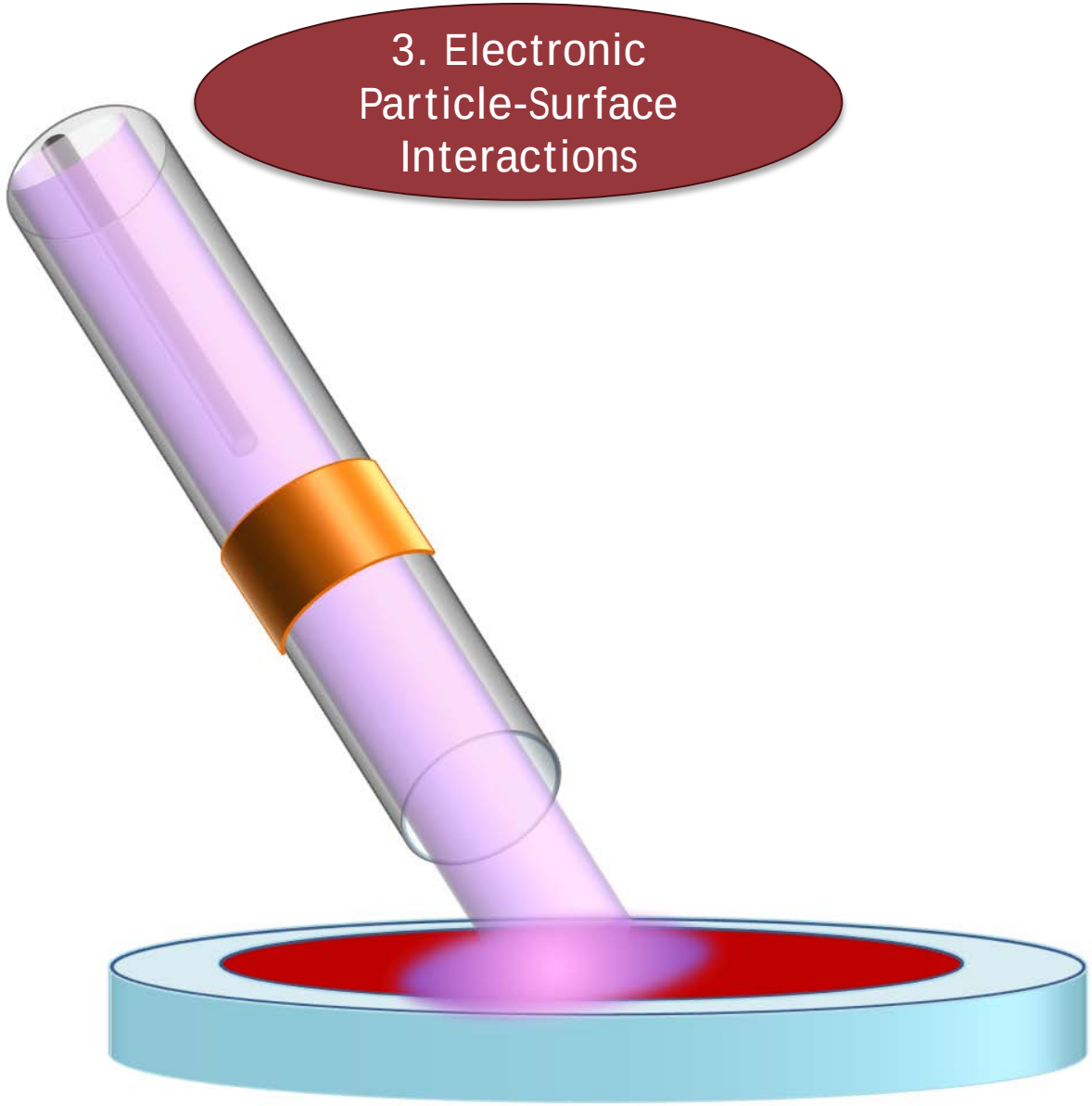
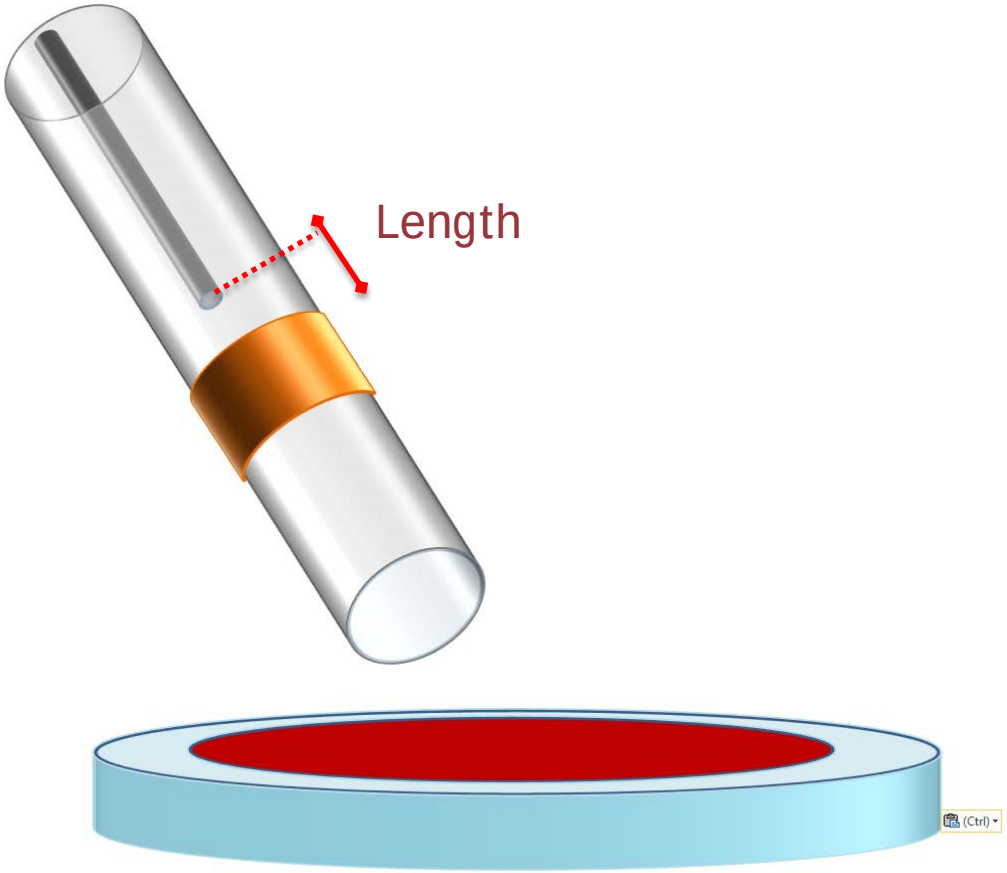

Canada
Brazil
Argentina
Peru
Bolivia
Paraguay
Uruguay
Chile
Colombia
Venezuela
Panama
Cuba
Dominican Republic
Jamaica
Belize
Guyana
Suriname
French Guiana (France)
Trinidad and Tobago
Guadeloupe (France)
Martinique (France)
Bahamas
Cape Verde
Gambia
Guinea Bissau
Sierra Leone
Guinea
Mauritania
Mali
Niger
Nigeria
Chad
Sudan
Ethiopia
Kenya
Somalia
Dem. Rep. of Congo
Angola
Zambia
Namibia
Botswana
Zimbabwe
Mozambique
South Africa
Madagascar
Mauritius
Swaziland
Algeria
Libyan Arab Jamahiriya
Egypt
Morocco
Spain
France
Germany
Poland
Belarus
Ukraine
Romania
Turkey
Iraq
Saudi Arabia
Yemen
Islamic Republic of Iran
Afghanistan
Pakistan
Turkmenistan
Kazakhstan
Mongolia
China
India
Myanmar
Thailand
Cambodia
Malaysia
Philippines
Indonesia
Australia
Sri Lanka
Maldives
Bangladesh
Bhutan
Nepal
Taiwan, China
Lao People's Dem. Rep.
Dem. People's Rep. of Korea
Rep. of Korea
Hunger Map 2011
Category
1
2
3
4
5
Undernourished
<5%
5-9%
10-19%
20-34%
≥35%
Incomplete data
WFP
World Food Programme

What can we alter to affect LTP ionization?



Q1: Does electrode length affect fragmentation?

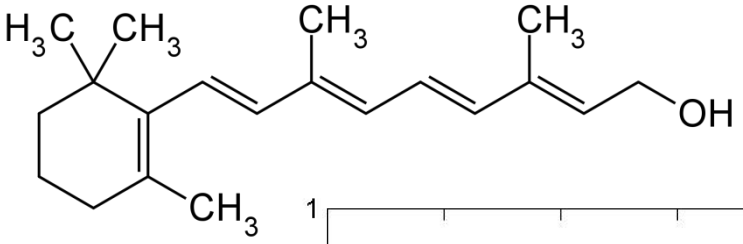


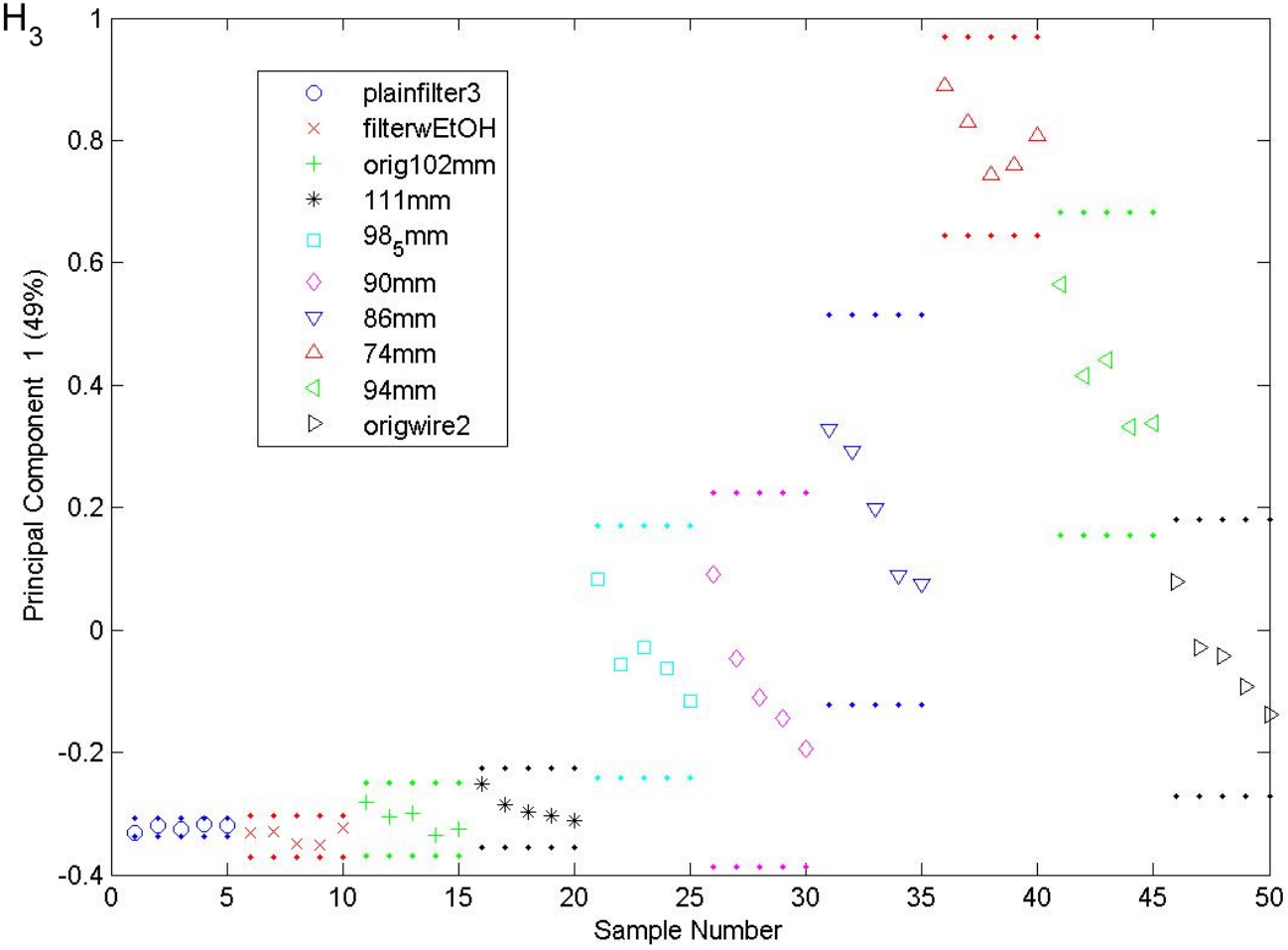
Length (mm)	d (mm)
111	-12.5
102	-3.5
98.5	0
94	4
90	8
86	12
74	24

** Experiment Performed on Bruker

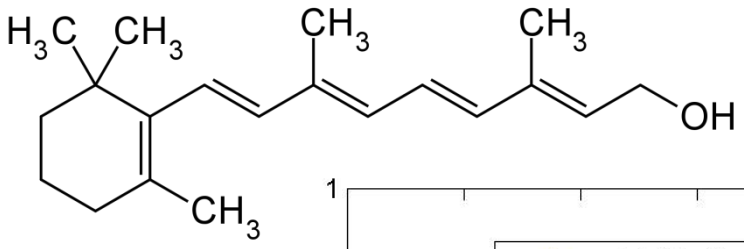
Total electrode length: 108mm

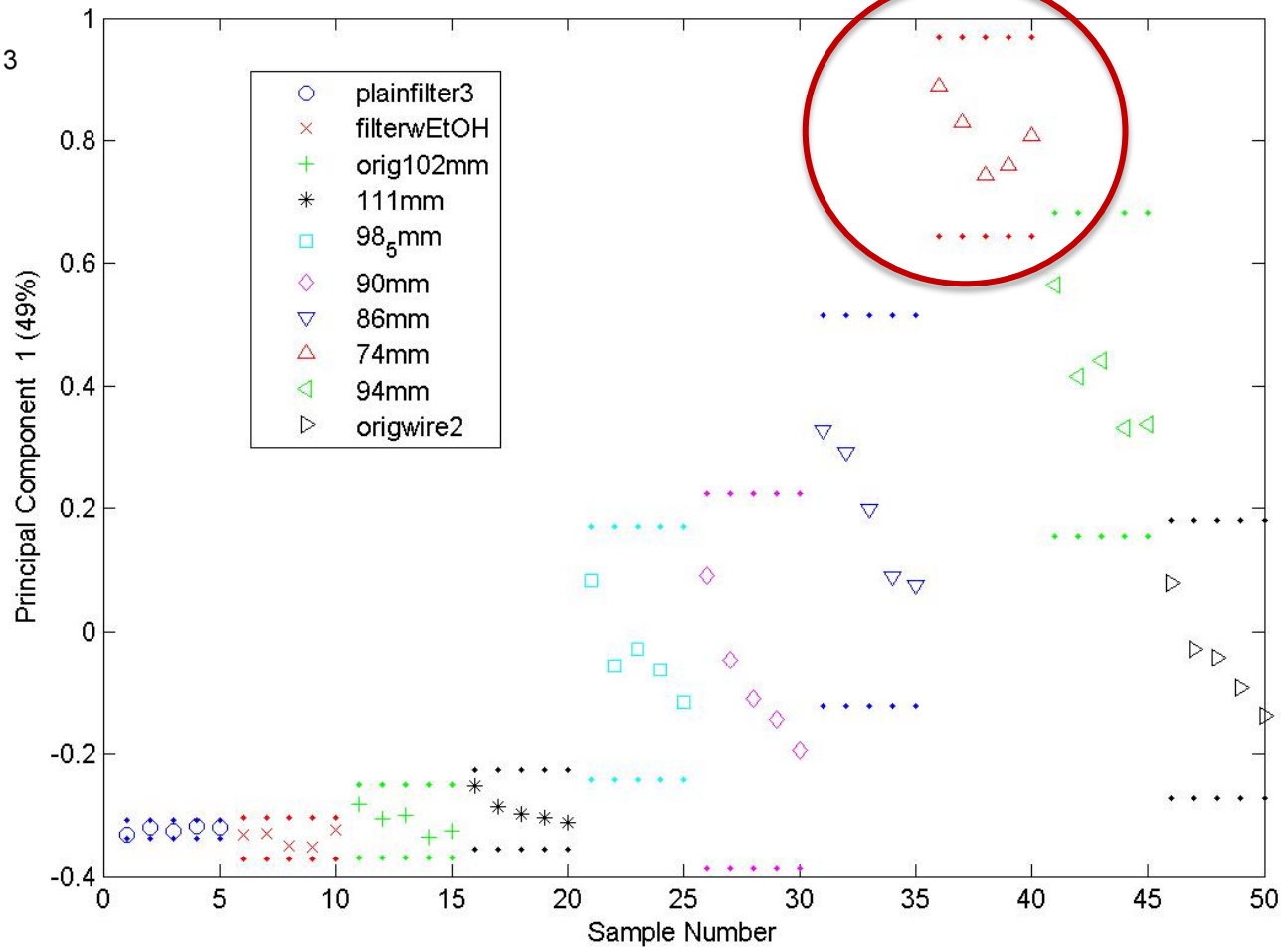
Principal Component #1 Scores: Vitamin A



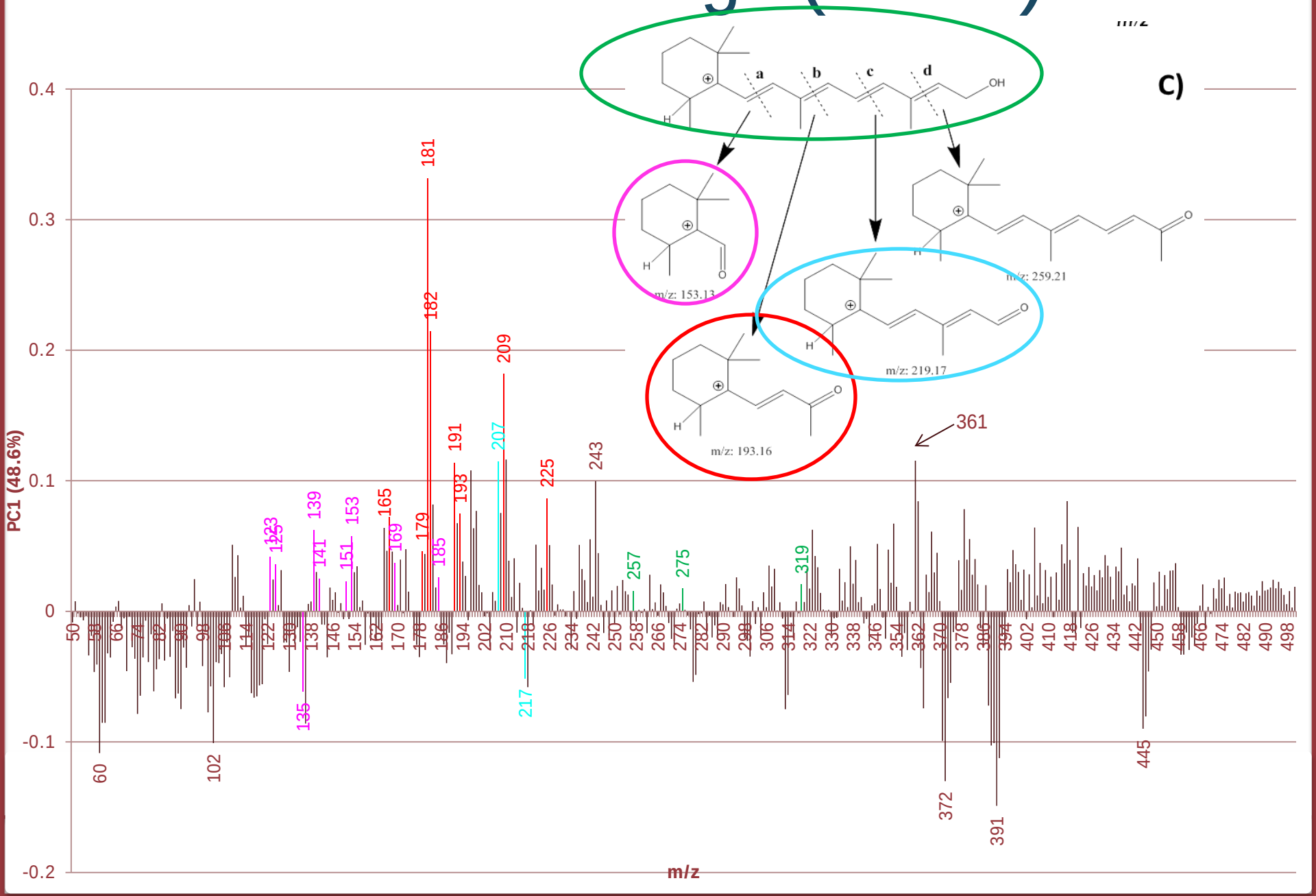


Principal Component #1 scores indicate length of electrode does affect Vitamin A fragmentation

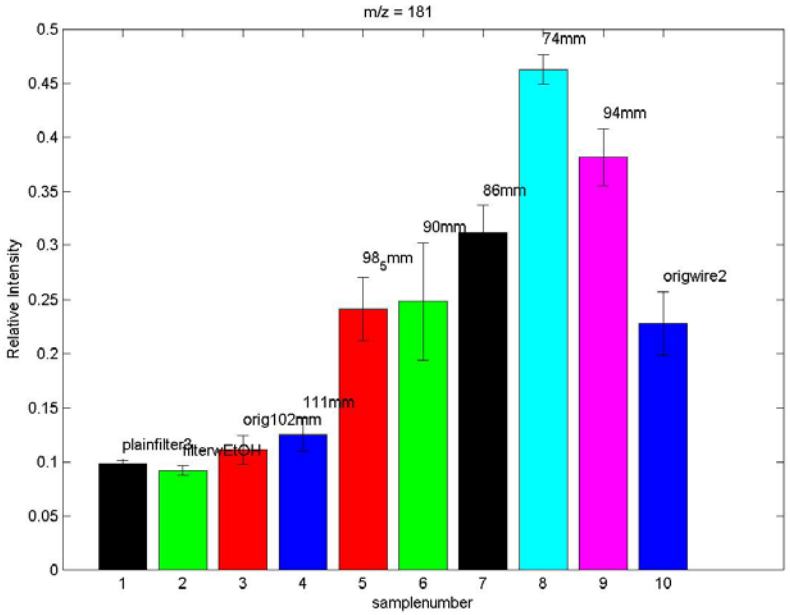


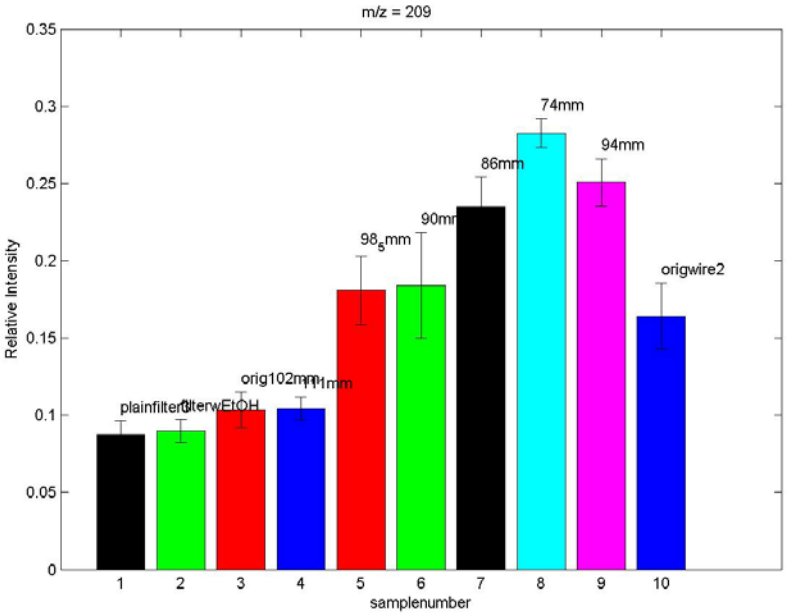


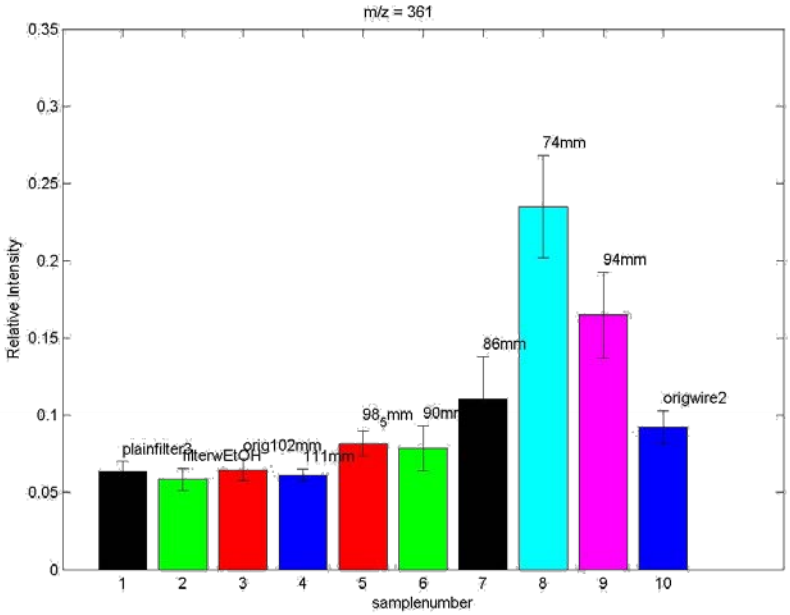
PCA- Loadings (zoom)

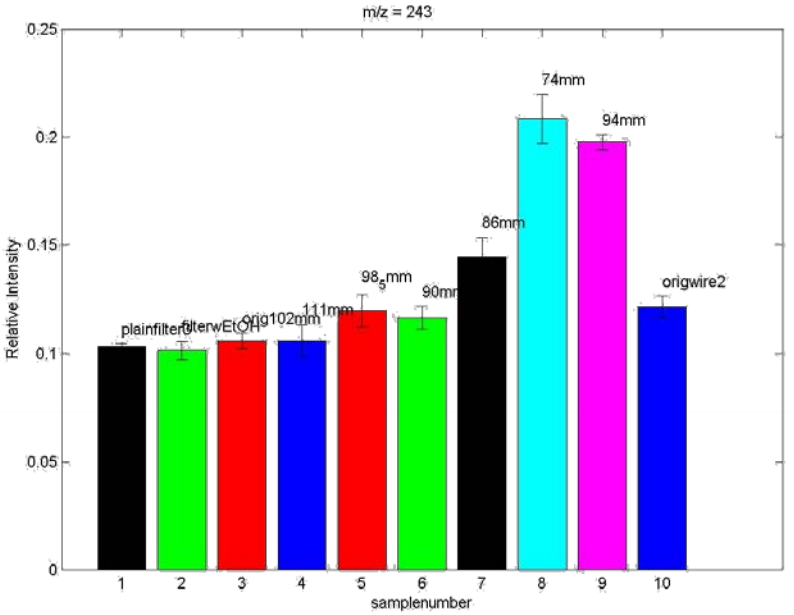


Loadings Comparison- positive

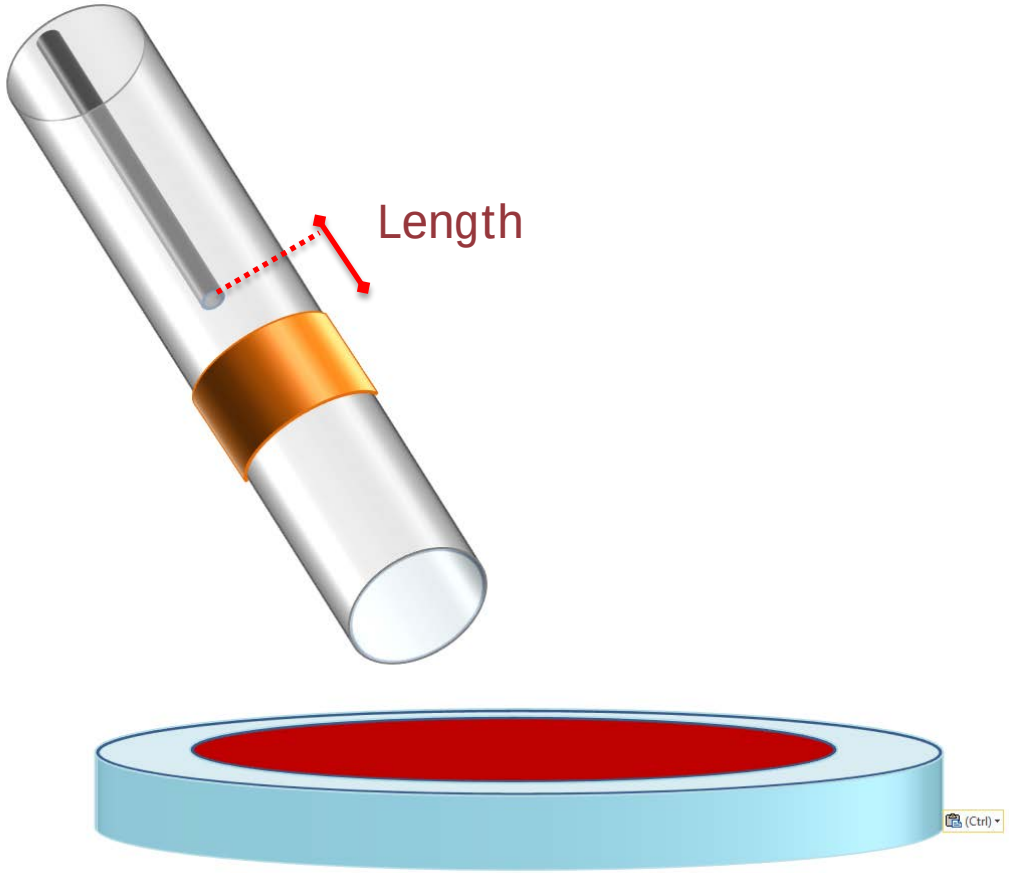








Q2: How *exactly* does electrode length affect fragmentation and is the effect universal?



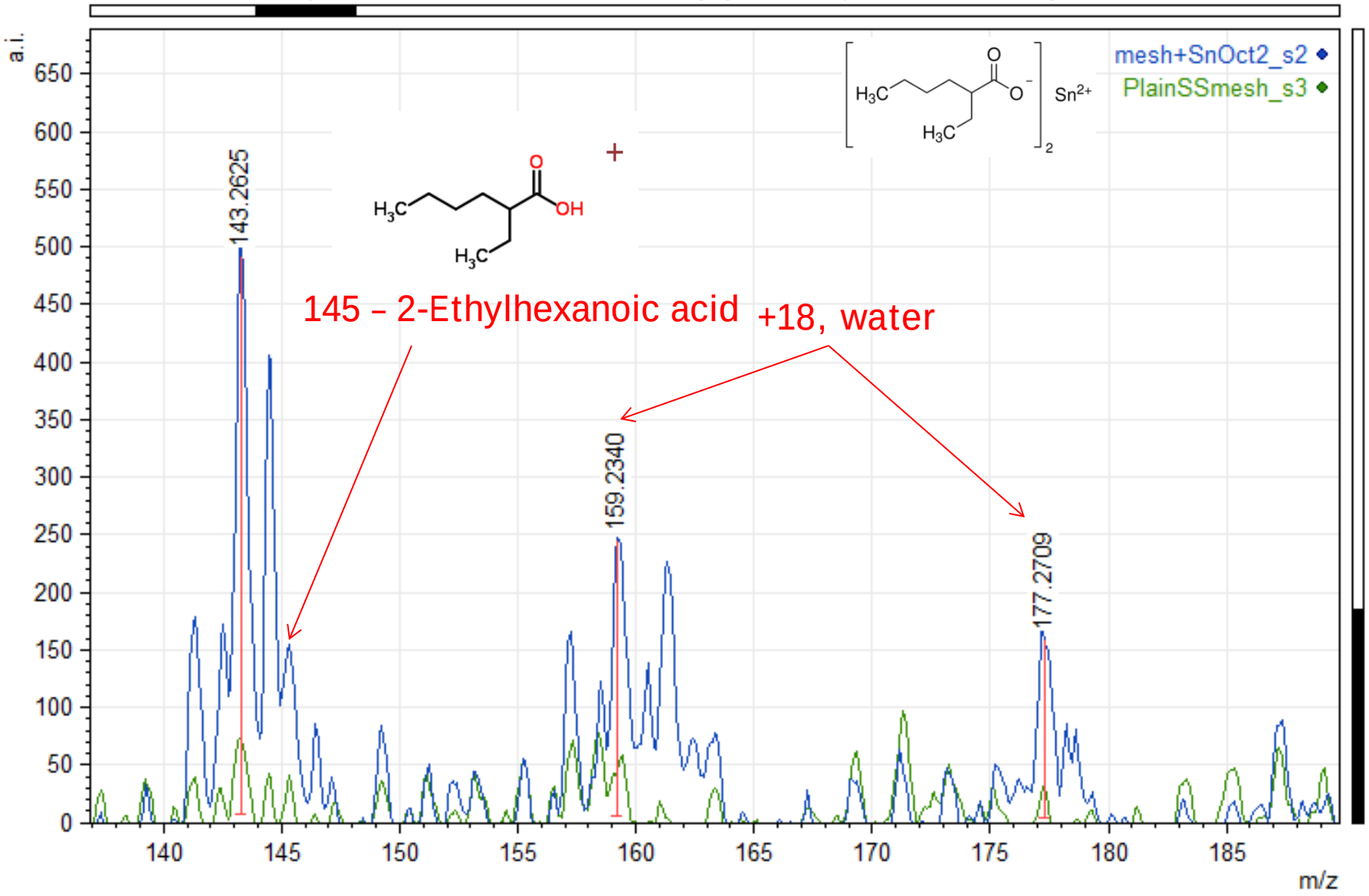
Length (mm)	d (mm)
111	-12.5
102	-3.5
98.5	0
94	4
90	8
86	12
74	24

** Experiment Performed on Advion

Total electrode length: 108mm

SS mesh coated with $Sn(Oct)_2$

(Stannous Octoate, Tin(II) 2-ethylhexanoate)



Future Work

Sputter degradation of Poly(ethylene teraphalate) polymers with static secondary ion mass spectrometry

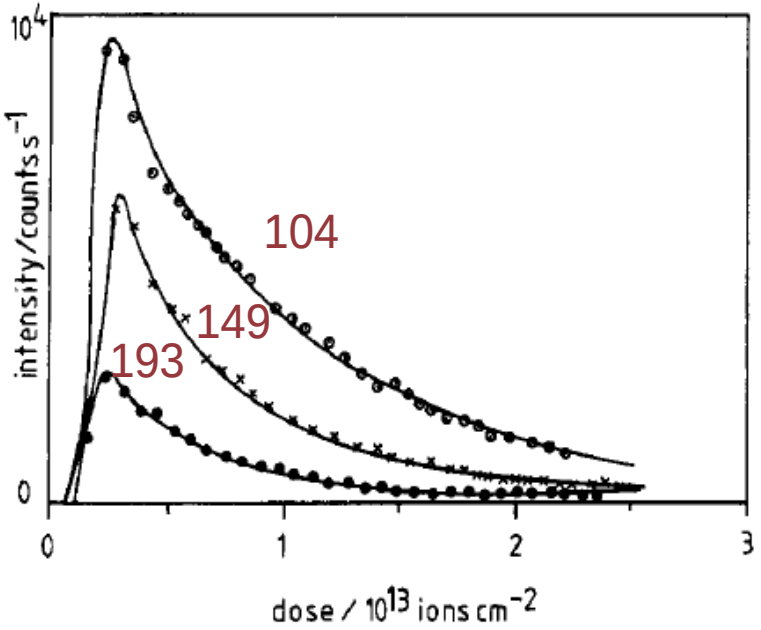
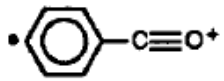
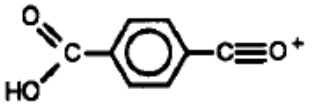
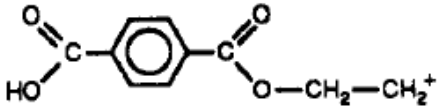
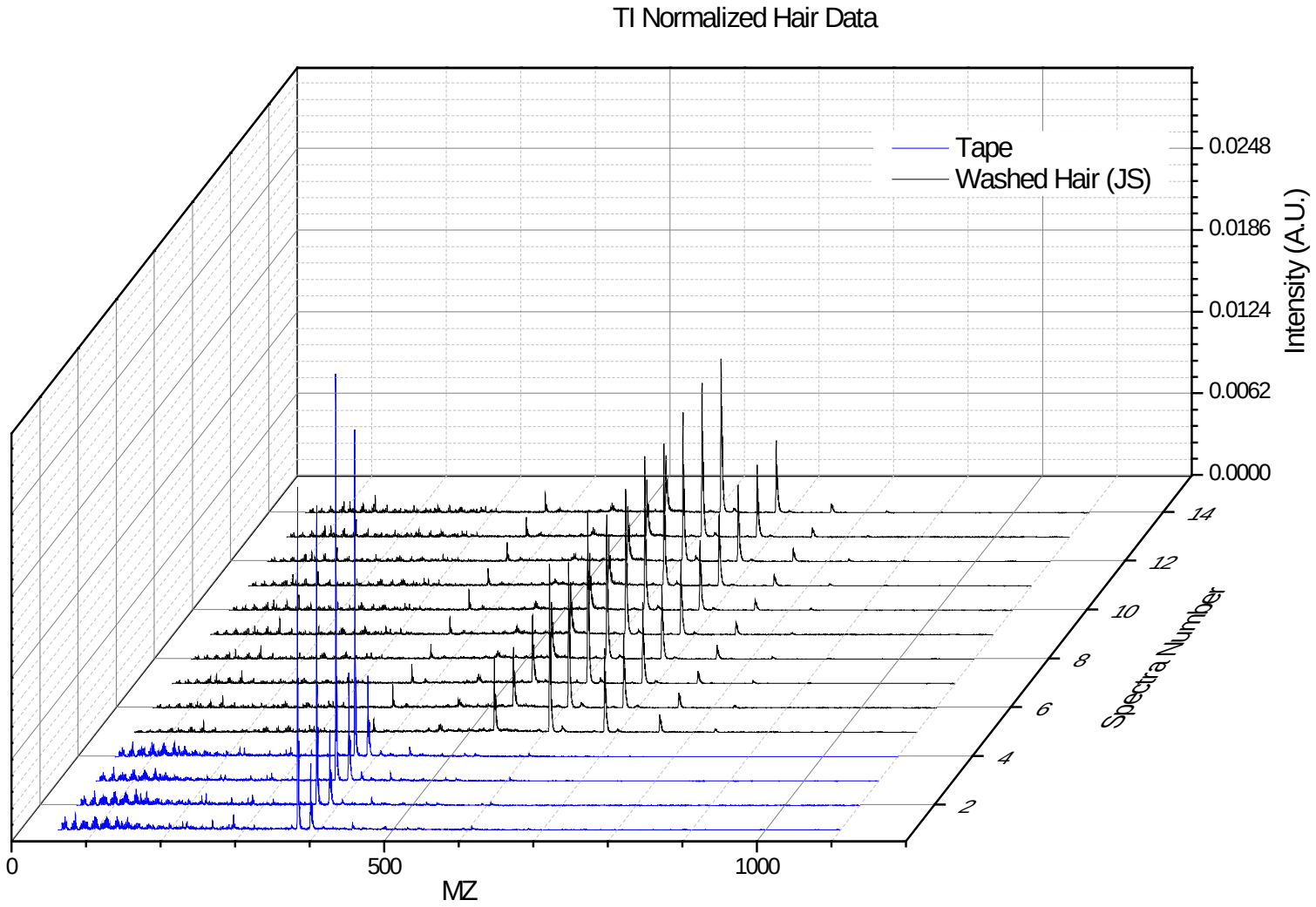


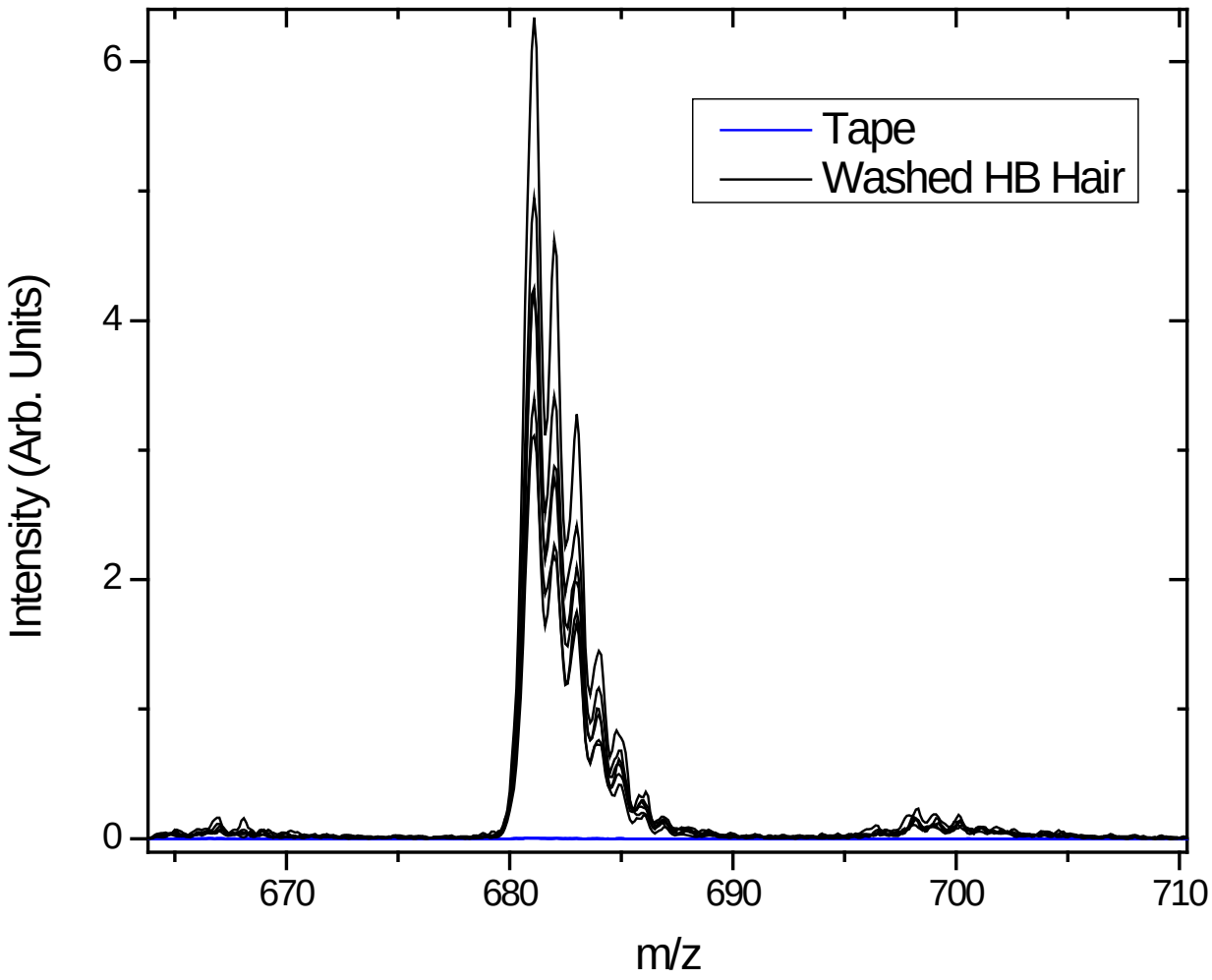
Table I. PET Cluster Ion Identities

ion, m/z	structure
77	
91	
104	
149	
193	

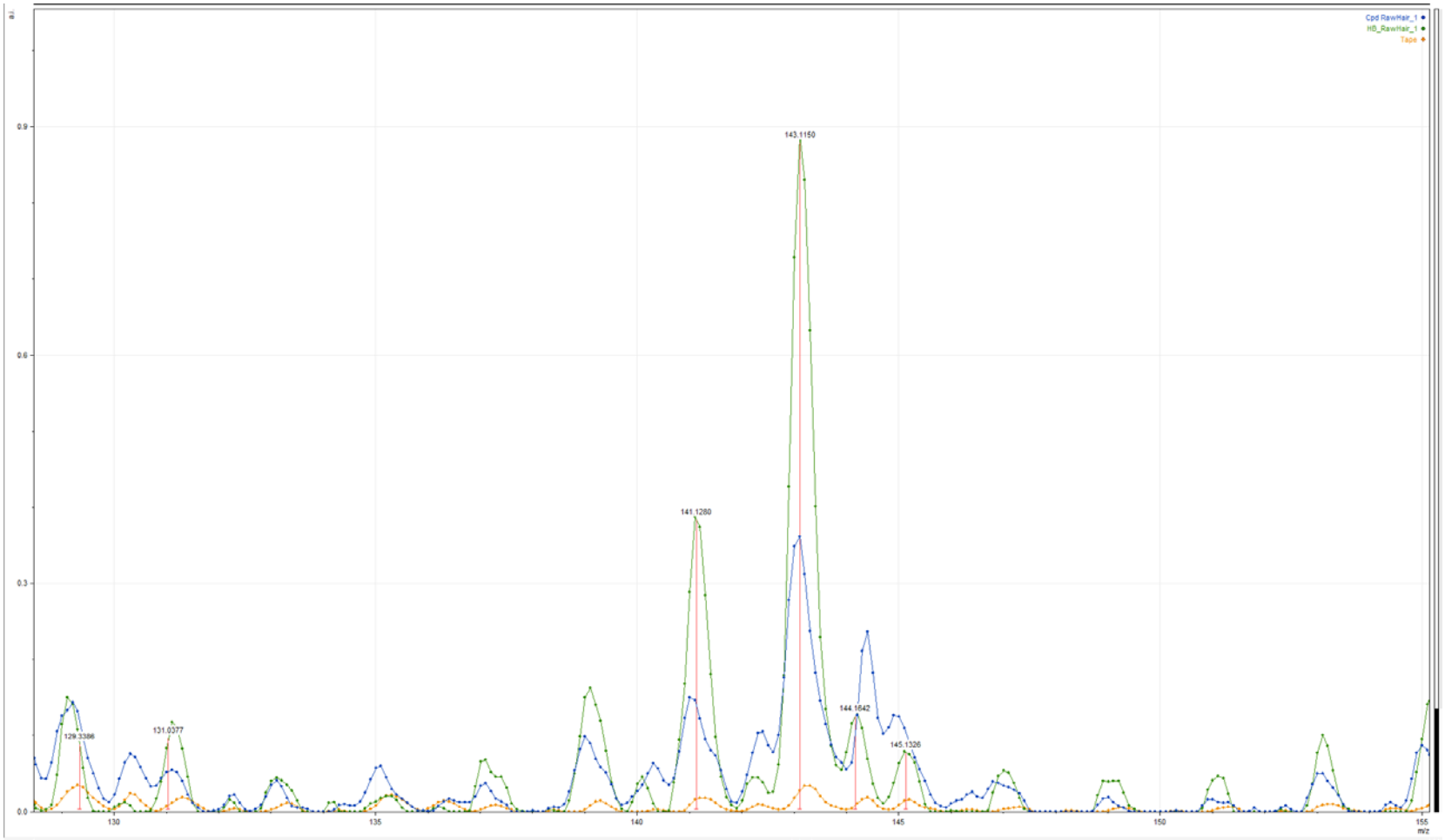
ANALYTICAL CHEMISTRY, VOL. 63, NO. 6, MARCH 15, 1991

Key Issue: Instrument contamination


TI Normalized Hair Data
Tape
Washed Hair (JS)
0.0248
0.0186
0.0124
0.0062
0.0000
Intensity (A.U.)
14
12
10
8
6
4
2
Spectra Number
0
500
1000
MZ


Intensity (Arb. Units)
6
4
2
0
670
680
690
700
710
m/z
Tape
Washed HB Hair

Hair : AgCl??


Cpd RawHair_1
HB_RawHair_1
Tape
143.1150
141.1280
144.1642
145.1326
131.0377
129.3386
0.9
0.6
0.3
0.0
130
135
140
145
150
155
m/z

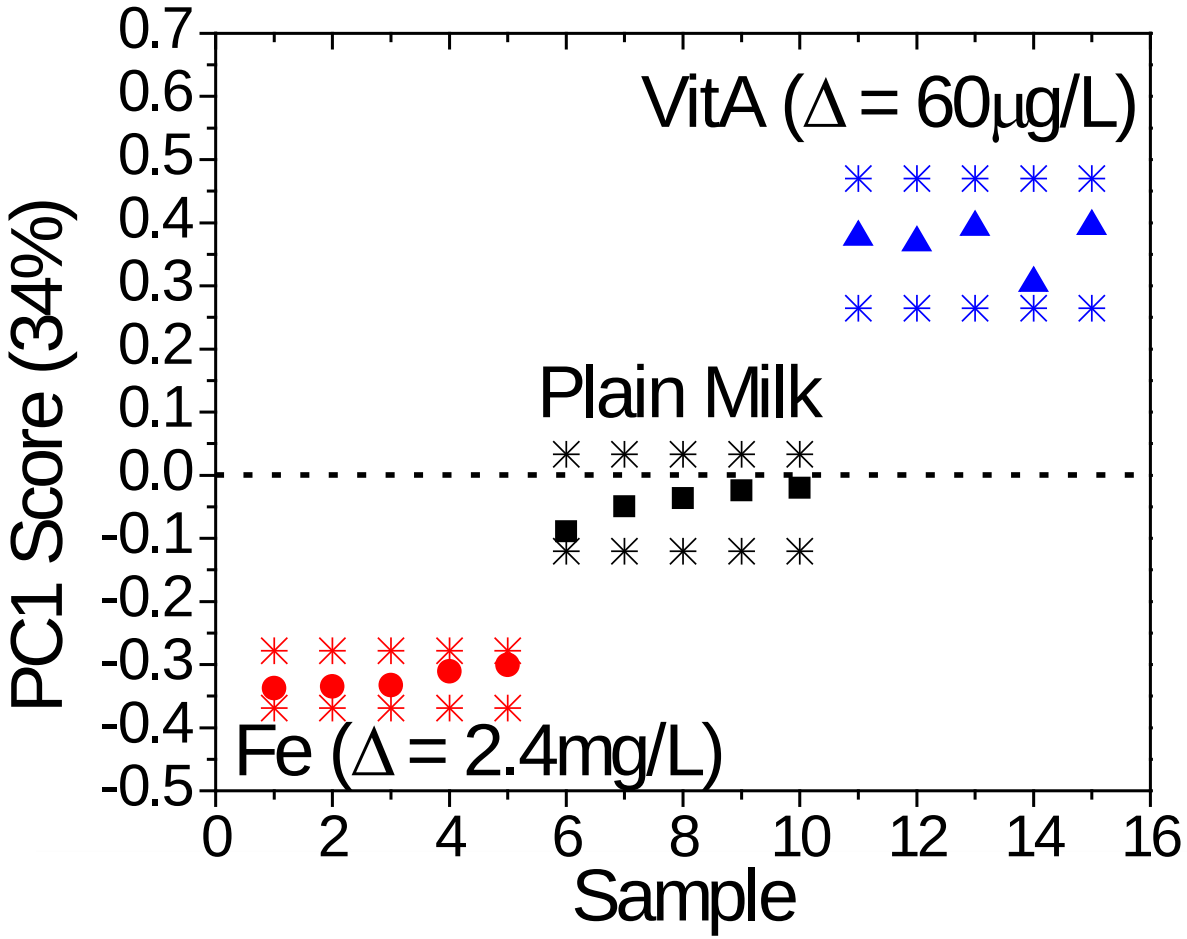
Disruptive Technology for Measuring Nutrients and Nourishment

Buddy Ratner, Jeanette Stein, Edward Lo,
Sam Herschbein David Castner

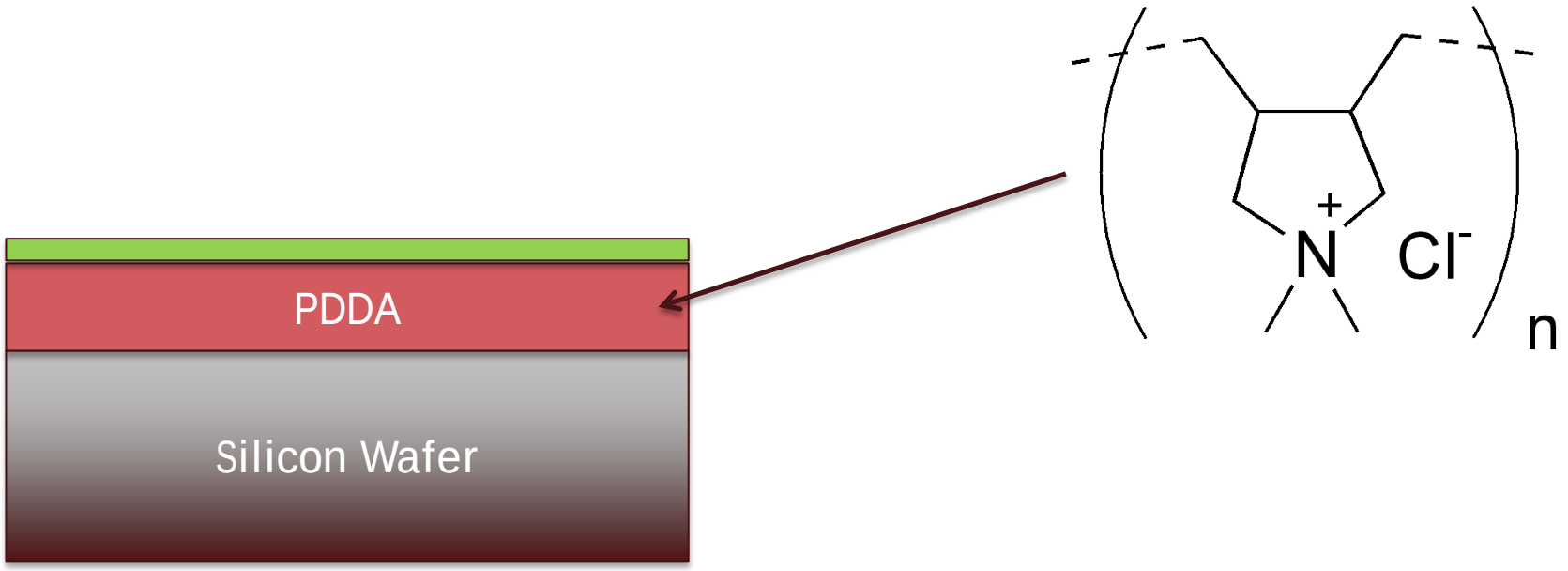
University of Washington
Department of Bioengineering
Seattle Washington 98195

BILL & MELINDA GATES foundation

PPAMS was able to distinguish changes in vitamin A and iron content in unpasteurized, raw cow's milk



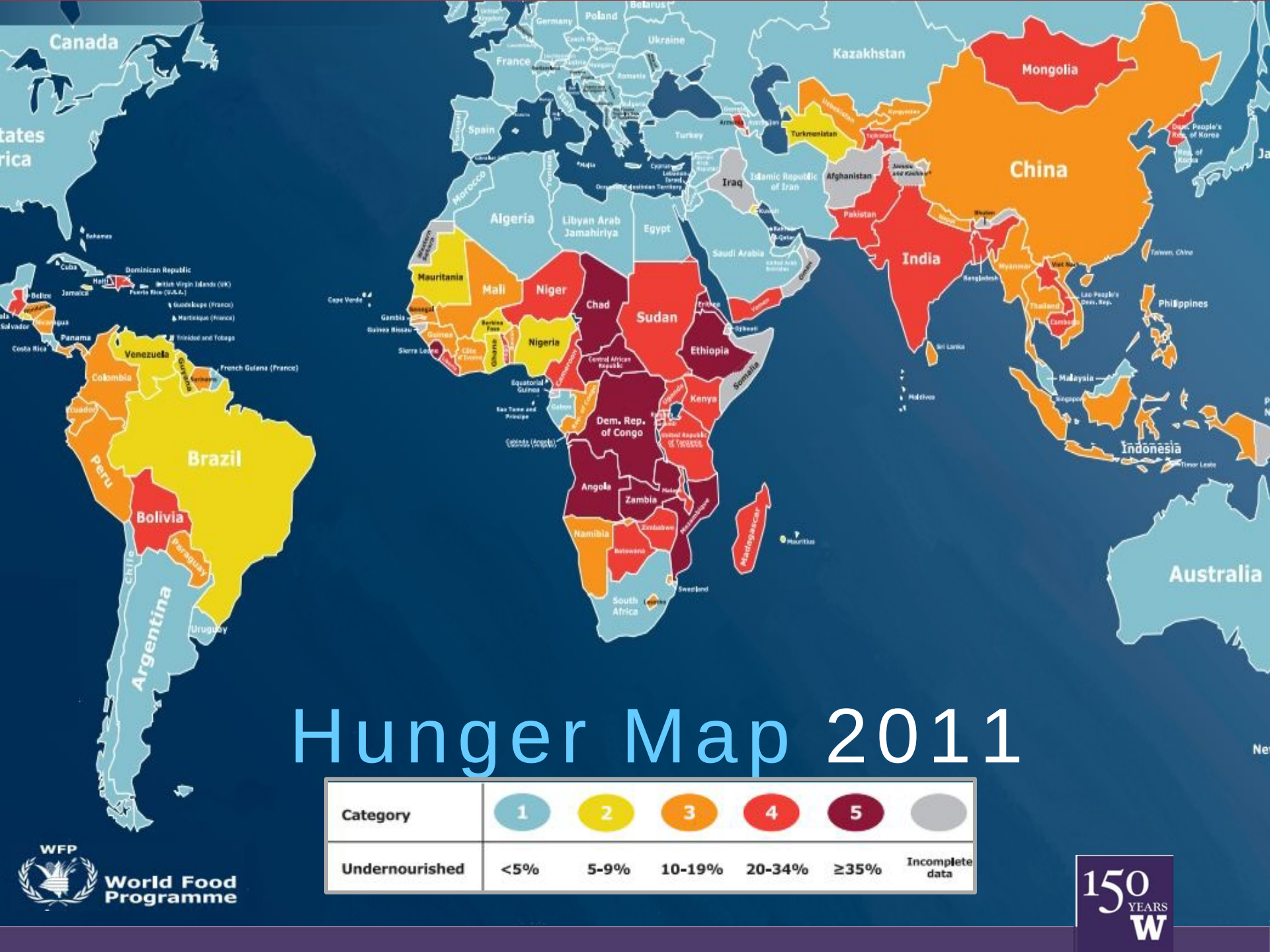
Collabration with Dr. Castner's group on Polydiallyldimethylammonium chloride (PDDA) and PET polymer samples



What future applications can we utilize the PPAMS for?

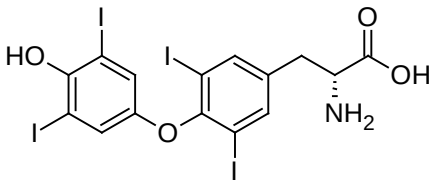
(Funding would be nice too.)

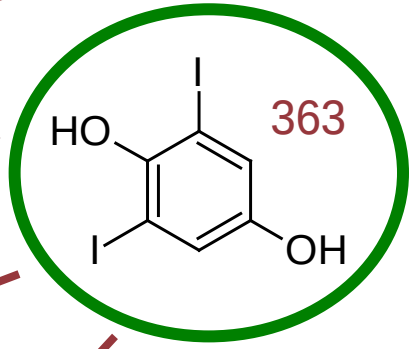
New Directions

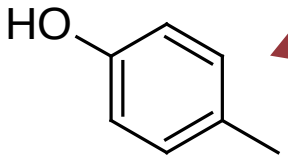

Canada
Kazakhstan
Mongolia
China
India
Pakistan
Afghanistan
Iraq
Islamic Republic of Iran
Saudi Arabia
Turkmenistan
Turkey
Spain
France
Germany
Poland
Ukraine
Belarus
Morocco
Algeria
Libyan Arab Jamahiriya
Egypt
Mauritania
Mali
Niger
Chad
Sudan
Nigeria
Ethiopia
Somalia
Kenya
Dem. Rep. of Congo
Angola
Zambia
Namibia
South Africa
Madagascar
Brazil
Peru
Bolivia
Paraguay
Argentina
Chile
Uruguay
Venezuela
Colombia
Ecuador
Panama
Costa Rica
Cuba
Haiti
Dominican Republic
Jamaica
Belize
French Guiana (France)
Philippines
Indonesia
Malaysia
Australia
Hunger Map 2011
Category
1
2
3
4
5
Undernourished
<5%
5-9%
10-19%
20-34%
≥35%
Incomplete data
WFP
World Food Programme
150 YEARS

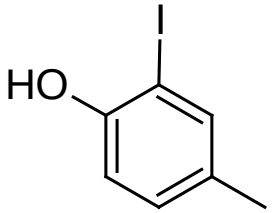
Positive ion PPAMS and PPAMS/MS spectra on a in methano

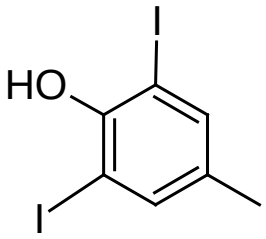
MS/MS 363: Thyroxine Ring



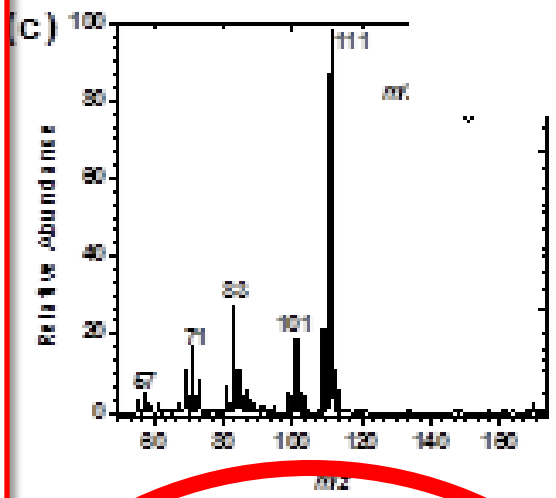




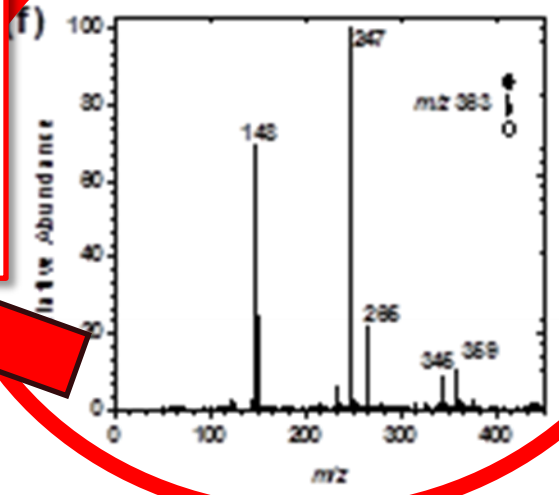


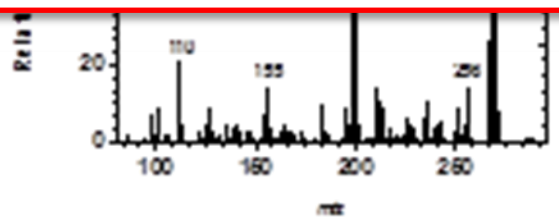


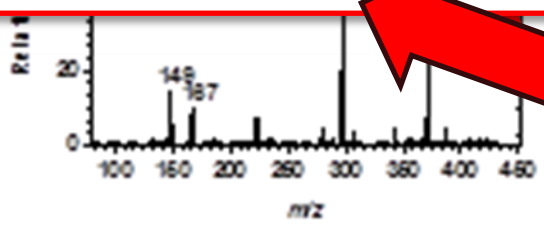
MS/MS for



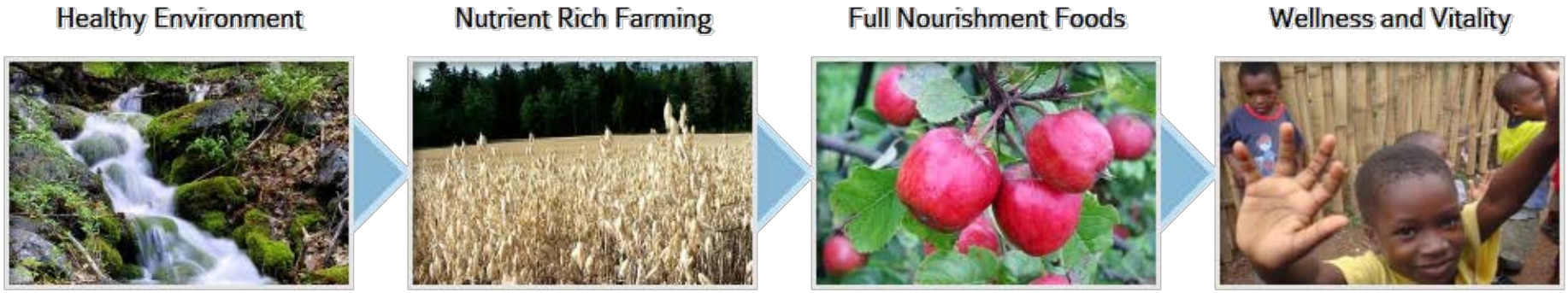
MS/MS for Thyr







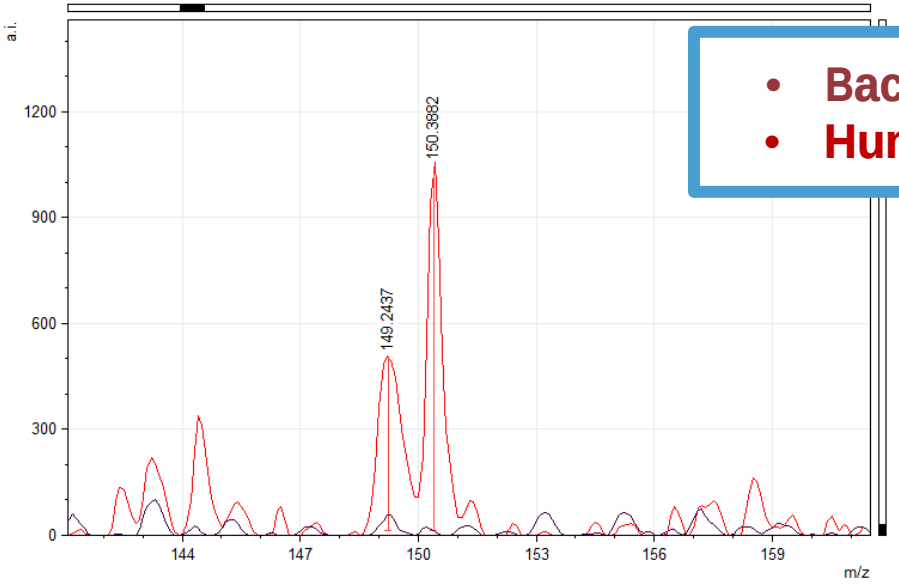
Moving towards studying the Nutrient Economy with the Ashoka Foundation

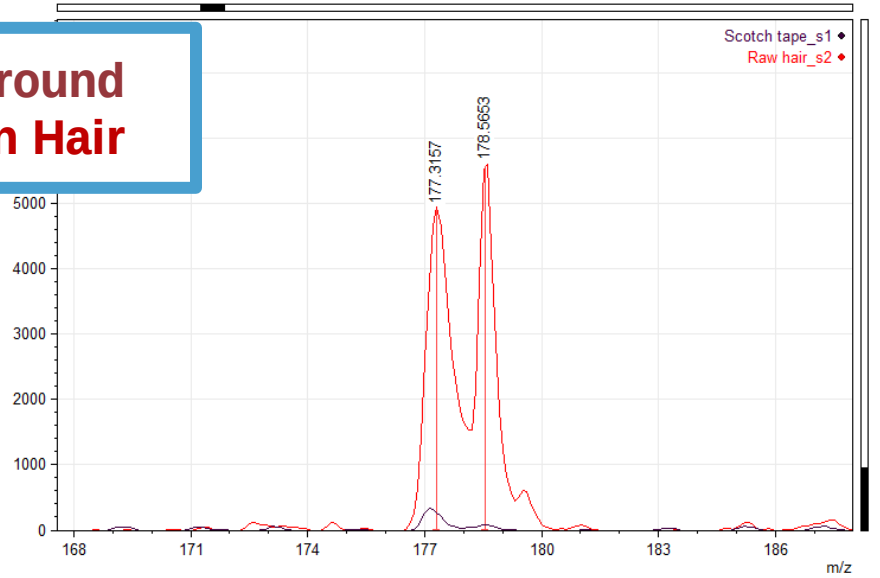


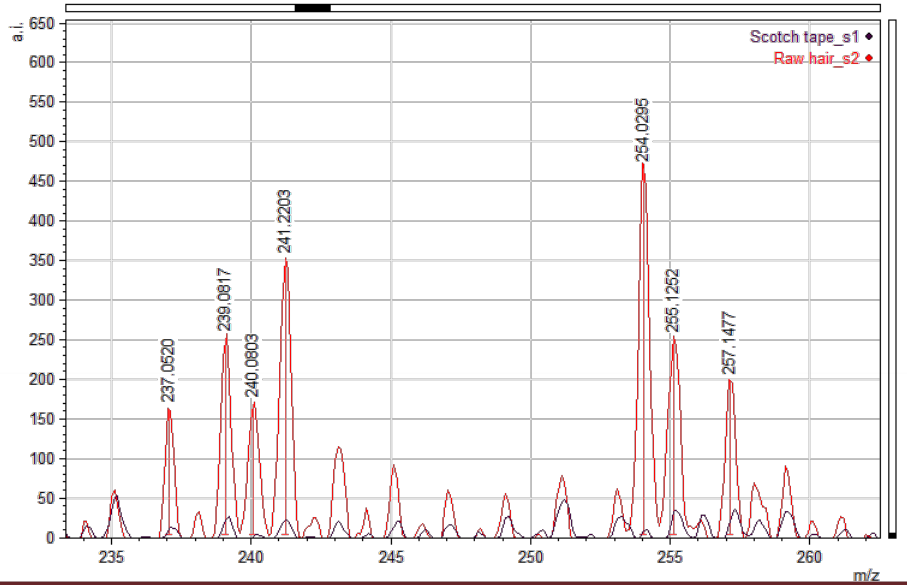
- What are the markers for wellness? How many different markers can we identify and what affect do they have on our lives/economies?
- Are the nutrient programs that we have in place adequate to address issues?
- What is the bioavailability of the nutrients in our foods?
- What is the economic impact of these changes? In developed and underdeveloped nations?

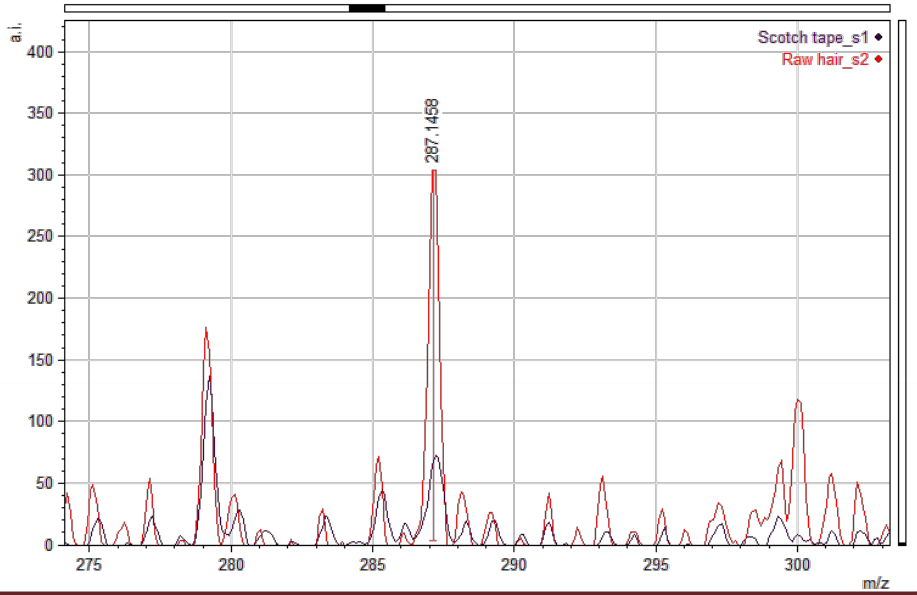
Several peaks were found to be present only in the sample of human hair

- **Background**
- **Human Hair**

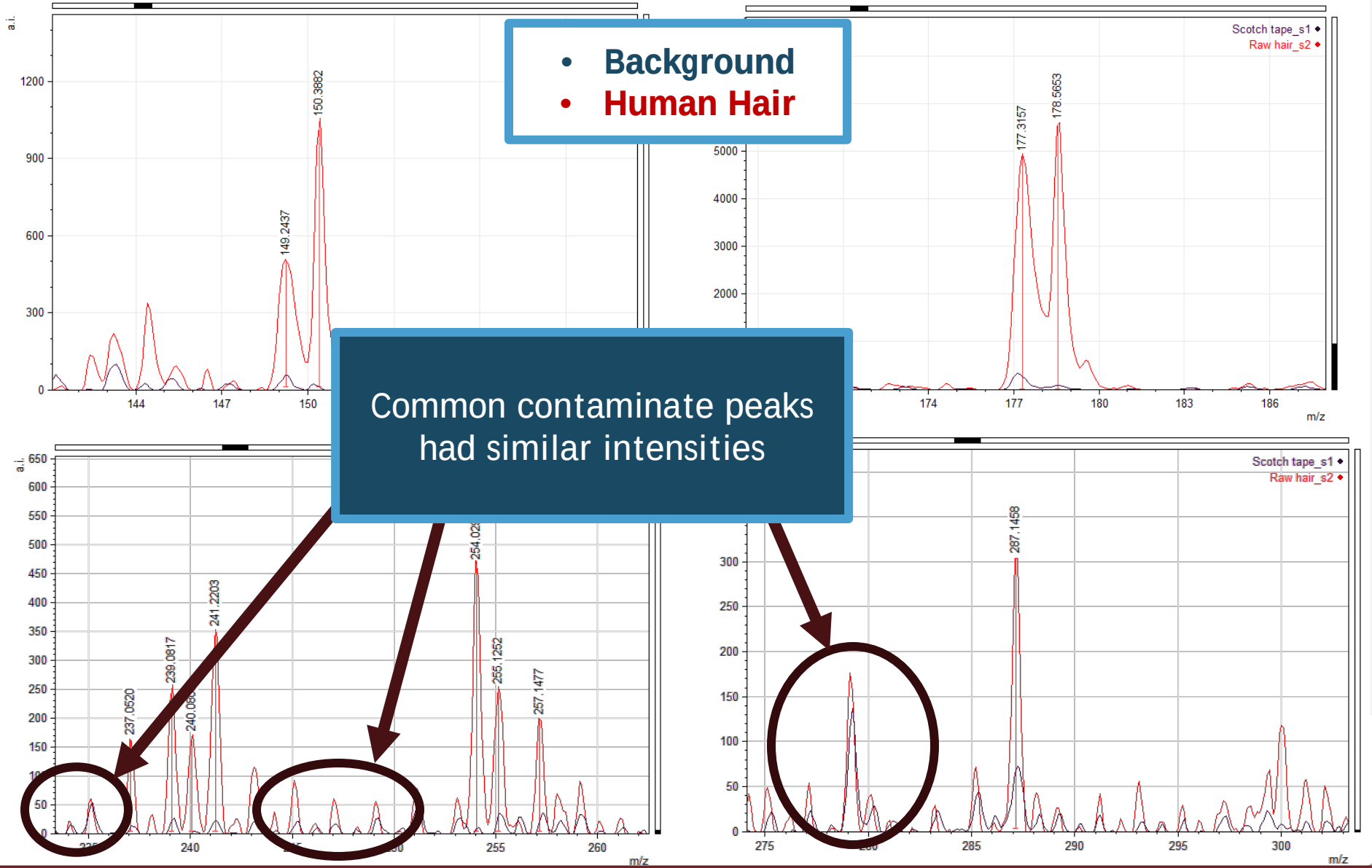








Several peaks were found to be present only in the sample of human hair



Detection of Neglected Tropical Diseases

- For the elimination of lymphatic filariasis (LF, also known as elephantiasis) by 2020
- The control of:
  - Onchocerciasis (river blindness),
  - Soil-transmitted helminthic (STH) infections (ascariasis, trichuriasis, and hookworm disease)
  - Schistosomiasis.