SELÇUK ÜNİVERSİTESİ

The 12th International & 17th National Congress on Quality Improvement in Clinical Laboratories

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Quality Management of Mass Spectrometry in Clinical Laboratories

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Outline

- Need and use mass spectrometry in clinical laboratory
- LC-MS/MS Maintenance
  - System suitability test
  - Calibrator accuracy and calibration curve slope
  - Internal standard (IS) peak area
  - Quality control
  - Retention and/or Relative retention time to IS
  - Ion ratio
- In practice

Need MS

- To have less method inherent pitfalls;
  - Cross reactivity
  - Anti-reagent ab, heterophilic ab
  - Low detection level
  - Drug intereferences…
- Minimise analytical uncertainty
- Minimise cost effect (?)

4 Mass Type Common in Use in Clinical Laboratories

ICP-MS

GC-MS

LC-MS/MS

Maldi TOF

GC-MS, LC-MS/MS Applications

Analysis	GC-MS	LC-MS/MS
Amino Acid Disease	♥♥	♥♥♥♥♥♥♥
Organic Aciduries	♥♥♥♥♥	♥♥♥♥
Fatty acid oxidation defects	♥♥♥♥♥	♥♥♥♥♥
Carbohydrates	♥	♥
Phospholipids	♥	♥
Steroids	♥♥♥♥	♥♥♥♥♥♥♥♥♥
Volatile organic compounds	♥♥♥♥♥♥	♥
Ethanol, methanol	♥♥♥♥♥♥	♥
Drugs	♥♥♥	♥♥♥♥♥
Unknown drug/substance	♥♥♥♥♥♥♥♥	♥♥♥♥♥
Forensic toxicology	♥♥♥♥♥♥♥	♥♥♥♥♥
Food and residual substances	♥♥♥♥♥	♥♥♥♥♥

More, better?

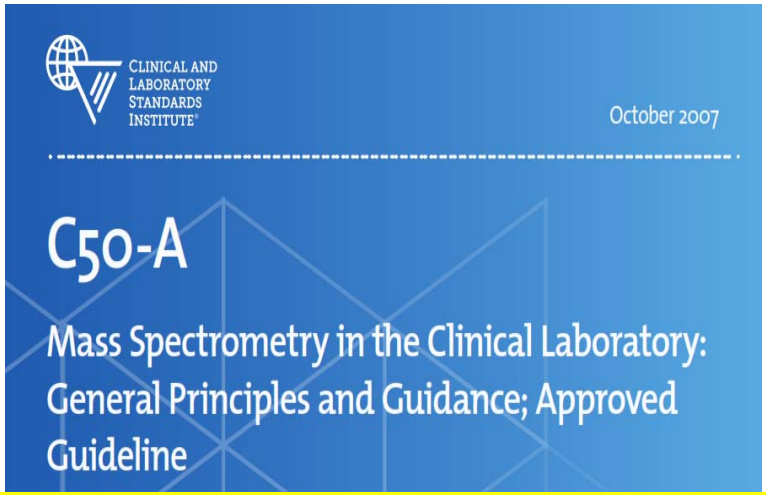
- MS methods are more;
  - Versatile
  - Sensitive
  - Specific
  - Multiplex analytes

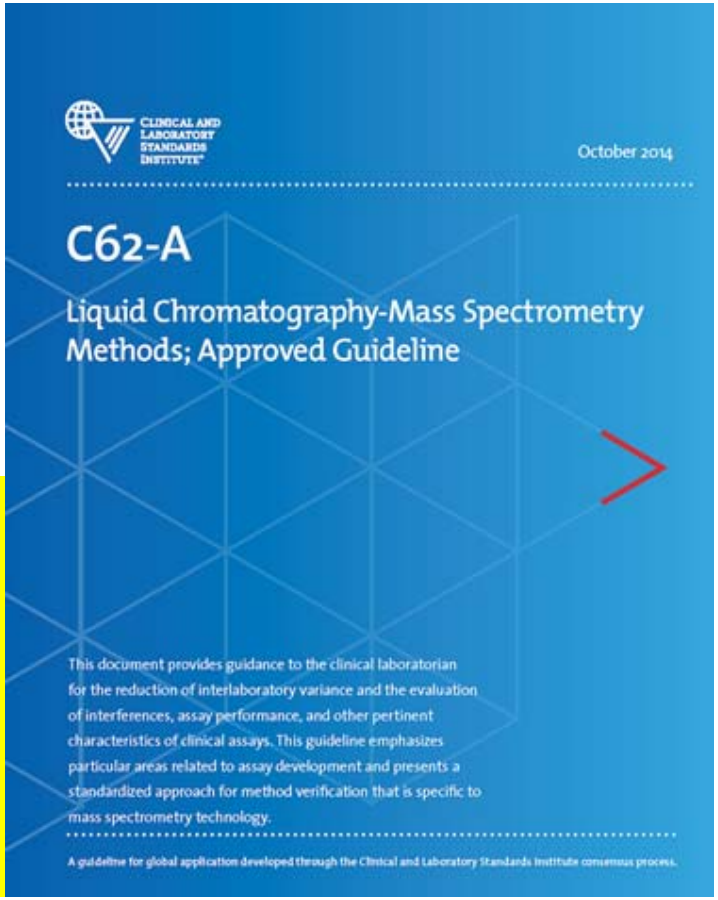
- Low detection limits
- 'In House'

  - CHALLENGES (need more attention any routine analyzers)
    - Systematic quality management
    - Continous attention

Rules, Regulations

Centers for Disease Control and Prevention (CDC) Clinical Laboratory Improvement Amendments (CLIA), Council of American Pathologist (CAP), European CE the directive 98/79/EC 'rather vague guidance for in house developed tests'





The goal of this guideline is to provide a basic understanding of the technology and how it should be used in the clinical laboratory with an emphasis on:

- **advantages and disadvantages;**
- **precautions required in its use;**
- **quality control awareness;**
- **assay verification/validation;**
- **approaches to reporting results; and**
- **communication of the data.**

"All in one" reagents (kits) available

Solutions for Clinical Diagnostics

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HPLC Complete Kits

ClinTox® Human Biomonitoring

Benzene and Derivatives

- ortho-Cresol and Phenol in Urine
- Hippuric Acid and Methylhippuric Acids in Urine
- t,t-Muconic Acid in Urine

Polycyclic Aromatic Hydrocarbons (PAH)

- 1-Hydroxypyrene in Urine - On-Line Analysis

- HPLC Complete Kits
- ClinRep® Diagnostics
- ClinRep® TDM
- ClinTox® Human Biomonitoring
- LC-MS/MS

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Shop By

Product Type

- Kit (4)

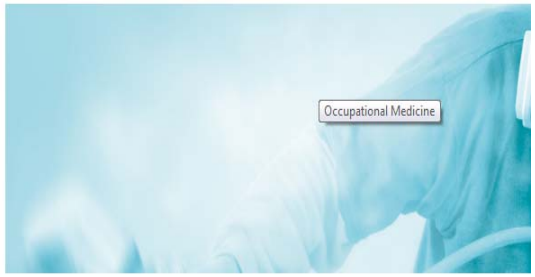
Method of Analysis

- HPLC (4)

Parameter

- 1-Hydroxypyrene (1)
- Hippuric acid (1)
- m-Methylhippuric acid (1)
- Mandelic acid (1)
- o-Cresol (1)
- o-Methylhippuric acid (1)

Occupational Medicine



4 Item(s) Sort By Position Show 20 per page

1-Hydroxypyrene in Urine - HPLC

Here below we report the complete catalog of product families of this category (click on an item in the catalog to see a list of individual products)

- Clinical Chemistry /Endocrinology
- Clinical Pharmacology
- Forensic Toxicology
- Chemioterapics
- Lyophilised Matrix Calibrators
- Chemical Standards
- Lyophilised Matrix Controls
- Analytical and Preparative Instrumentation
- Accessories and Consumables

73 Organic Acids LC-MS/MS
Analysis Kit

ZİVAK

VMA, HVA, 5-HIAA LC-MS/MS
Analysis Kit

Verification/Validation

- IVD-CE MS kits;
  - Validated in one/ a few instruments
  - Problem is huge number of different MS and chromatography system. All systems show variable performance.
  - ISO-15189 standards suggested for verification.
  - Full Verification (CLSI C62-A); LOD, LOQ, linearite, uncertainty, interferences, accuracy

Pre-Validation

- **Method Development**
- **Pre- validation (e.g. CLSI EP10)**

Validation

- **Imprecision**
- **Recovery (measurand, IS)**
- **Accuracy, method comparison**
- **Linearity**
- **Sensitivity (LOQ, LOD)**
- **Spesificity, interferences**
- **Specimen type**
- **Assay calibration**

Post-Validation monitoring

- **Proficiency Testing**
- **System Performance Monitoring**

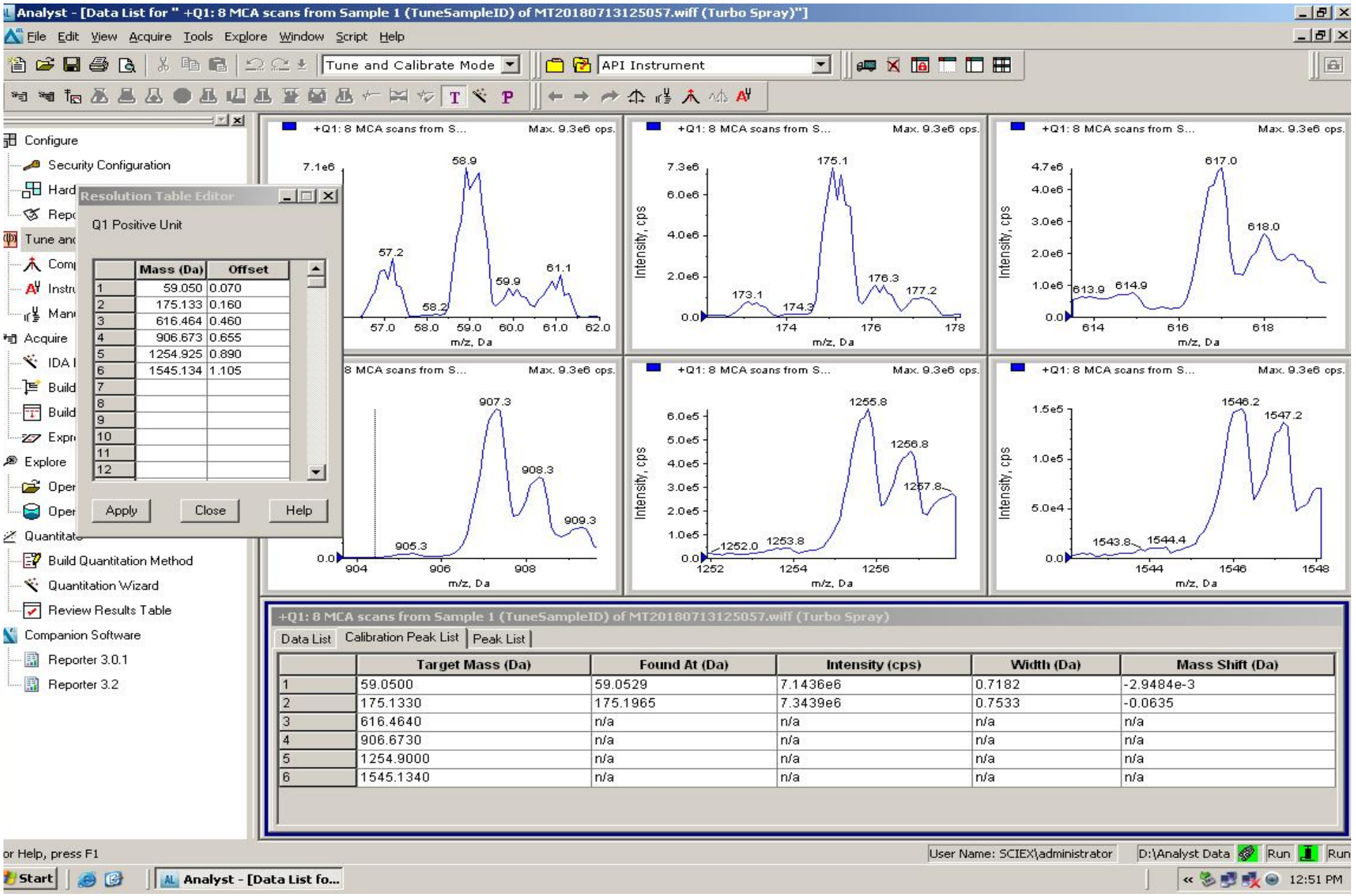
LC-MS/MS Quality Assurance Parameters

- System suitability test
- Calibrator accuracy and calibration curve slope
- Internal standard (IS) peak area
- Retention and/or Relative retention time to IS
- Ion ratio monitoring
- Quality control

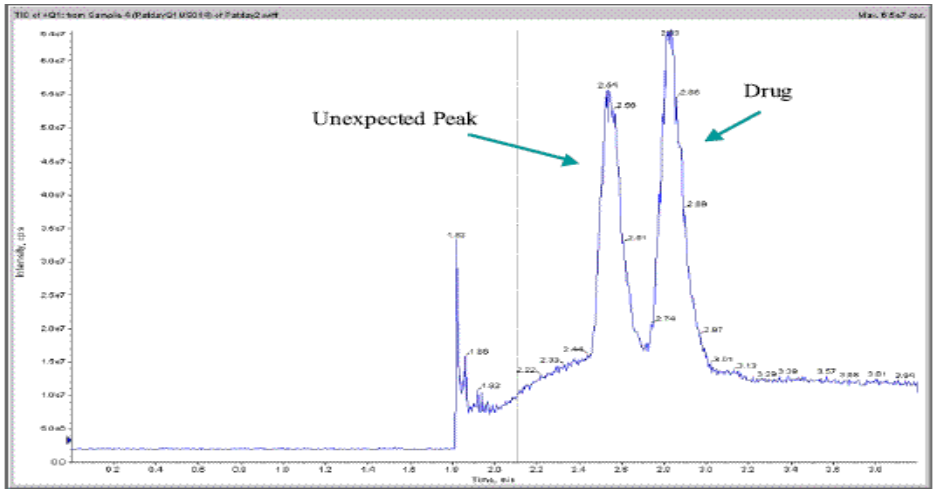
System Suitability Test (SST)

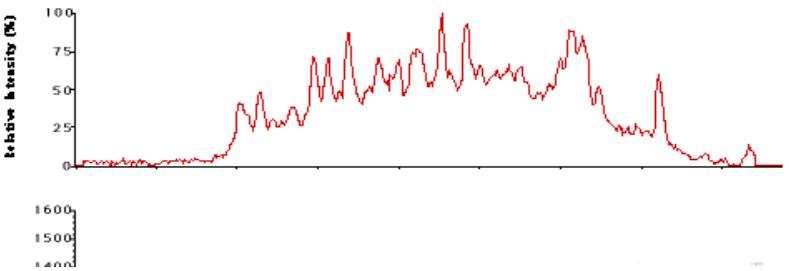
- This is a check to make sure your MS is performing properly.
- Infuse mass detector caffeine or reserpine, different from your compound.
- The tune shows when your MS is in need of cleaning or in maintenance
  - Peak shape, abundance,
  - shift (not more than 0,2 amu)
  - Widht (0.7 ± 0.1 amu for half the height resolution)
- CLSI –C62A Recommendations
  - Acceptance criteria should be set (RT, peak height and width, ion ratio, signal/noise ratio)
  - 3 samples should be evaluated prior batch analysis after instrument maintenance
  - Ion ratio of replicates CV <6%
  - S/N ratio >10/1 at lower limit of measuring level.

Polypropylene Glycol



System Suitability Samples





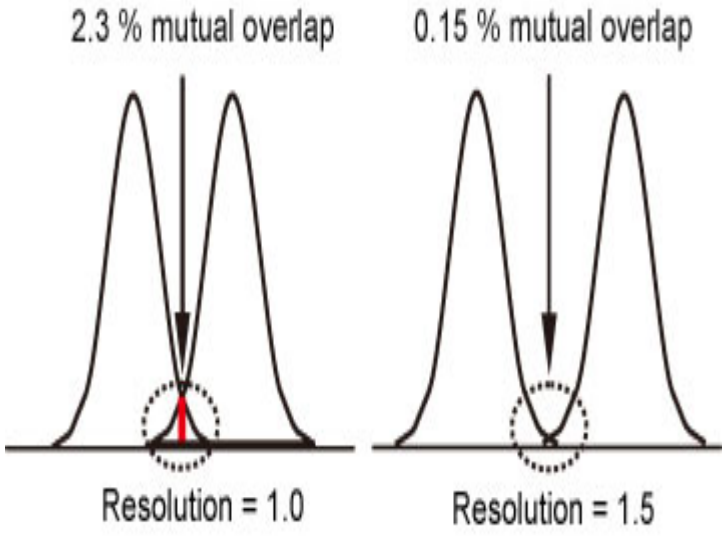
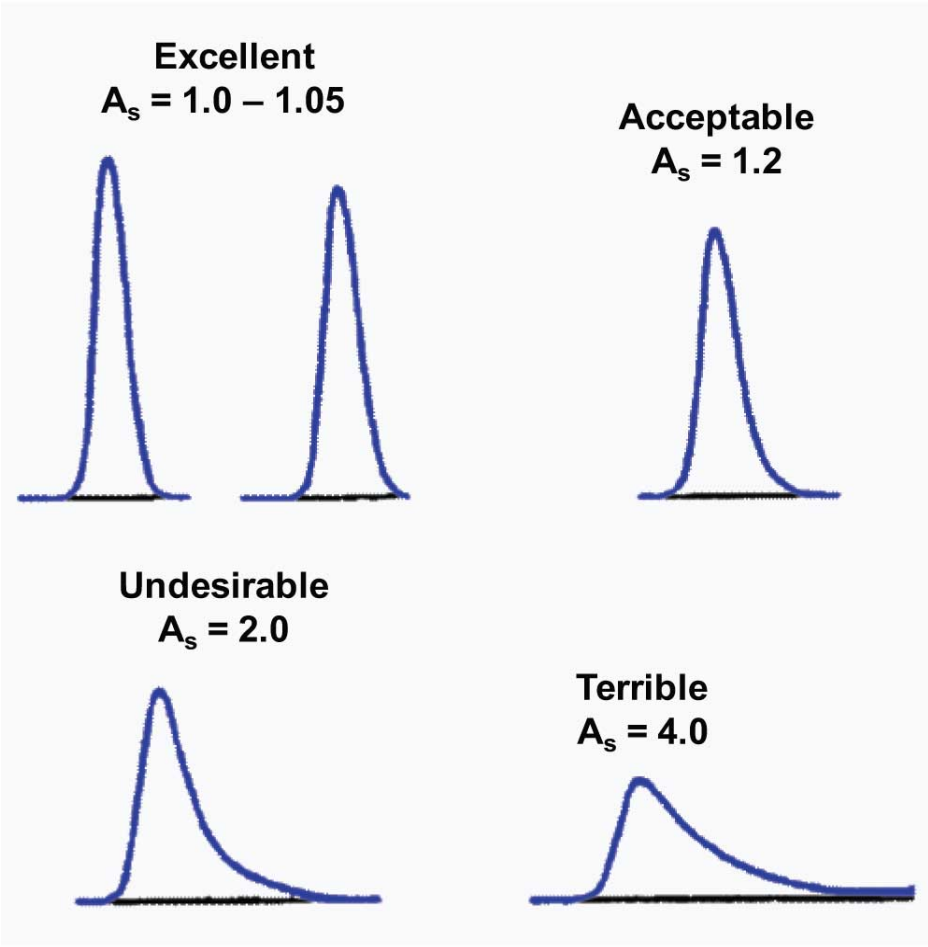


Fig. 2 Resolution and Peak Separation



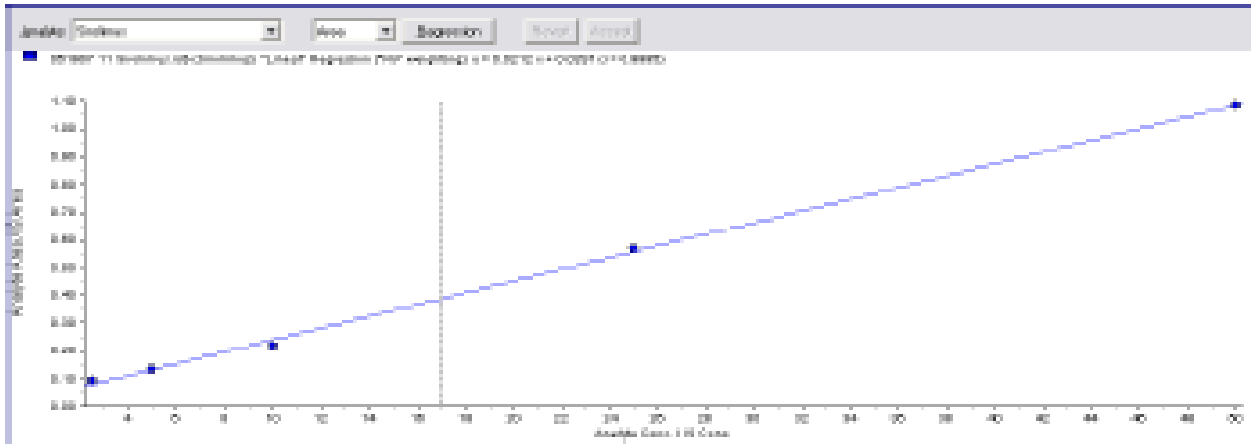
System Suitability Test (SST)

- Possible problems:
  - Shift in RT,
  - Peak asymmetry,
  - Change in peak intensity
  - Detection additional peaks
  - Ion ratio change
  - Decrease S/N ratio

- Possible Causes:
  - Mobile phase change, degradation, evaporation
  - System failure, malfunction,
  - Column change, deterioration
  - Temperature fluctuations
  - New interferent
  - MS maintenance, cleaning requried

Standards (Calibrators)

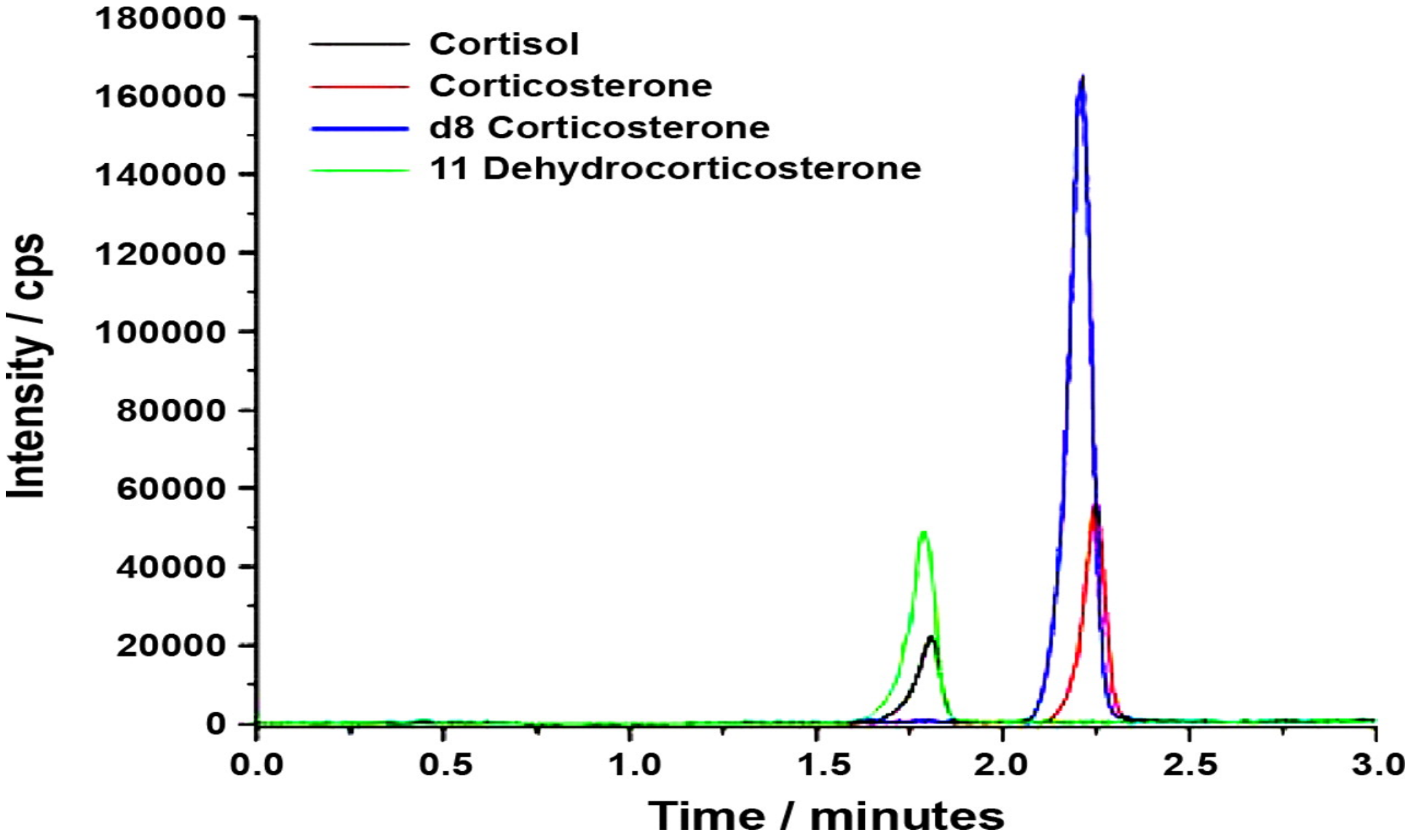
- Multipoint is better than single point. If you are using single-point calibration, the quality control (QC) material should be used.
- To have a new calibration set on every single run 'the best'
- There must be a procedure for not accepting a point on the calibration curve. Thus, it is understood whether there is an error in a calibrator.



Calibrators, Calibration Curve

- CLSI-C62A recommendation
  - Allowable bias +15% for all calibrators above LOQ, +20% for LOD
  - Calibration slope r^2>0,995
- Possible problems
  - Nonlinearity, change in apppropriateness of linear fit
  - Unacceptable bias for one or multiple calibrators
- Possible causes
  - Calibrator deterioration
  - Loss of detector sensitivity
  - Insufficient volume of injection
  - Pipetting error
  - Poor preoperative recovery

Calibrator and Internal Standard Signal Monitoring

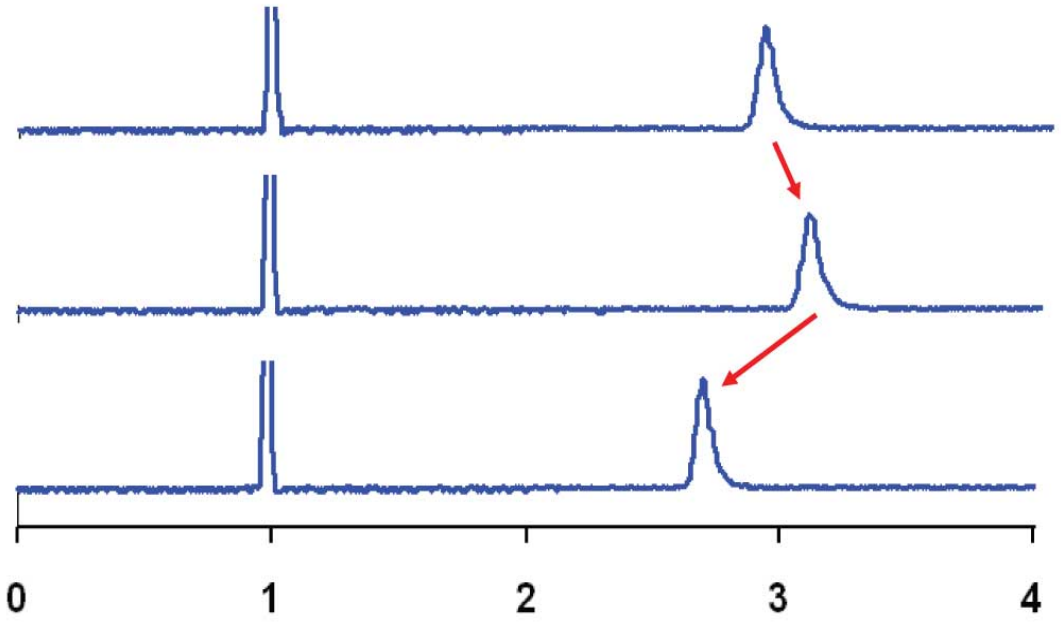


Internal Standard Peak Area

- CLSI-C62A recommendation
  - Acceptable range for IS peak area should be defined during method validation
  - IS peak areas should be comparabile with calibrators and samples in same run
- Possible problems
  - Sporadic IS shift throughout run or in only one sample
  - Gradual shift
  - Severe shift
- Possible causes
  - Instrument drift/charging
  - Poor preoperative recovery
  - Mistakes in the preparation of IS
  - Unacceptable ionisation suppression (matrix effect?)
  - Insufficient volume of injection
  - IS degradation

Retention time (RT)

- Careful to watch your retention time of your analytes
- Deuterated internal standards should come out at exactly the same time as your analyte

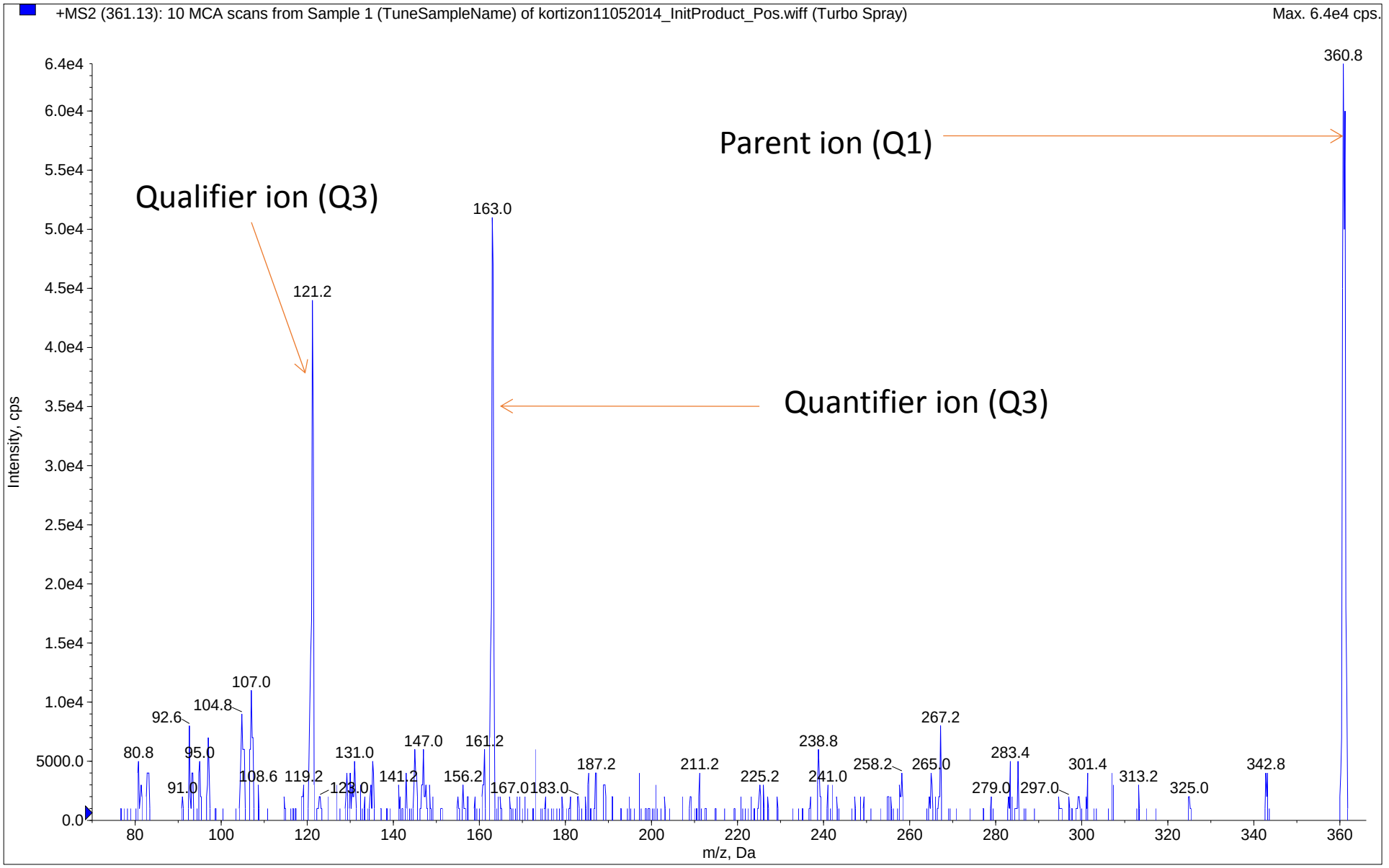


RT diff< 2.5 %

Rt or RRt to IS

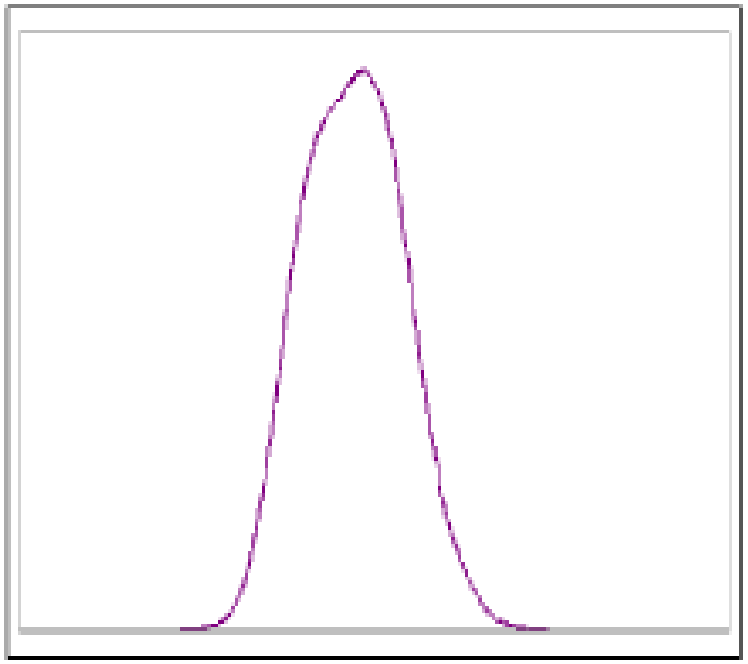
- CLSI-C62A recommendation
  - Should be within +2.5% of the mean Rt/RRt of the calibrators in the same or between batches
- Possible problems
  - Sporadic shift in Rt
  - Gradual shift in Rt over time
  - Drastic shift
- Possible causes
  - Mobile phase change, degradation, evaporation
  - LC pump failure, malfunction,
  - Column change, deterioration
  - Temperature fluctuations

Ion Ratio Monitoring

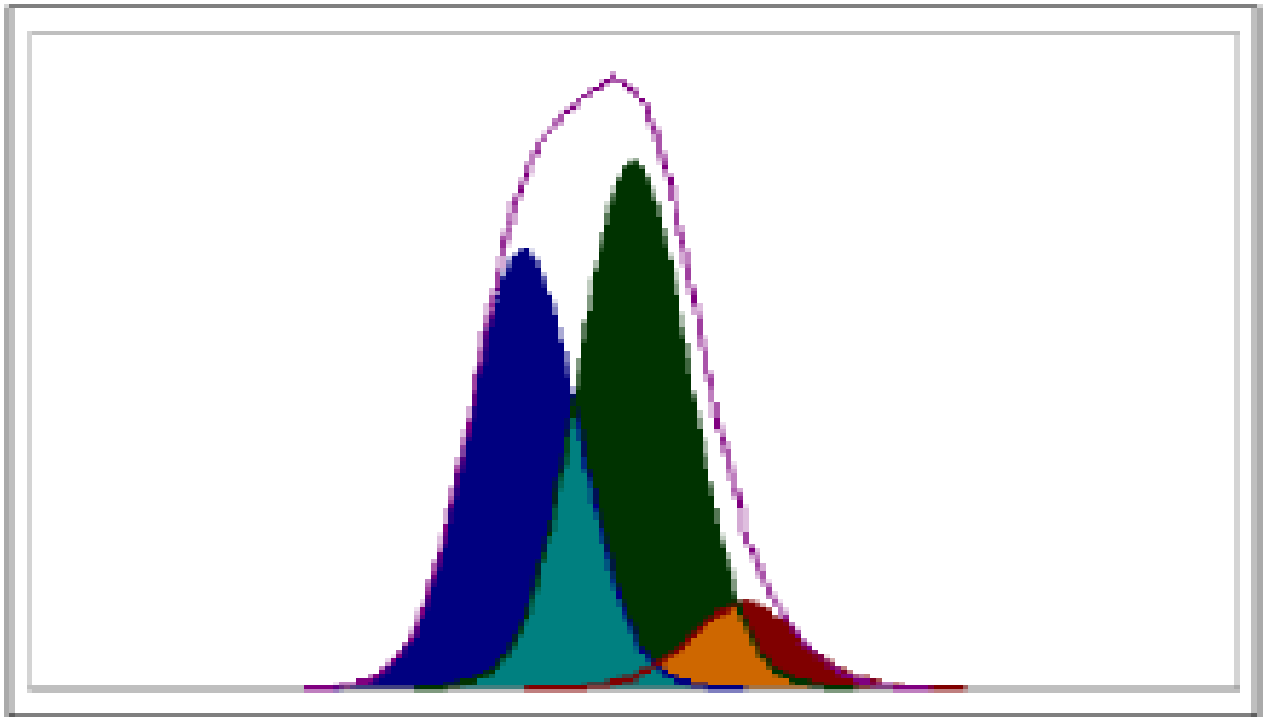


Interference peaks: reason to look at ion ratios

- Is this a right peak?
- How can we cut this peak?



- several peaks underneath
- Ion ratios are key to find hidden interferences.



Ion Ratios

- More ion ratios that are used the stronger the identification of compound.
- 3-4 ion ratios increase interval confidence.
- Ion ratios can be used for IS.
- If your parent compound 150, not pick 132 (possibly loss of 1 mol H_2O).
- Have well defined tolerance criteria (should be supported by literature and experimental).
- This tolerance should not be exceed 25% of calibrators.
- Most of the software can detect this.

Ion Ratios

	Sample Name	Epi	Norepi	Dop	Epi Ratio	Norepi Ratio	Dop Ratio	Epi IS Area	Norepi IS Area	Dop IS Area	Epi Qual	Norepi Qual	Dop Qual
1	Unextracted Standard	8.87	9.30	9.52	106.	95.4	99.3	4360000.	4360000.	22900000.	9.24	8.87	9.45
2	Cal 10	8.49	9.44	7.46	99.4	99.4	99.0	2930000.	2930000.	16200000.	8.44	9.38	7.39
3	Cal 25	23.9	25.5	23.4	101.	95.3	99.5	3270000.	3270000.	17300000.	24.2	24.3	23.3
4	Cal 100	97.8	102.	98.2	99.8	97.1	99.1	2920000.	2920000.	16000000.	97.5	99.5	97.3
5	Cal 200	201.	199.	201.	108.	101.	100.	2790000.	2790000.	16200000.	301.	200.	302.
6	Control 1	15.8	43.8	79.4	108.	92.9	99.6	2250000.	2250000.	16700000.	16.7	40.7	79.0
7	Control 2	89.7	172.	414.	104.	94.4	102.	2290000.	2290000.	15500000.	93.3	163.	421.
8	Negative Control	0.0264	0.234	0.00229	118.	11.4	726.	2880000.	2880000.	15000000.	0.0305	0.0267	0.0188
9	11 Sp 04-2	2.63	29.0	121.	108.	97.4	98.7	2790000.	2790000.	15700000.	2.88	29.1	119.
10	12 Sp 04-2	1.70	39.4	486.	118.	96.3	100.	2100000.	2100000.	15100000.	1.66	38.0	500.
11	13 Sp 04-2	3.38	32.1	161.	115.	93.8	102.	2740000.	2740000.	14900000.	3.61	30.1	165.
12	14 Sp 04-2	5.36	30.9	154.	108.	96.6	100.	2660000.	2660000.	15600000.	5.26	29.9	155.
13	15 Sp 04-2	0.553	2.10	36.1	168.	109.	98.3	2690000.	2690000.	15200000.	0.693	2.38	35.5
14	16 Sp 04-2	0.0796	0.931	1.17	1480.	40.5	103.	2360000.	2360000.	13800000.	0.252	0.378	1.21
15	17 Sp 04-2	0.676	3.45	20.6	139.	91.8	99.4	2000000.	2000000.	16300000.	1.22	3.17	20.4
16	18 Sp 04-2	0.739	12.0	42.1	168.	98.2	100.	2520000.	2520000.	15200000.	1.18	11.8	42.3
17	19 Sp 04-2	2.57	58.1	119.	117.	108.	99.7	2360000.	2360000.	11000000.	3.02	62.6	119.
18	20 Sp 04-2	7.08	54.3	231.	108.	92.7	99.3	2840000.	2840000.	15600000.	7.50	50.3	229.
19	21 Sp 04-2	8.61	22.3	102.	102.	98.0	101.	2530000.	2530000.	15000000.	8.00	21.8	103.
20	22 Sp 04-2	8.86	84.1	391.	108.	91.6	101.	2450000.	2450000.	14000000.	9.15	86.2	395.
21	23 Sp 04-2	1.52	23.8	129.	118.	91.0	101.	2290000.	2290000.	10600000.	1.61	21.6	130.
22	24 Sp 04-2	0.274	13.3	61.6	168.	96.2	100.	2210000.	2210000.	12100000.	0.424	11.9	61.7
23	25 Sp 04-2	0.884	7.43	33.3	139.	97.5	100.	2060000.	2060000.	12200000.	0.963	7.24	33.5
24	26 Sp 04-2	7.58	37.7	149.	108.	99.3	103.	2470000.	2470000.	15500000.	7.81	37.8	152.
25	27 Sp 04-2	6.75	46.3	152.	102.	102.	98.8	1460000.	1460000.	7940000.	6.87	47.3	160.
26	28 Sp 04-2	1.86	16.4	62.6	111.	90.4	101.	3190000.	3190000.	17500000.	2.07	14.8	63.0
27	29 Sp 04-2	3.82	18.4	97.7	108.	104.	100.	2400000.	2500000.	15300000.	4.07	18.1	97.9
28	30 Sp 04-2	1.93	12.9	34.6	104.	106.	100.	2620000.	2670000.	15300000.	1.94	13.7	34.6
29	31 Sp 04-2	0.302	4.09			71.8						2.84	8.8

Ion Ratio

- CLSI-C62A recommendation
  - Acceptable range should be established during method validation
  - Mean ratio of calibrators should not alter significantly within or between runs
  - If signal of qualifier ion is >50%, the ion ratio in the patient samples should be +20% from that of the mean ratio of the calibrators.
- Possible problems
  - Ion ratio outside of the acceptable range for individual patient sample
  - Significant change in mean between runs
  - Ion ratio outside of acceptable range for samples near LOQ
- Possible causes
  - Integration failure of precursor or product ion
  - Interfering substance in an individual patient sample
  - Reagent or system change (new interferent?)
  - Loss in assay sensitivity resulting inadequate signal for qualifier ion

Quality Controls

- QC and calibration materials indirectly evaluate the accuracy and precision of patient samples.
- QC procedures must be designed to find immediate errors (operator performance, instrument failure...)
- Controls preparation should go through as much as sample preparation.
- Should be the same matrix as sample.
- Multiple sets of controls (low, normal and high) help with troubleshooting issues.
- If there is an abnormal control use after than a negative control (carryover)

NIST

National Institute of Standards and Technology

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cap

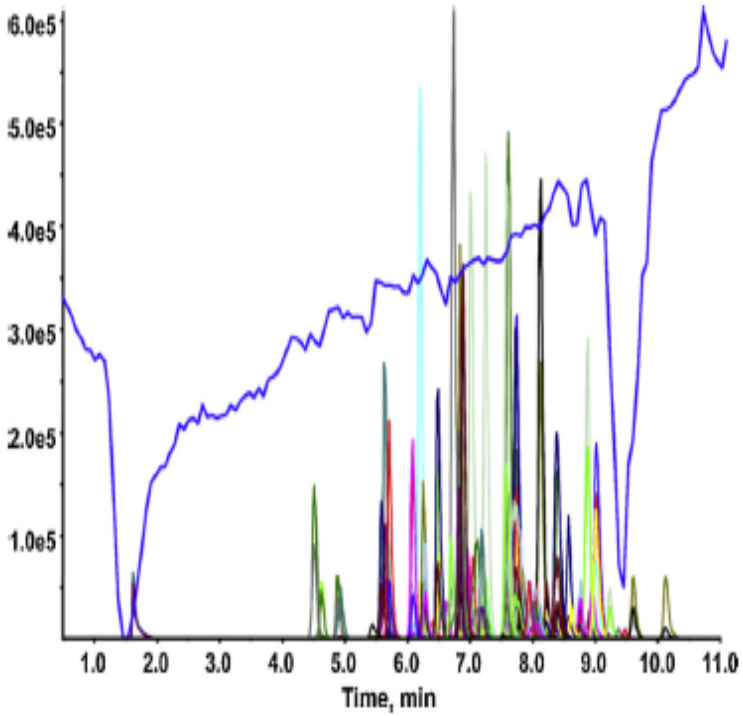
Accuracy Based Surveys

Quality Control

- CLSI-C62A recommendations
  - 3 QC levels should be tested in each batch
  - Establish own acceptable QC mean ± SD (not supplier provided range)
  - Evalute new lot QC according to CLSI C24
  - Document every failed QC
- Possible problems
  - Random QC failure
  - Gradual QC shift
  - Severe QC shift
- Possible causes
  - QC deterioration
  - Loss of detector sensitivity
  - Insufficient volume of injection
  - Pipetting error
  - Poor preoperative recovery

Ion Suppression

- When other compounds in your matrix steal the charge from the analyte of interest in the ion source
- Cause signal decrease or increase
- Test for ion suppression in your assay as a whole by running a blank matrix in the analyte of interest

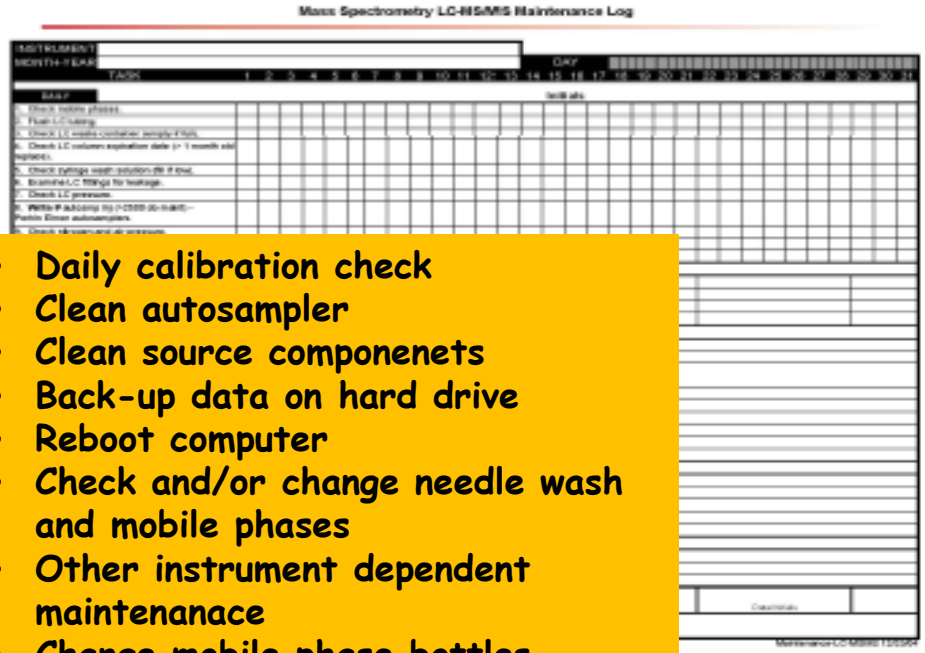


By using an effective gradient, you may avoid ion supression

External Quality Assessment

- EQA programs available CAP or American Proficiency Instute (API), CDC for new born screening...
- EQA may not be available for all LC-MS/MS tests;
  - New test
  - Analyte instability
  - Interlaboratory differences (difficult harmonisation)
    - Different source of calibrators
    - Different sample preparation techniques
    - Selection of ions or ion transitions
    - ....

Maintenance logs: Best friends



- Daily calibration check
- Clean autosampler
- Clean source componenets
- Back-up data on hard drive
- Reboot computer
- Check and/or change needle wash and mobile phases
- Other instrument dependent maintenanace
- Change mobile phase bottles

- Maintenance records can help you find a problem before it occurs
- Logs must be filled out Daily (or each working day).
- It tracks preventative as well as corrective maintenance
- Manufacturer's recommendation should be checked for what they would recommend as maintenance

In a real lab world;
in practice

Assuring to give true results

- various mass spec specific criteria for routine testing
- some helpful tips and tricks to make day-to-day operations go more smoothly;
  - Consumables
  - Instruments
  - Sample preparation
  - Preventative maintenance

Reagents, solvent, column...

- Reagents and solvents must be of the appropriate grade
- Each time a new reagent should be carefully checked before use
- Each mobile phase bottle should be clearly labelled (date, content, who prepared….)
- Have a written procedure for just about everything you do in the lab (operation, preparing calibrators, maintenance logs, detecting potential carryovers…)
- Before a new column is put into place, it needs to be evaluated for performance.
- Before start make sure you have enough correct mobile phase and autosampler needle wash.
  - NEVER top off mobile phase bottles
  - NEVER use detergent to clean bottles

Basic instrumentation tips

- make sure the instrument's all warmed up and the source is at the right temperature
- inject only analyte containing mobile phase before injecting calibrators and patient samples.
- make sure that the retention time or peaks come out the same time.
- keep up on your maintenance and documentation

Sample Preparation Tips

- very important to accurately and consistently pipette patient sample and calibrators
- need to be very precise in adding your internal standard (critical step for quantitation).
- to double check to make sure that not only using the correct reagent but purity of reagent.
- make sure you have enough internal standard for all the samples.

Preventative maintenance

- Regularly calibration check
  - Caffein (since stable compound) dissolved in methanol, infuse directly into MS.
  - Check parameters;
    - Peak abundance
    - Peak width
    - Right mass
- Clean autosampler and MS components (source)
- Back-up your data
- Check your needle washes and your mobile phases
- Other instrument dependent maintenance.

SELÇUK ÜNİVERSİTESİ

Thanks for patience