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Selecting and planning for the right mass spectrometer

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Learning Objectives

After this presentation, you should be able to:

- explain what specifications are important to consider when selecting a mass spectrometer
- explain what ancillary components are required for installation of a mass spectrometry system

Overview

- factors to consider when choosing instrumentation
- which type of instrument do you require?
- what else do you need?
- what resources are available?

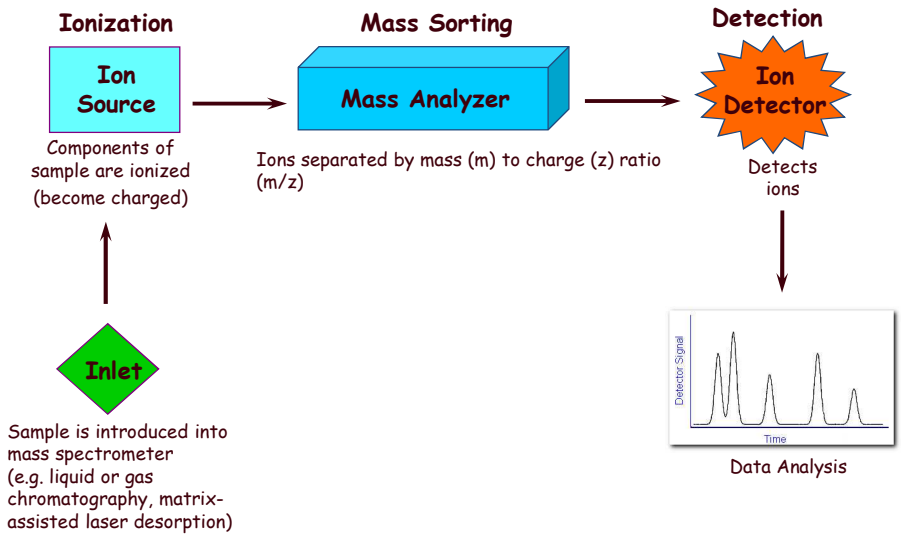
Initial factors to consider when choosing instrumentation

- what do you actually need for the applications you wish to implement?
  - analytes you wish to measure
  - quantitative vs qualitative methods
  - targeted vs untargeted methods
  - accurate mass determination required
  - sensitivity
  - throughput
  - robustness requirements

Initial factors to consider when choosing instrumentation (cont)

- what expertise do your technologists possess?
  - mass spectrometer has to be optimized for every single analyte you want to measure
  - not a "plug and play" technology
- what is the cost – direct and indirect – of implementation?

What are the components of a mass spectrometry system?



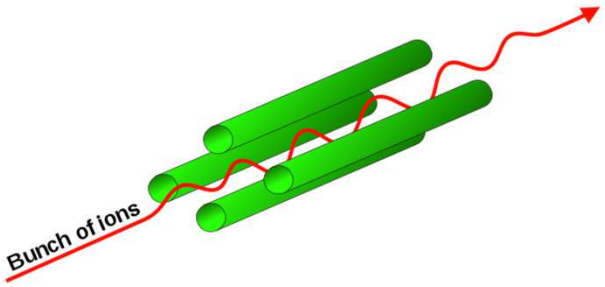
Mass Analyzers

Types of mass analyzers:

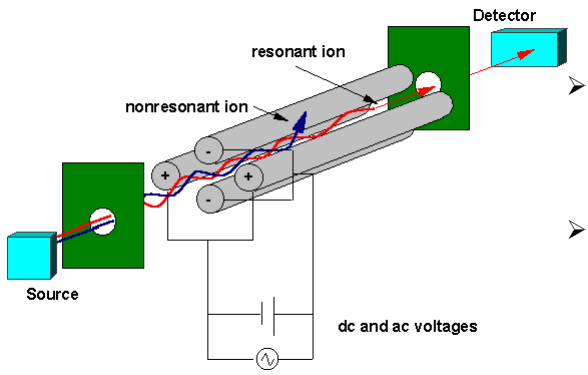
- single quadrupole
- triple quadrupole
- quadrupole ion trap
- time of flight/quadrupole time of flight
- fourier transform ion cyclotron resonance (FTICR)

What is a quadrupole?

- four metal rods set parallel to each other
- each opposing rod pair is connected electrically and a radio frequency (RF) voltage is applied between rod pairs
- direct current voltage is superimposed on RF voltage
- only ions with certain mass to charge ratio (m/z) will move through quadrupole at the specific voltages



What is a quadrupole?



- allows one m/z to be monitored or to scan for a range of m/z by varying the voltages
- other ions will have unstable trajectories and will collide with the rods

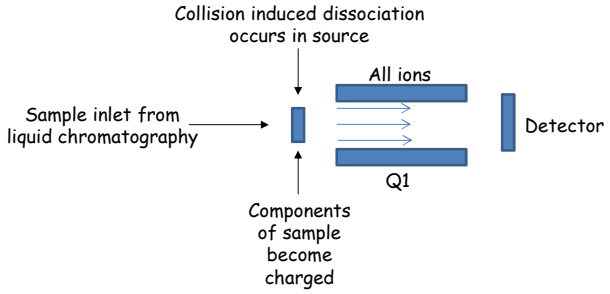
http://www.chemicool.com/definition/quadrupole_mass_spectrometry.html

What is a mass to charge ratio or m/z?

- the mass, m, of an analyte is the molecular weight
  - e.g. testosterone has a molecular weight of 288 g/mol
- when testosterone is ionized in positive mode, it becomes protonated (H^+) so gains a hydrogen and a positive charge, or z
  - mass (m) = 289 (i.e. m+1 = $[M+H^+]$ = 288 + 1 = 289)
    charge (z) = +1

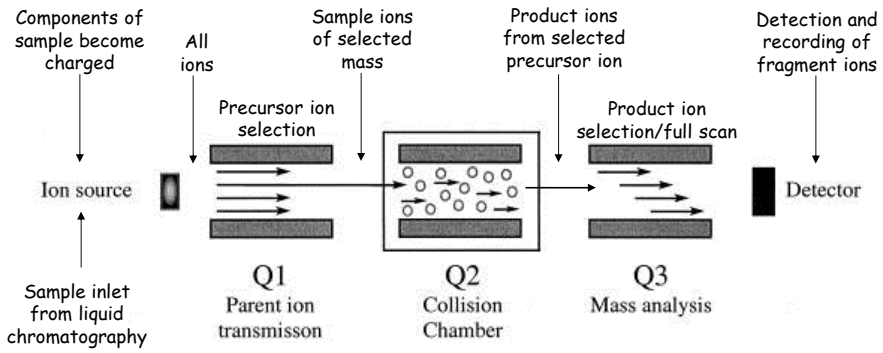
    mass to charge ratio (m/z) is $\frac{289}{1}$ = 289

Single quadrupole mass spectrometer



- only ions of desired mass to charge ratio reach detector when using optimized voltages for analyte of interest
- all analytes with that mass will be detected
- can also scan across a mass range by varying voltages
- not as specific as other instruments

Triple quadrupole mass spectrometer



Q1. Ions of interest are selected (precursor/parent ions)
Q2. Fragmented into smaller product ions
Q3. Product ions separated by mass (m) to charge (z) ratio (m/z)

- also known as a tandem mass spectrometer (MS/MS)
- very selective so best for quantitative analysis
- poor scanning capabilities

Quadrupole ion trap mass spectrometer

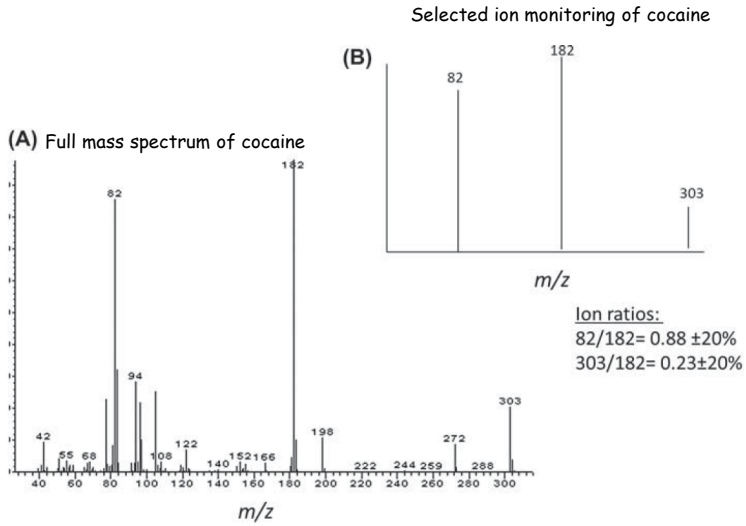
- quadrupole used to generate a field that functions to "trap" ions without destroying them
- ideal for qualitative analysis and elucidation of ion structure
- not as useful for quantitative analysis due to capacity limitations of the trap
- can be used to produce product ion spectra if used with MS/MS
  - an extra layer of selectivity

How is triple quadrupole mass spectrometry commonly used in the clinical laboratory?

- small molecules
  - steroid hormones
    - testosterone, estradiol, 25-hydroxyvitamin D
  - thyroid hormones
    - free T4, free T3, T4, T3
  - therapeutic drug monitoring
    - cyclosporine, tacrolimus, sirolimus, busulfan, voriconazole, posaconazole, ketoconazole, itraconazole
  - toxicology
    - drug confirmations (opioids, amphetamines, cocaine metabolite etc), comprehensive drug screens
- proteins/peptides
  - thyroglobulin, insulin-like growth factor 1 (IGF-1)

What are the commonly used different modes of operation using these instruments?

Selected Ion Monitoring (SIM)



Ion ratios:
82/182= 0.88 ±20%
303/182= 0.23±20%

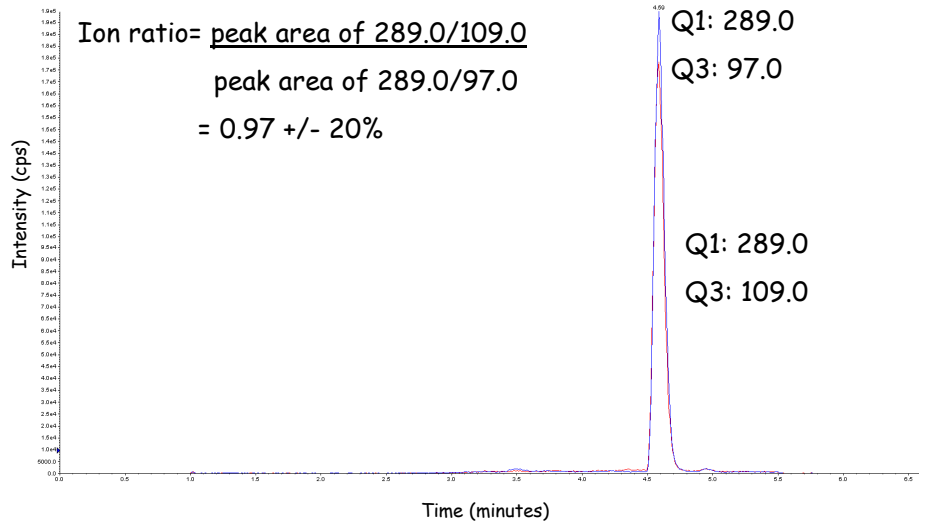
Wu AHB et al, 2012: 50(8):733-742,Clin Toxicol

Selected Ion Monitoring (SIM)

- typically employed in clinical laboratories using GC- or LC-MS
- targeted method
- monitoring fragmentation pattern of specific ions
- usually monitor 3 ions (may include molecular ion and fragment ions)
- use ratios between relative abundance of ions to ensure specificity
- ion ratios consistent across calibrators, controls and patient samples
- improves sensitivity, selectivity and precision of method

Selected Reaction Monitoring (SRM)

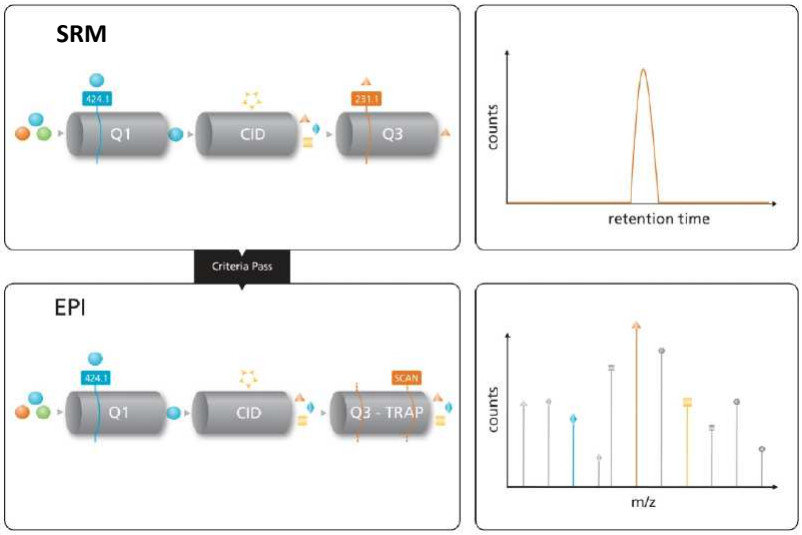
Ion ratio= peak area of 289.0/109.0
peak area of 289.0/97.0
= 0.97 +/- 20%



Selected Reaction Monitoring (SRM)

- typically employed in clinical laboratories using LC-MS/MS
- targeted method
- monitoring of precursor/product ion pairs – transition
- usually monitor 2 transitions per analyte and internal standard
- use ratio between 2 transitions to help determine if there are interferences in the LC-MS/MS method – ion ratios
- ion ratios consistent across calibrators, controls and patient samples
- improves sensitivity, selectivity and precision of method

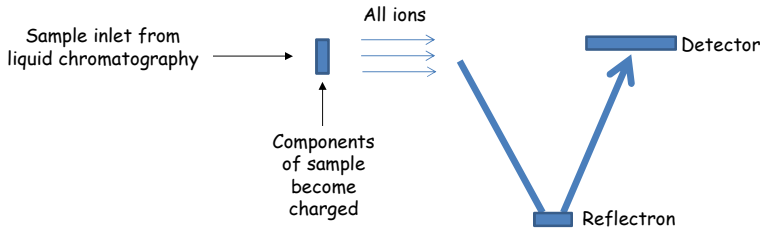
SRM and product ion spectra



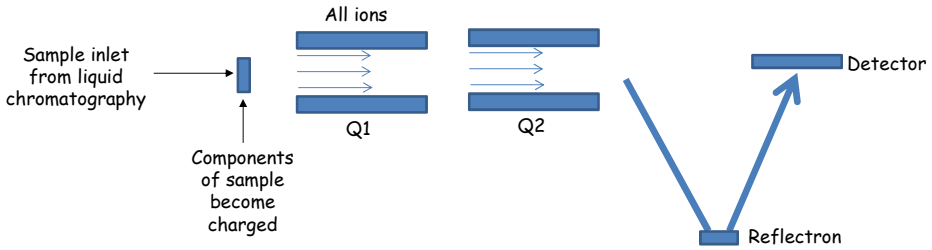
Courtesy of AB SCIEX

High resolution mass analyzers

Time of flight MS (TOF-MS)



Quadrupole time of flight MS (QTOF-MS)

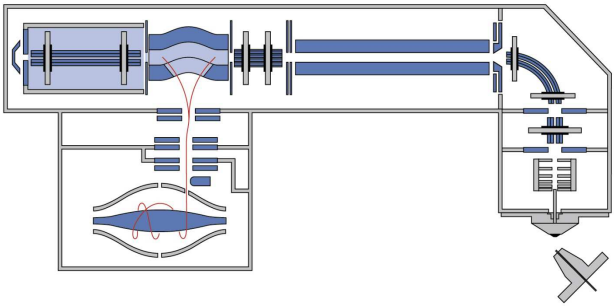


Time of flight MS (TOF-MS)

- based on time it takes for an ion to travel a specific path length when the same force is applied to all ions
- lighter ions arrive at detector earlier than heavy ions
- theoretically TOF-MS has no m/z range limit
- linear dynamic range limitations due to detector saturation
- useful for accurate mass determination
- not as useful for quantitative analysis unless using QTOF-MS

Fourier transform ion cyclotron resonance MS

- FTICR-MS (Orbitrap technology uses similar principles)



- ions trapped in a cell inside a strong magnetic field and move in circular orbits in a plane perpendicular to magnetic field
- RF electrical potential is applied to transmitter plates causing trapped ions to be excited into larger circular orbits
- frequency of motion of ion is inversely proportional to its mass

What defines a mass analyzer as "high resolution"?

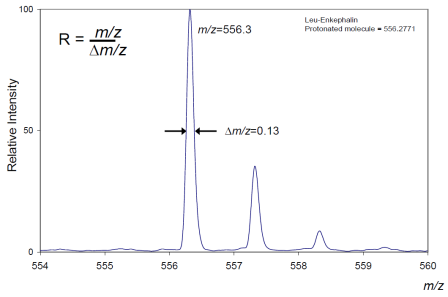
Mass Resolution

The ability to distinguish between ions differing slightly in m/z ratio

Can be calculated in two different ways:

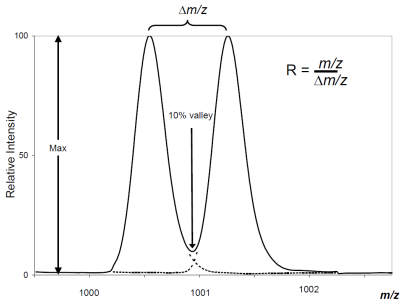
Δm/z is the full width of the peak at half its maximum height (FWHM).

Resolution = 556.3/0.13 = 4279



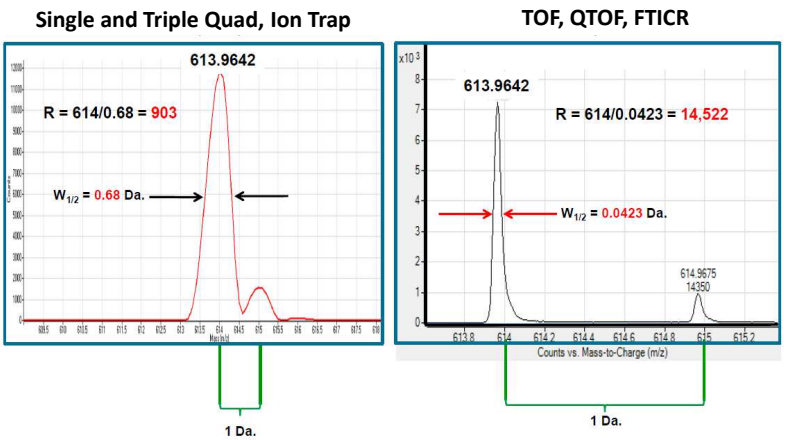
m/z of lowest mass peak is divided by the difference in m/z of the peaks (Δm/z).

Resolution = 1000/1= 1000



CLSI C50-A document

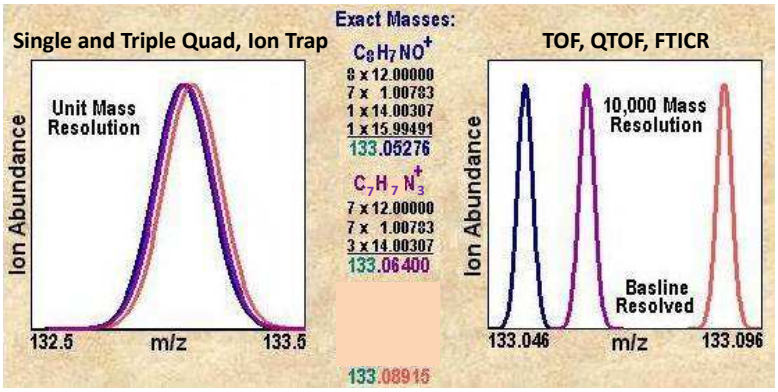
Mass Resolution



Courtesy of Agilent Technologies

Nominal Mass vs Exact Mass

- nominal mass = mass of a molecule calculated using atomic masses of each atom taken as integers
- exact mass = calculated mass based on adding up the masses of each isotope in a molecule



http://www.epa.gov/esd/chemistry/ice/faq.htm#wiaem

What are the commonly used different modes of operation using these instruments?

TOF-MS

- full scan of all ions in sample
- extract chromatogram to obtain accurate mass
- database search to identify compound as well as matching LC retention time

QTOF-MS

- full scan of all ions in sample and set criteria to trigger MS/MS
- extract chromatogram to obtain accurate mass
- database/library search to identify compound based on fragmentation pattern, accurate mass, ion ratios, LC retention time

FTICR

- full scan and full scan fragmentation of all ions in sample
- extract chromatogram to obtain accurate mass
- database/library search to identify compound based on accurate mass, fragmentation pattern and LC retention time

How is high resolution mass spectrometry commonly used in the clinical laboratory?

- toxicology
  - comprehensive drug screens
- microbiology (with MALDI ionization source)
  - identification of bacteria, fungi and mycobacteria
- proteins/peptides
  - thyroglobulin, insulin-like growth factor 1 (IGF-1)

Which type of instrument do you require?

	LC-MS	LC-MS/MS	LC-TOF-MS	LC-QTOF-MS	FTICR
Specificity	++	+++	++	+++	+++
Sensitivity	++	+++	++	+++	+++
Resolution	Low	Low	High	High	Highest
Mass Accuracy	~0.1 units	~0.1 units	~0.01 units	~0.01 units	~0.0001 units
Operational difficulty	++	+++	+++	++++	++++
Suited for which Applications?	Targeted Quant	Targeted Quant	Targeted or untargeted Qual	Targeted or untargeted Quant	Targeted or untargeted Quant
Cost	$$	$$$-$$$$	$$	$$$$	$$$$$

DO NOT FORGET THE COST OF A SERVICE CONTRACT – SIGNIFICANT $$$

(Quant: quantitative analysis; Qual: qualitative analysis)

Other considerations for implementing mass spectrometry

- mass specs are heavy! and large!
- and they generate heat

- need to move cabinets?
- electrical supply
- gas supply – nitrogen, argon
- exhaust
- UPS or back up power
- roughing pump and oil (and disposing of oil)

Other considerations for implementing mass spectrometry (cont)

- mass spectrometry vendor should be able to give you a site guide documenting the requirements for the instrument

- optional (but really nice!) - interface between mass spectrometer and laboratory information system

All of these components can add $$$ to the cost of implementation!

What resources are available?

- colleagues already running mass spectrometry methods
  - invaluable resource
  - can give you "real world" experience with instrumentation
- mass spectrometry vendors
- attend conferences specializing in mass spectrometry
  - e.g. MSACL, ASMS, AACC/MSSS
- literature search
  - can see what instrumentation other clinical laboratories use to measure specific analytes in clinically relevant concentration ranges

Conclusions

- make a list of what your laboratory needs ahead of time with regards to sensitivity, robustness, throughput etc
- mass analyzers vary in specificity, sensitivity, cost and ease of use – should be chosen wisely in terms of desired applications
- don't forget the "extras" such as gas and electrical supply, exhaust, service contract etc as the cost is significant

References/Resources

- Clinical and Laboratory Standards Institute (CLSI). *Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline.* CLSI document C50-A
- Clinical and Laboratory Standards Institute (CLSI). *Liquid-Chromatography-Mass Spectrometry Methods; Approved* Guideline. CLSI document C62-A
- Wu AH, Gerona RR, Armenian P, French D, Petrie M, Lynch KL. *Role of liquid-chromatography-high-resolution mass spectrometry (LC-HR/MS) in clinical toxicology.* Clin Toxicol. 2012. 50(8):733-742
- SelectScience Mass Spectrometers Buying Guide. http://www.selectscience.net/mass_spectrometry_buying_guide.aspx (Accessed November 7th 2017)
- French D, Terrazas E. *The successful implementation of a licensed data management interface between a Sunquest laboratory information system and an AB SCIEX mass spectrometer.* J. Pathol. Inform. 2013. 4:1
- https://www.aacc.org/publications/cln/articles/2015/february/implementing-mass-spec-part-one
- https://www.aacc.org/publications/cln/articles/2015/may/implementing-mass-spectrometry-in-the-clinical-lab

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Making the most of vendor visits and discussions with colleagues

MSACL US 2018
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Wednesday, January 24th

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Learning Objectives

After this presentation, you should be able to:

- Describe questions that should be asked of vendors and colleagues when choosing a mass spectrometer

Overview

- questions to ask vendors
- questions to ask colleagues
- have vendors test actual samples
- site visit to vendors
- selection of vendor

Questions to ask vendors

- request for proposals (RFP) – your institution may already have one
- cover all aspects of instrument requirements

Questions to ask vendors (cont)

- request for proposals (RFP) – your institution may already have one
- cover all aspects of instrument requirements
  - dimensions of instrument
  - venting,
  - electrical requirements,
  - software, hardware (mass spectrometer)
  - liquid chromatography system (pumps, autosampler, column oven, degasser)
  - universal power supply (UPS)
  - nitrogen supply (with or without separate air compressor)
  - service availability
  - interface with laboratory information system
  - training

Questions to ask vendors (cont)

- request for proposals (RFP) (cont)
- cover all aspects of instrument specifications
  - mass range
  - ionization options
  - scan rate
  - minimum dwell time
  - speed of positive/negative polarity switching

Questions to ask vendors (cont)

- sensitivity of available instruments for analytes of interest
- specificity of available instruments for analytes of interest
- availability and cost of training
  - is any training included in purchase price of instrument?
- speak with engineer who will install instrument in your laboratory
  - have them come for a site visit if possible
- contact information for clinical laboratories already using their instrumentation

Questions to ask colleagues

- ease of use
  - software particularly
- maintenance schedule
- instrument downtime
- if colleague is in your geographical area
  - service availability – how quickly does an engineer arrive on site once service call has been placed
  - how responsive is the sales representative?

Unbiased evaluation of each instrument

- design an experimental set of samples that can be sent to each vendor under consideration
- unbiased evaluation of instruments for analytes you want to measure
- choose or make clinically relevant samples
  - patient samples, or spiked samples, or multi-analyte samples or samples with different analyte concentrations
- carry out sample preparation in the same way for each set

Unbiased evaluation of each instrument (cont)

- provide solutions for optimization of mass spec
- provide a column and detailed HPLC protocol
  - include mobile phase composition, gradient and injection volume
- provide the transitions to be monitored
  - can be found in the literature
- ask for the specific data so you can compare
  - (e.g. peak areas, signal to noise ratio, chromatograms etc)

(Laha, Henderson and Hoofnagle, Judging a book by its data: Planning experiments to fully evaluate prospective instrument vendors, poster presentation, MSACL 2016)

Site visits to vendors

- have vendor walk through daily, weekly, monthly maintenance
- possible to have different users with different privileges?
- how many samples fit in autosampler – do you need to use vials and/or plates?
- develop a data acquisition method and use it
  - set up a run
- develop data analysis method and use it
  - analyze data from the run

Selection of vendor

- hardest decision you will have to make!
- compare instrument requirements/specifications (from RFP)
- compare results from patient and other samples
- most important features to compare
  - performance for current and future analytes of interest
  - reliability of instrument
  - service availability and responsiveness
  - availability of training in method development skills
  - responsiveness of sales representative

Decision making

- how you feel during decision making process

- how you feel after you make a decision

Conclusions

- make a list of what your laboratory needs ahead of time with regards to sensitivity, robustness, throughput etc
- ask all vendors the same questions concerning instrument requirements and specifications
- give all vendors the same blinded samples to run
- rank each instrument based on what is most important to you
- you will find that each system has pros and cons that are comparable
- hardest decision you will have to make!

References/Resources

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Acknowledgements

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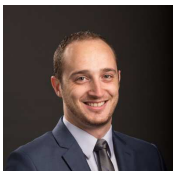

Joe El-Khoury

Acknowledgements

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Financial considerations for purchasing a mass spectrometer

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Yale SCHOOL OF MEDICINE

Disclosures

- No Conflict of Interest to Disclose

Learning Objectives

- Describe the clinical and monetary benefits of purchasing a mass spectrometry system
- Explain the financial components that have to be considered when purchasing a mass spectrometry system
- Calculate the return on investment for the purchase of a mass spectrometer
- Participate in effective negotiations with vendors
- Describe the options for financing a mass spectrometry system purchase
- Get a mass spectrometry system for free*

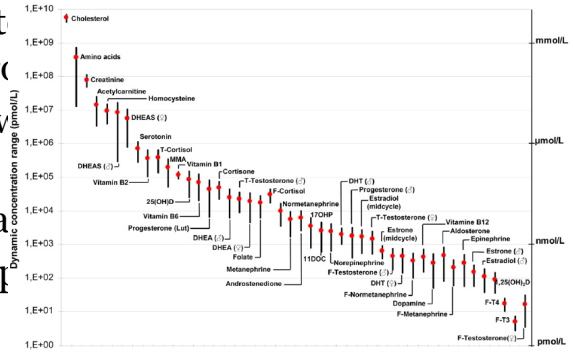
Polling Question #1

- Why are you thinking of buying a mass spectrometry system?

A. Better quality
B. Improved sensitivity over immunoassays
C. Improved turn-around-time from sendout
D. To save money
E. No alternative option for testing on market

Clinical and Quality Benefits (Not just the money!)

- Why LC-MS/MS?
  - Highly specific and accurate quantitation
  - Pot ... on (pr
  - Lov
  - No ana
  - Imp



Vogeser, M. et al. Exp Clin Endocrinol Diabetes 2007; ***115: 559-570.***

Monetary Benefits

- Cheaper vs sending out to reference lab
- Potential for outreach business by acting as reference lab
- Cheaper reagents than other assays (although initial cost of instrument is high)

How do you convince your administrator that a mass spectrometer is a good investment?

- Speak their language
- Answer the following questions:
  - What's the ROI (Return on investment)?
  - How much capital do you need?

Return-On-Investment

- a performance measure used to evaluate efficiency of an investment

$$ROI = \frac{(\text{Gain from Investment} - \text{Cost of Investment})}{\text{Cost of Investment}}$$

- Typically reported in % or ratio of profit-to-investment

Cost of an LC-MS

One off costs	Example $ amounts
– Mass spectrometer	400,000
– Liquid chromatography system	40,000
– Nitrogen generator	25,000
– UPS	8,000
– Interface to LIS	30,000
– Electrical work	3,000
– Duct/venting work	10,000
– Removal of benches	200
– And others	
	Total $516,200 (per mass spectrometer)

Cost of an LC-MS

Recurring costs	Example $ amounts
– Service contract (1st year is free – covered by warranty)	30,000/year/instrument
– Reagents and consumables	see next slide
– Personnel (for 2 technologists in CA)	225,000/year
– And others (Regulatory…etc)	
Total	~$255,000/year
5-year total	~$1,275,000

Polling Question #2

- Have you purchased a used LC-MS system?
  - Yes
  - No

What are the gains from investment in mass spectrometry?

- Example: immunosuppressants by immunoassay
- Predicted reagent cost by LC-MS/MS: $3 per patient sample

Test	Current methodology			New methodology	Difference
	Cost per test	Yearly volume	Yearly cost	Estimated yearly cost	
Cyclosporine	$17.00	3,000	$51,000	$9,000	-$42,000
Sirolimus	$19.00	1,700	$32,300	$5,100	-$27,200
Tacrolimus	$17.00	23,000	$391,000	$69,000	-$322,000

Total savings $391,200/year

5-year total $1,956,000

What are the gains of investment in mass spectrometry? (cont)

Recoup costs	Example $ amounts
– Bill for in-house test	$225/sample
– Realistically recover ~ 25%	$56.25/sample
• cyclosporine	$168,750/year
• sirolimus	$95,625/year
• tacrolimus	$1,293,750/year

Total $1,558,125/year

5-year total $7,790,625

Note: billing depends on outpatient vs inpatient test volume at your institution

Return on Investment calculation (ROI)

• Example: buy 2 LC-MS/MS systems to run immunosuppressants (cost estimate over 5 years)

- LC-MS/MS one-off cost $1,032,400
- Yearly costs $1,275,000
- Savings per test (gain) $1,956,000 on reagents
- Recoup cost (gain) $7,790,625 on billing

$$ROI = \frac{(\text{Gain from Investment} - \text{Cost of Investment})}{\text{Cost of Investment}}$$

$$ROI = \frac{(\$9{,}746{,}625 - \$2{,}307{,}400)}{\$2{,}307{,}400} \times 100 = +322\% \text{ in 5 years}$$

But what if they won't give u $2.3 million dollars to spend?

Polling Question #3

- How do you acquire a mass spectrometer?

A. Capital Purchase
B. Long-Term Lease
C. Reagent Rental Agreement
D. Free
E. N/A - never bought one but I can't wait!!

Polling Question #4

- If you lease an MS, what do you at the end of term?

A. Purchase at Fair Market Value
B. Extend lease contract (as is)
C. Re-negotiate an extension
D. Return to vendor

How do you finance the initial instrument cost?

- Capital equipment budget
- Instrument lease
  - pay whole cost of instrument during monthly payments - lease to own ($2.3 mill. becomes $45,000/ month)
  - pay smaller monthly payment but do not own instrument at end of lease, but you have 3 good options:
    - Purchase it for market value (usually below $50,000 per instrument after 5 years)
    - Extend contract for another 2-3 years
    - Return to vendor

Tips for negotiation

1. Always submit RFP to at least 2 vendors (more is better)
2. Expand your training slots (at least 3/instrument purchased)
   - These are almost free give-aways for vendors
   - Also make sure that these are good for 5 years
3. Get written confirmation of all of requests– don't just take their word for it (e-mail is ok, doesn't have to be contract)

Polling Question #5

- Can you get a MS for free?

A. Yes
B. No

Tips for negotiation (Cont'd)

4. Shift the cost of development and validation time to the vendor
   - Can contract so that you don't get charged until validation is complete or 3 months after the MS is installed.

That's typically 1-3 months for straightforward methods– that's up to ~$150,000 in instrument costs alone!!

Speaker and Presentation Evaluations for French-El-Khoury using Survey Monkey

https://www.surveymonkey.com/r/7JGJZZK

Please let us know what training resources you need

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Yale SCHOOL OF MEDICINE

SLIDE 20