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Theory and Key Principles Series

Gas Chromatography (GC)

Session 7 – Processing GC Data

Introduction

Welcome to Shimadzu's Gas Chromatography Theory and Key Principles Series!

Presenter

Ollie Stacey

GC/GCMS
Technical
Specialist

- Part of Shimadzu team for >2.5 years
- Previous experience with TOF-GCMS
- Expertise in GCxGC and GCxGC-MS

Questions, feedback & certificates

Please use the panel on the left-hand side to ask **questions**.

We'll send an email within 48 hours of this session, which will include the link to a **survey**.

As well as giving you the opportunity to provide **feedback**, the survey includes a **quiz**, to test your knowledge, and the ability to request a **certificate**.

Theory & Key Principles Series – GC

– *Introduction to Gas Chromatography **

– *GC Columns **

– *The Split/Splitless Inlet **

– *Advanced Liquid Injection Techniques **

– *Alternatives to Liquid Injection **

– *Choice of Detectors for GC **

– **Processing GC Data**

– Maintenance & Troubleshooting

– Method Development

** Now available on demand at www.shimadzu.co.uk/webinars*

Processing GC Data

In this presentation:

- Sample types
  - Samples, standards, quality controls
- Compound types
  - Targets, standards, internal standards, surrogates
- Qualitative analysis
  - Component identification
- Quantitative analysis
  - Area percent
  - External standards
  - Internal standards
  - Standard addition

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Sample types

Sample types

Samples (unknowns)

Unknown samples for analysis – containing one compound, or a mixture of compounds, that we'd like to know answers to questions:

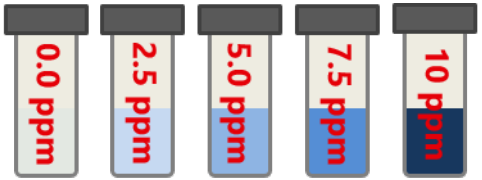
- *how pure is my product?*
- *what concentration is target analyte x at?*
- *is target analyte y present?*
- *what is the ratio between analyte x and y?*

Sample types

Standards

A standard contains a known concentration of target analytes.

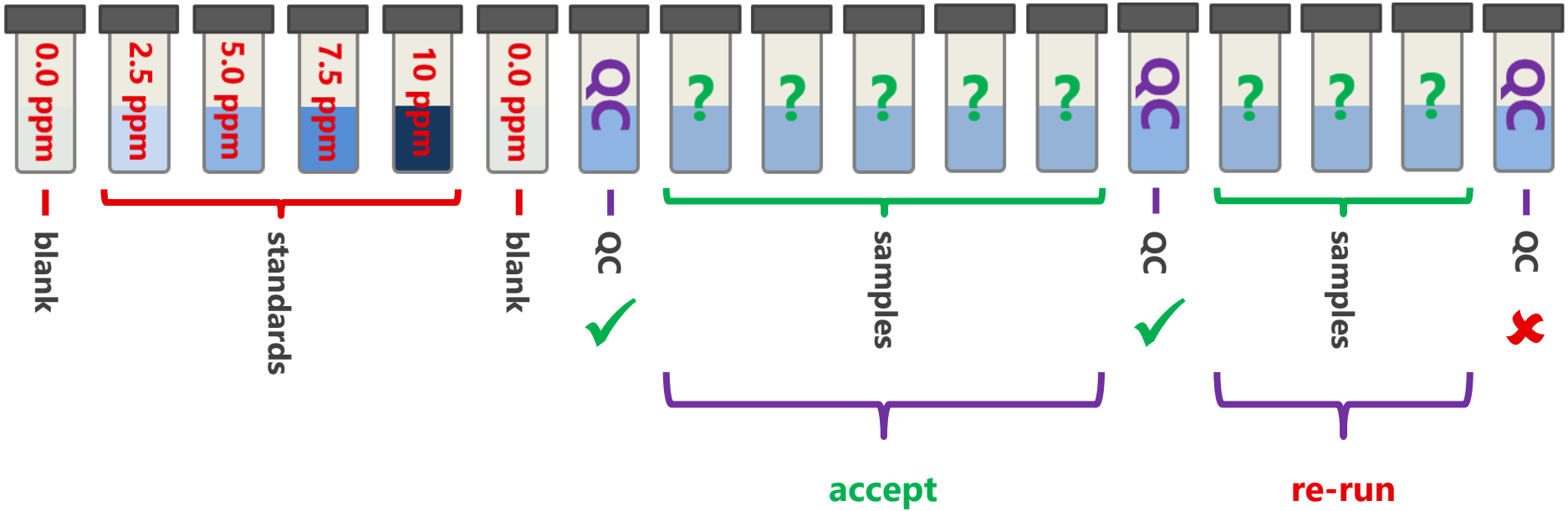
Usually a number of standards are required in order to determine the concentration of analytes in the samples.



Quality Controls (QCs)

Either matrix samples spiked with known concentration of analyte or a standard of a known concentration to confirm the system calibration is still valid.

Typical analysis batch



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Compound types

Compound types

Targets

- Compounds of analytical interest.
- Usually to determine how much is present.

Matrix

- Other compounds in the sample not of analytical interest.
- Matrix can cause increased noise or changes in target response.

Internal Standards (ISTDs)

- Spiked into a prepared sample (and standards & QCs).
- Used to account for instrument variability.
- Usually similar to target (i.e. xylene with benzene target).
- Can't be present in sample.

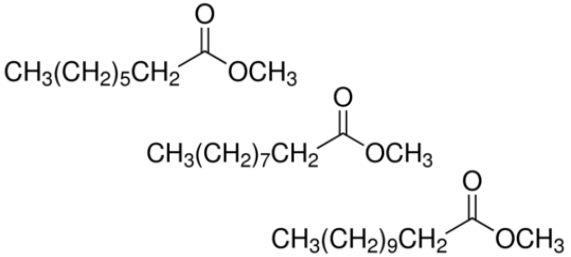
Surrogates

- Spiked into a sample before any preparation is performed.
- Used to determine recovery of targets during sample preparation.
- Must be as chemically-similar to the target as possible (i.e. isotope).

Compound types

Targets

C_8, C_{10} & C_{12} saturated fatty acids. [Require derivatisation to FAMEs].

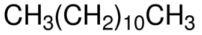


Matrix

Plant material.

Internal Standards (ISTDs)

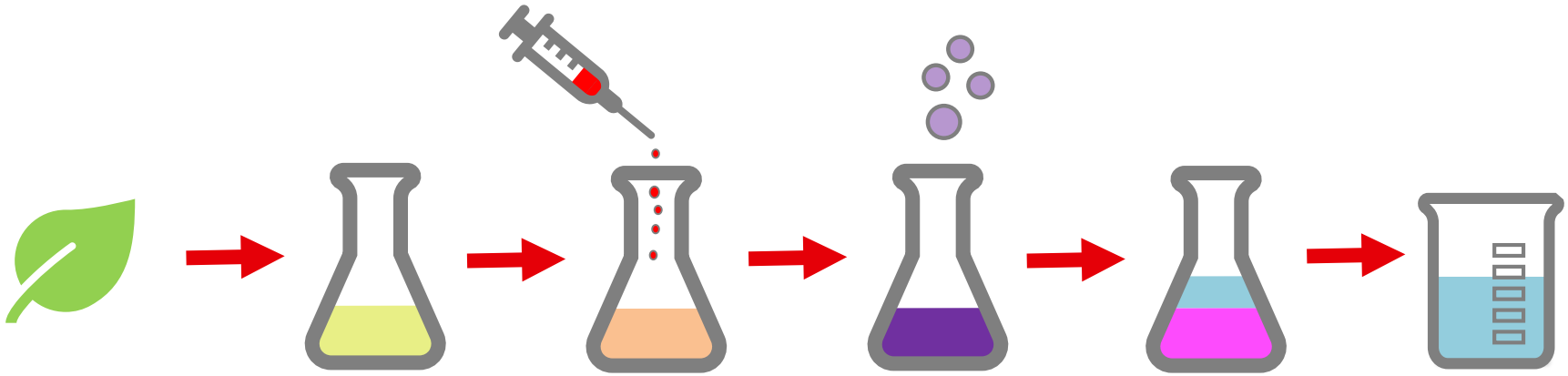
Commercially-purchased FAME reference standard i.e. C_{11}.
Potentially need to use a non-FAME compound, so n-C_{12} (dodecane).



Surrogates

If using GCMS, d_{15}-C8 fatty acid standard.
If using GC-FID, usually another fatty acid.

Sample preparation



Sample

Might be crushed or blended.

Dissolve

Suspended or dissolved in suitable solvent, such as water.

Surrogate Addition

Surrogate usually added at this stage.

Derivatisation

Derivatisation agent(s) added and sample incubated.

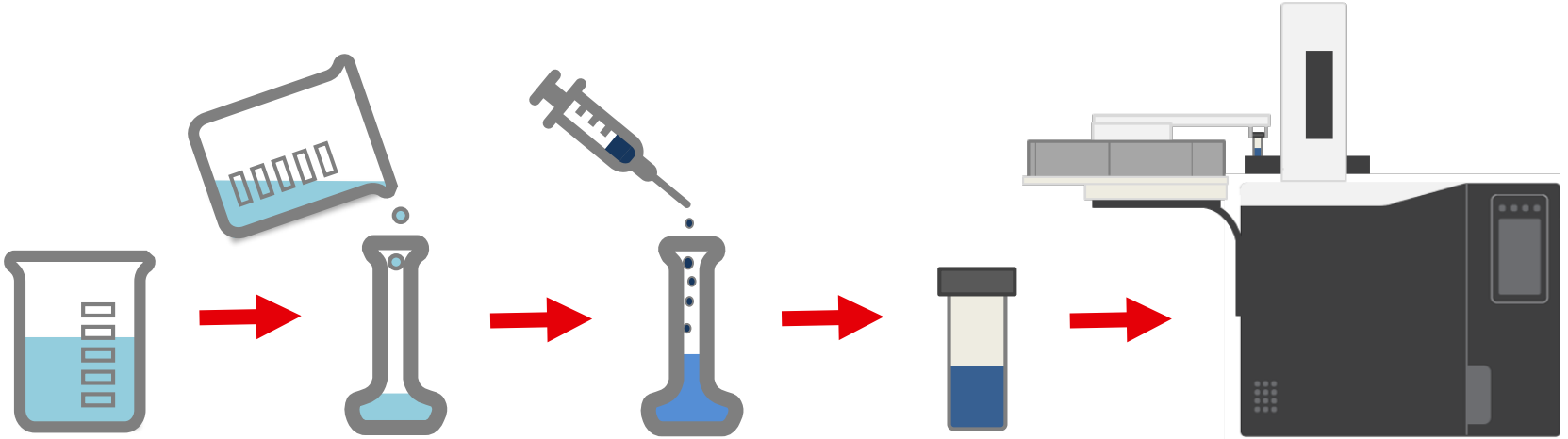
Extraction

Organic solvent, such as hexane added.

Separation

Organic phase collected.

Sample preparation



Derivatised Sample

Dilution

Sample measured into volumetric flask and diluted if required.

ISTD Addition

ISTD added and sample made up to volume of flask.

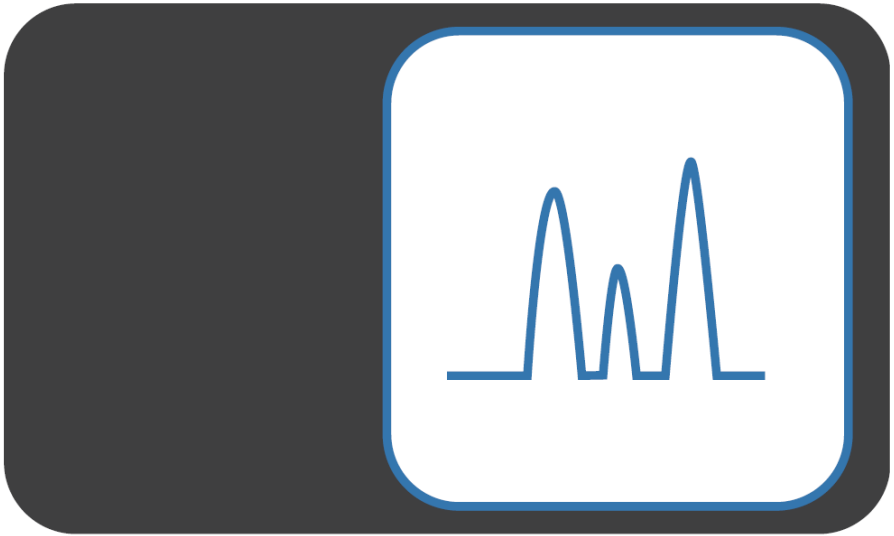
Decant

Sample transferred to vial.

Analyse

Sample analysed on GC.

Data acquisition



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Data processing

Qualitative vs. Quantitative

Qualitative analysis

- What's in my sample?
- Is compound *x* in my sample?

Quantitative analysis

- How pure is my product?
- How much of compound *x* is in my sample?

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Qualitative analysis

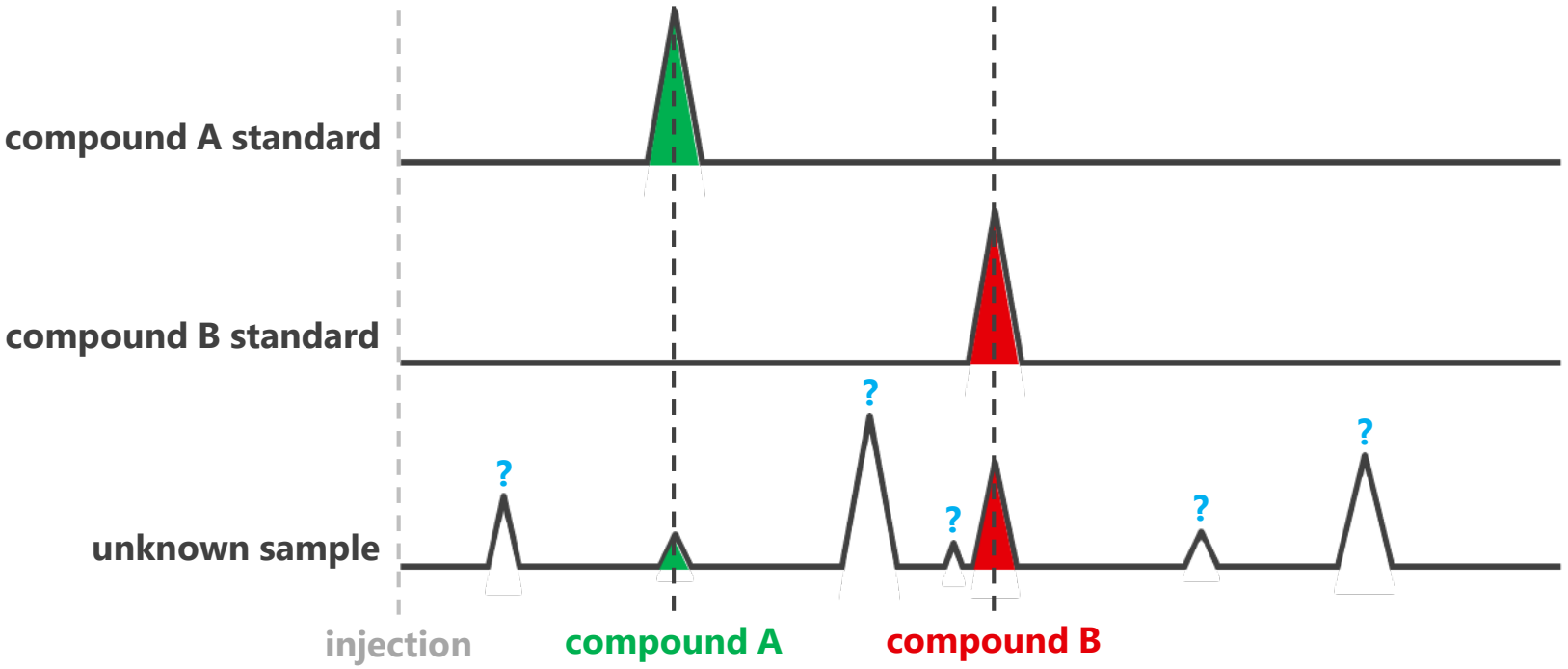
Qualitative analysis

For GC detectors, identity confirmation is based solely on retention time (RT).

To identify compounds within a sample, a standard containing that compound must be analysed.

When analysed under the same conditions, a specific compound always elutes at the same time.

Qualitative analysis

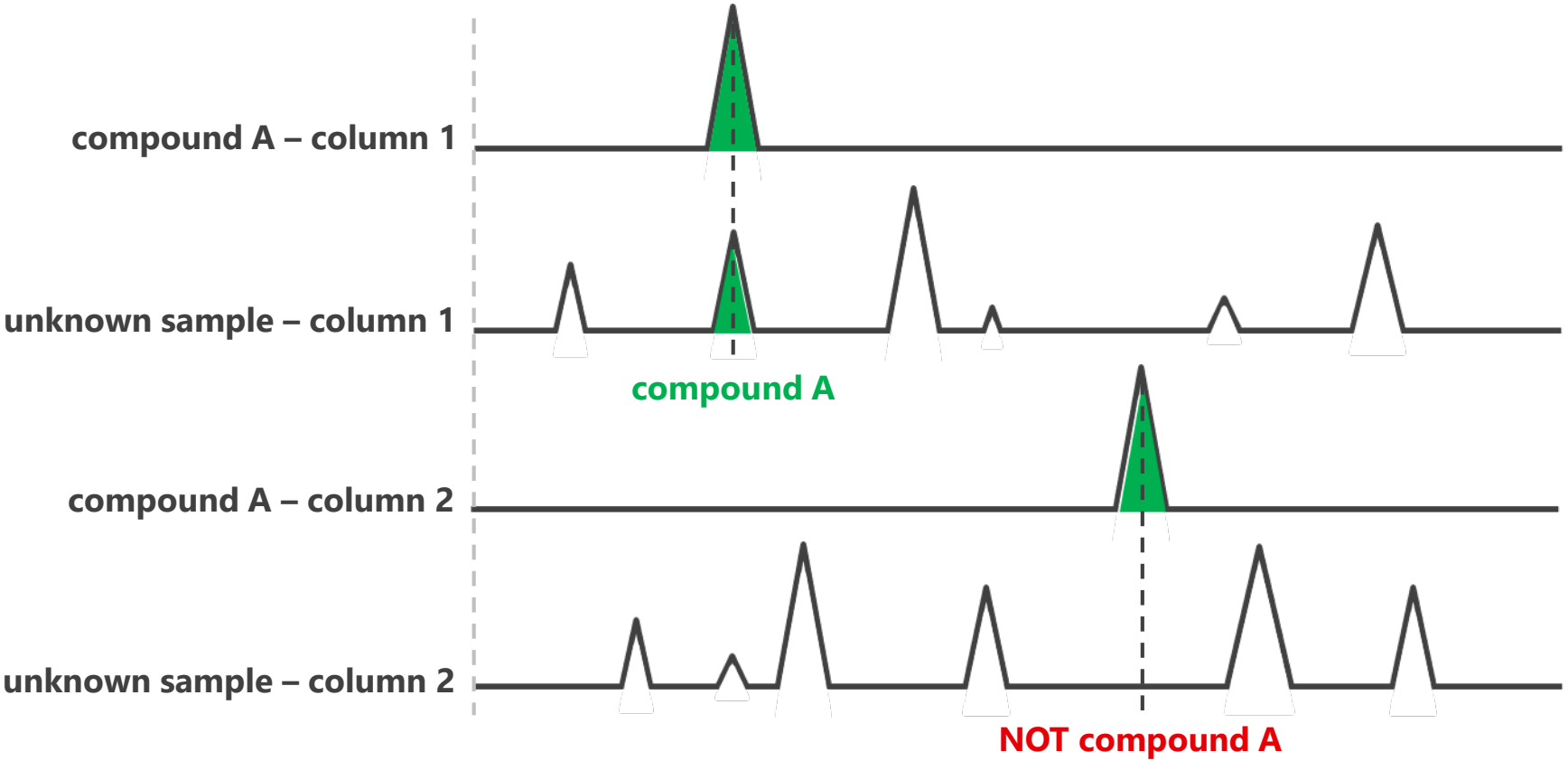


Qualitative analysis

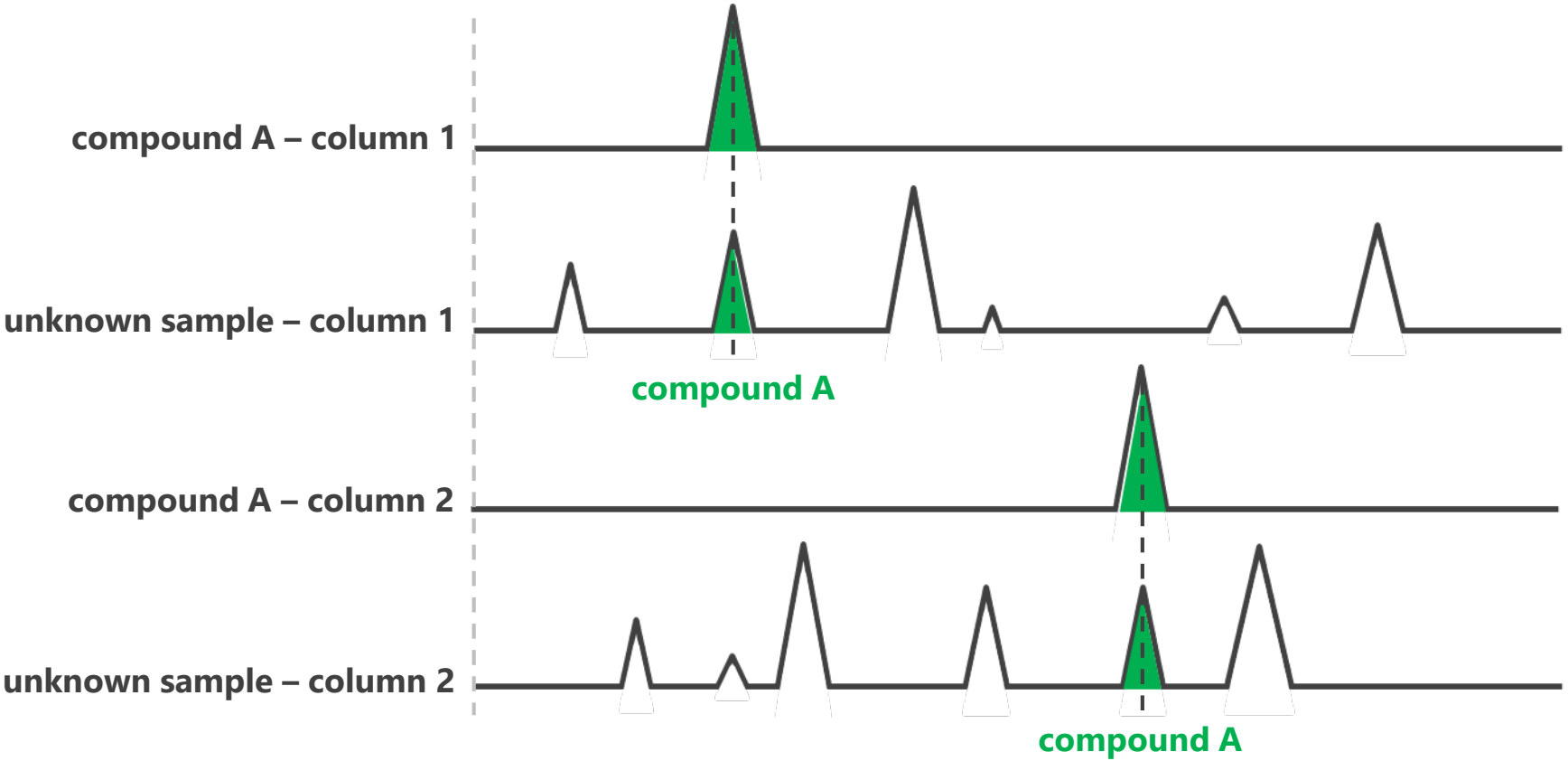
BUT…

Multiple compounds will possess the same retention times as that analyte, so confirmation must be sought.

Qualitative analysis



Qualitative analysis



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Quantitative analysis

Quantitative analysis

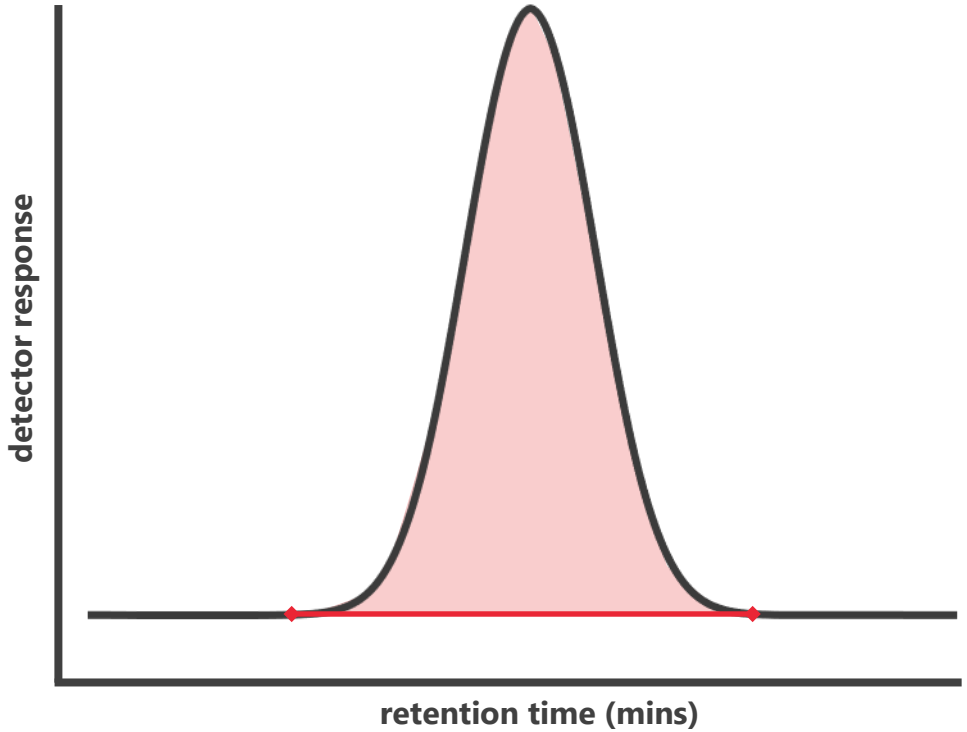
- Area %
- External Standard Calibration
- External Standard Calibration with Internal Standard (ISTD)
- Standard Addition

Integration

Integration determines peak area.

Most software packages use their own algorithm to perform integration.

Refer to your software's manual for details.



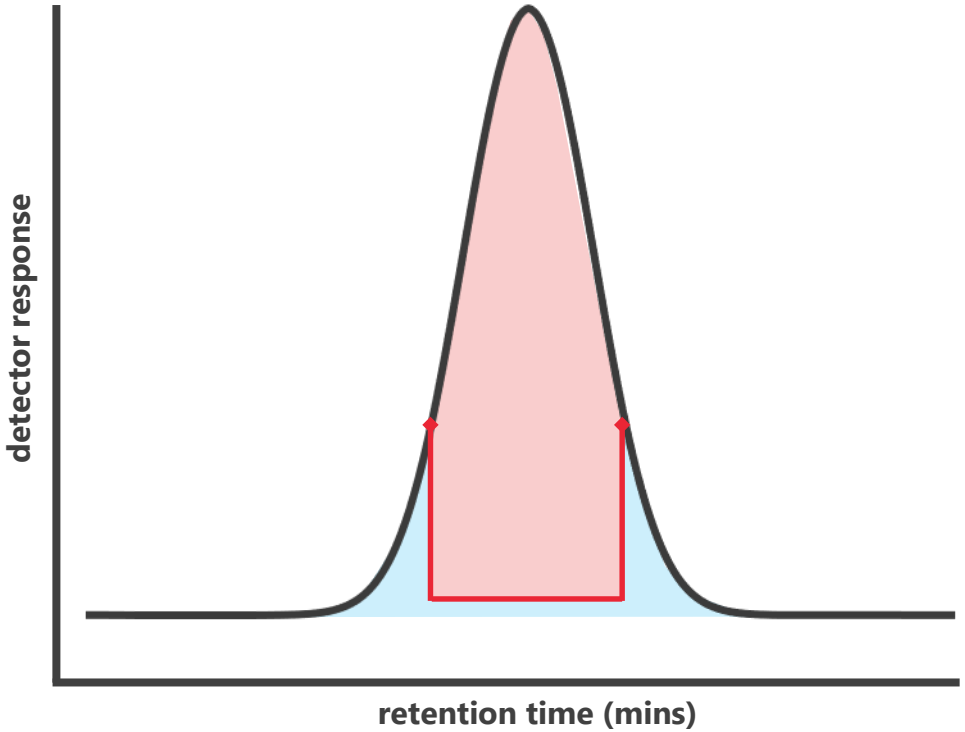
Integration

Integration determines peak area.

Most software packages use their own algorithm to perform integration.

Refer to your software's manual for details.

Incorrect integration = incorrect results



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Area %

Area %

peak area	350	450	1100	50	150	900	total area = 3000

compound A concentration = 15 %

Area %

Advantages	Disadvantages

• Simple analysis method
 	
• Requires no standard samples
	• Makes assumptions:
	  • All compounds have same response factor
	  • All compounds are detected
	
	• Doesn't give definitive amounts

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Corrected area %

Corrected area %

peak area	350	450	1100	50	150	900
RRF	1	1	2	2	3	2

corrected area = 1875

compound A concentration = 24 %

RRF = relative response factor

Corrected area %

Advantages	Disadvantages

• Simple analysis method	• Makes assumptions:
	  ~~• All compounds have same response factor~~
	  • All compounds are detected

• Requires no standard samples	• Doesn't give definitive amounts

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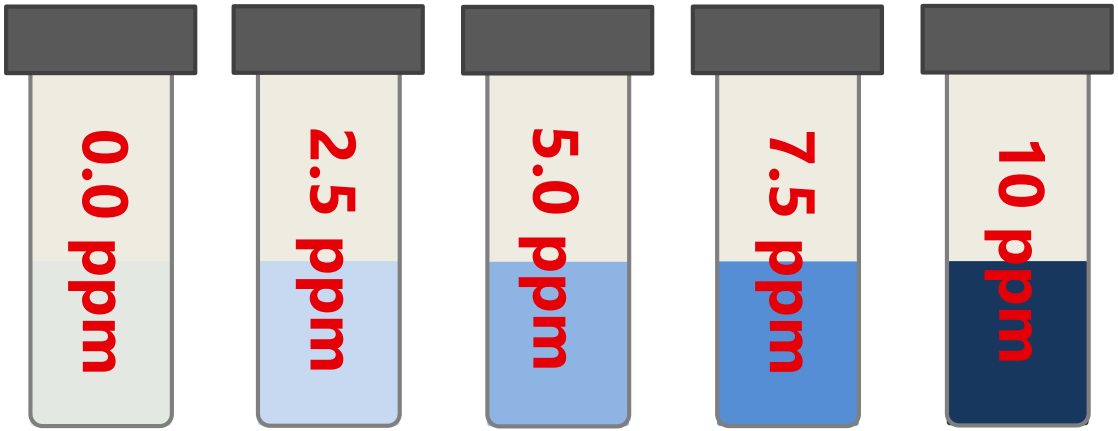
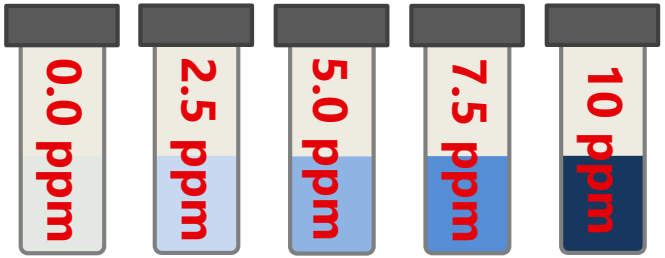
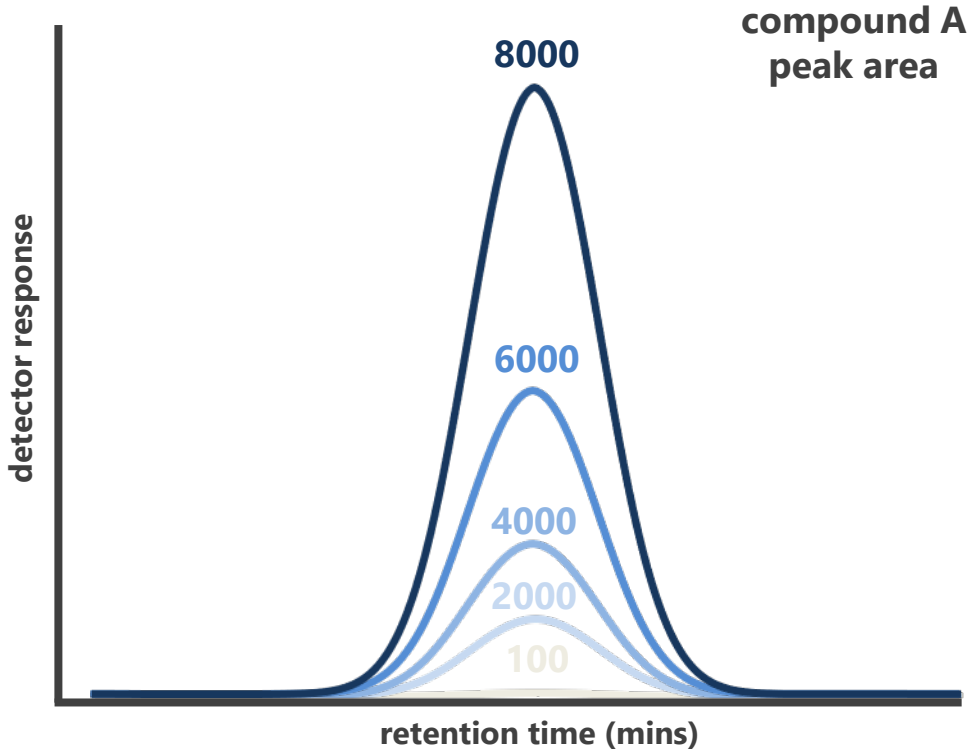
External standard calibration

External standard calibration

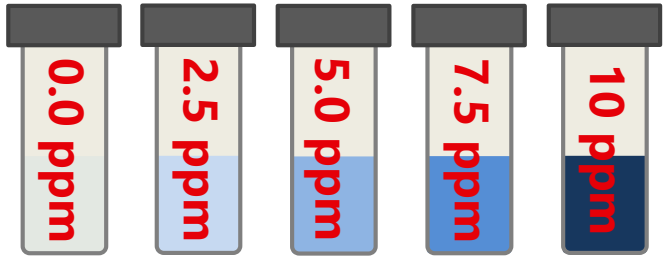


External standard calibration

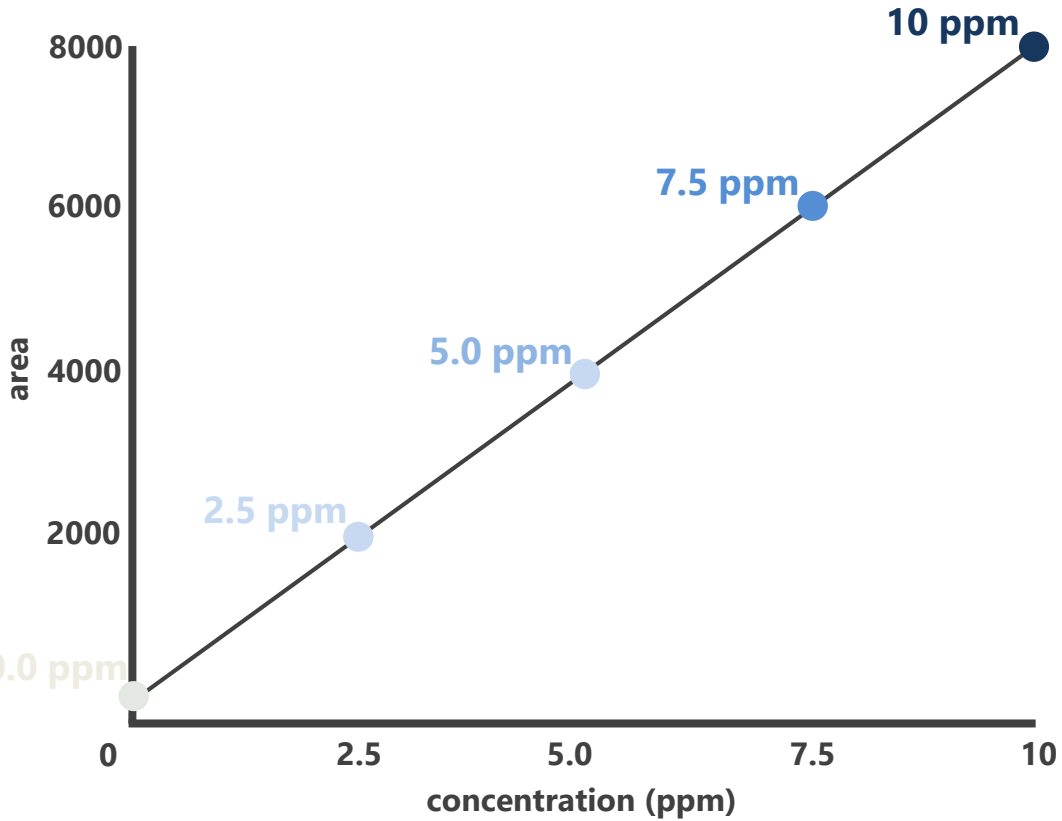




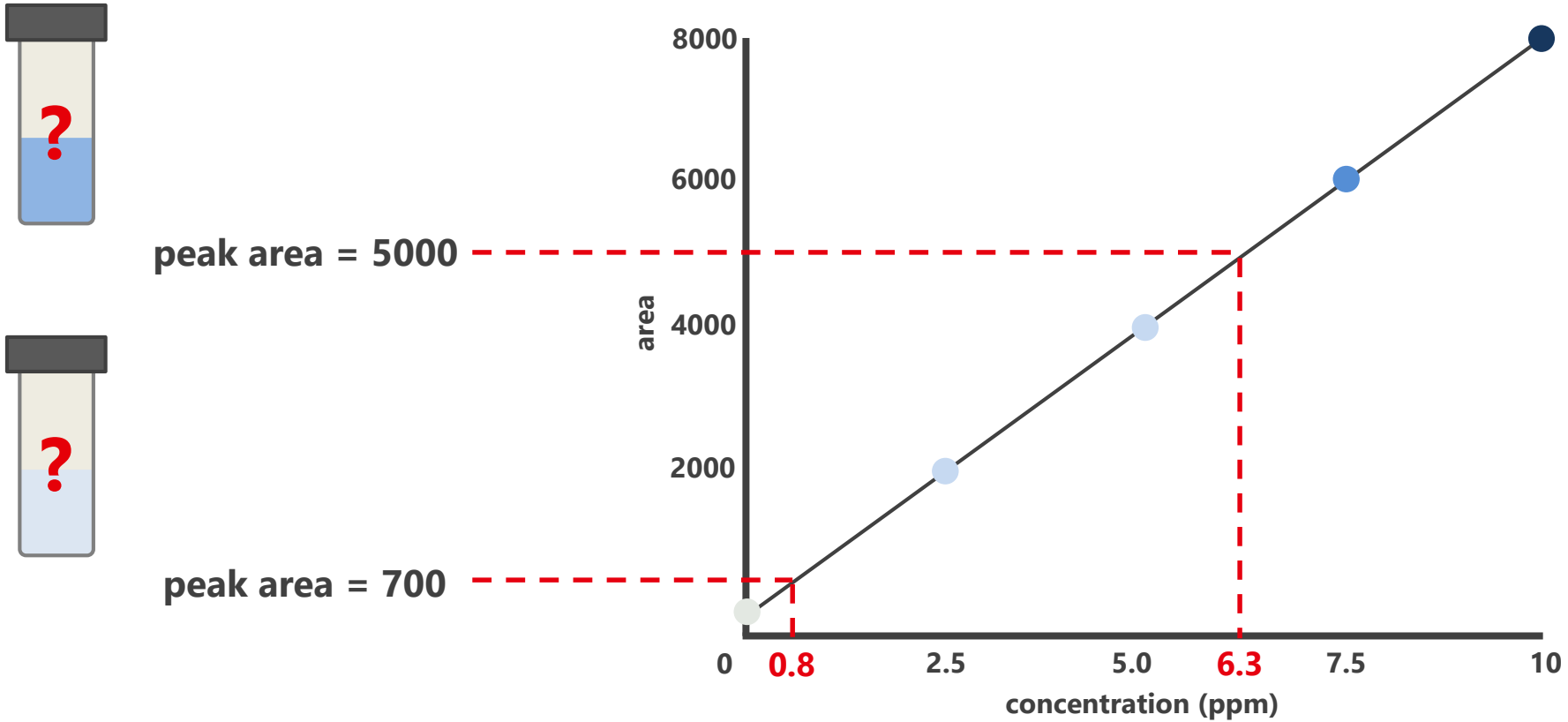
External standard calibration



concentration	peak area
0.0 ppm	100
2.5 ppm	2000
5.0 ppm	4000
7.5 ppm	6000
10 ppm	8000



External standard calibration



External standard calibration

Advantages	Disadvantages
• Quantity of compounds can be calculated • Must more robust than area %	• No correction for injection volume variation • Requires preparation of standards

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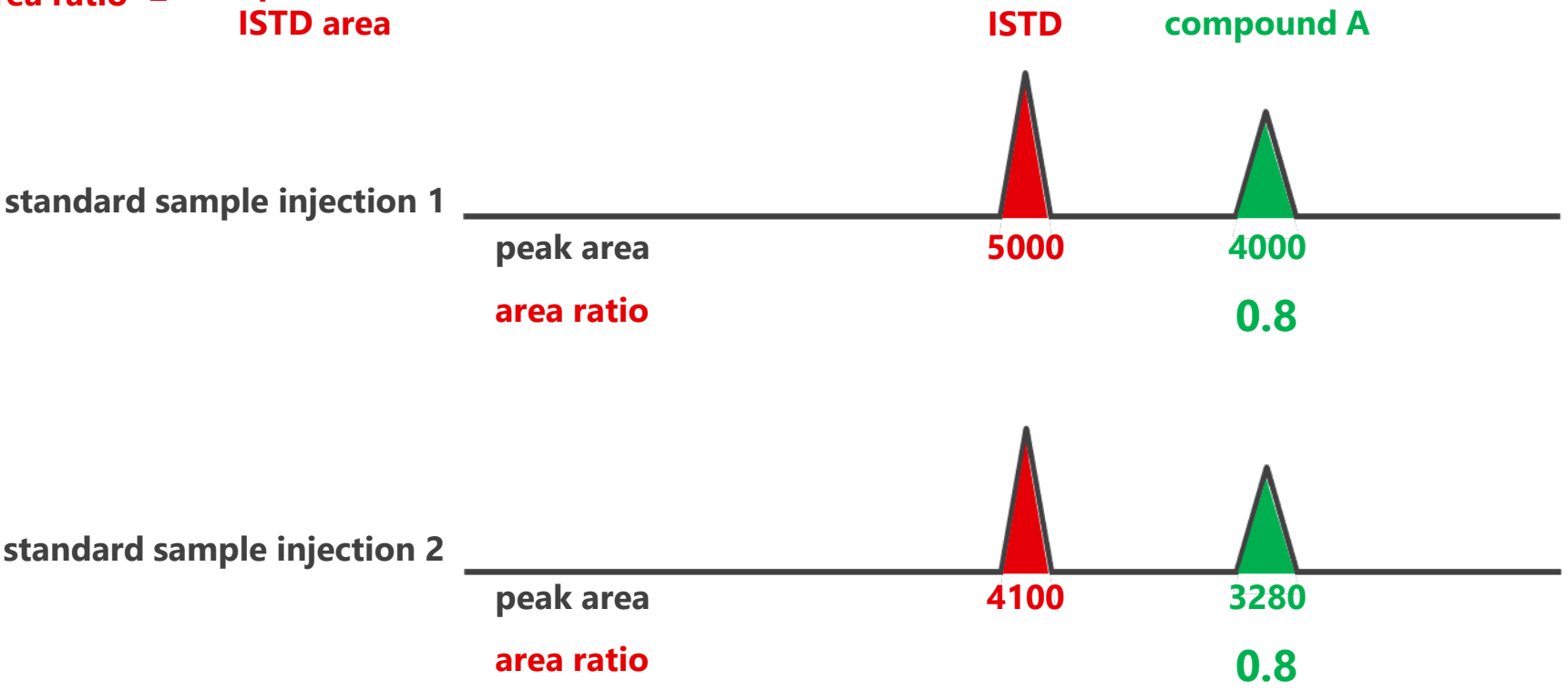
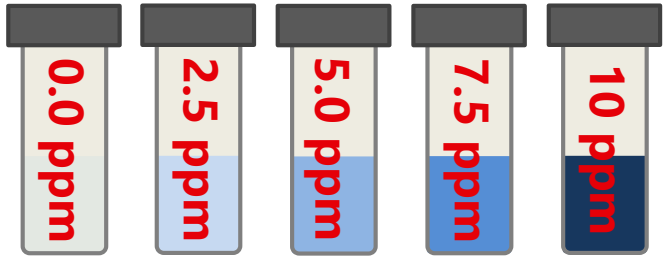
External standard calibration with ISTD

External standard calibration with ISTD

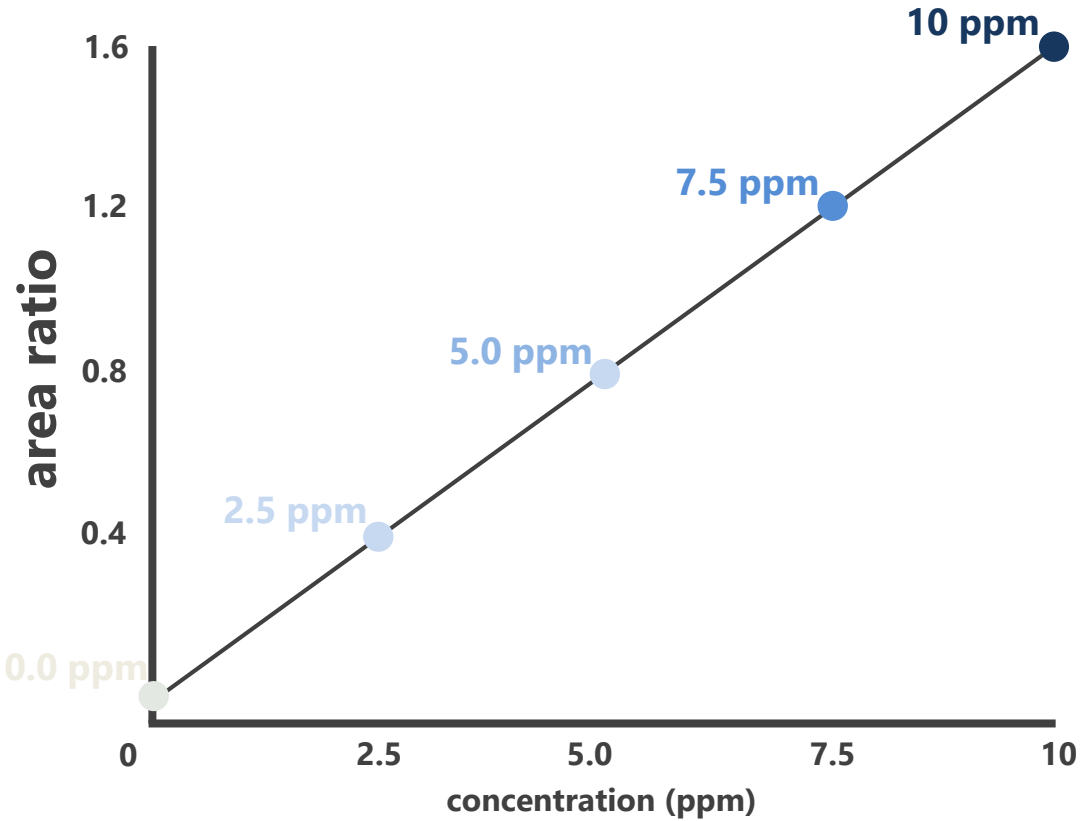
$$\text{area ratio} = \frac{\text{compound area}}{\text{ISTD area}}$$



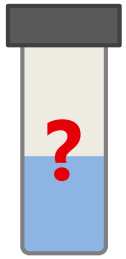
External standard calibration with ISTD

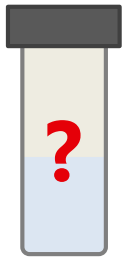


concentration	peak area	area ratio
0.0 ppm	100	0.02
2.5 ppm	2000	0.4
5.0 ppm	4000	0.8
7.5 ppm	6000	1.2
10 ppm	8000	1.6



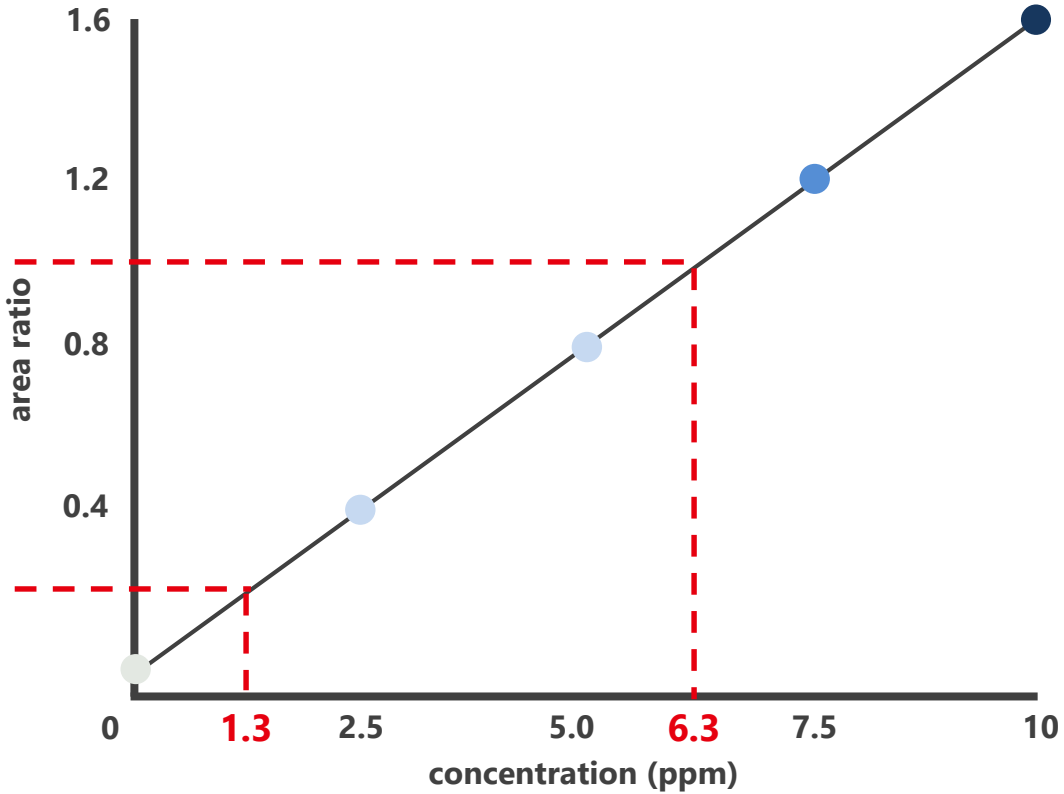
External standard calibration with ISTD





area ratio = 1.0

area ratio = 0.2



External standard calibration with ISTD

Advantages	Disadvantages
• Quantity of compounds can be calculated • More robust method of quantitation	• ~~No correction for injection volume variation~~ • Requires preparation of standards • Requires additional preparation

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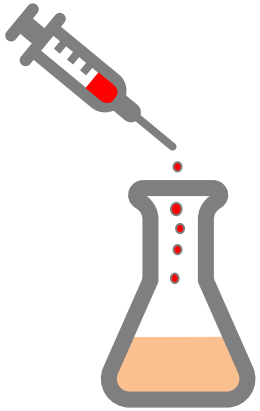
Recovery

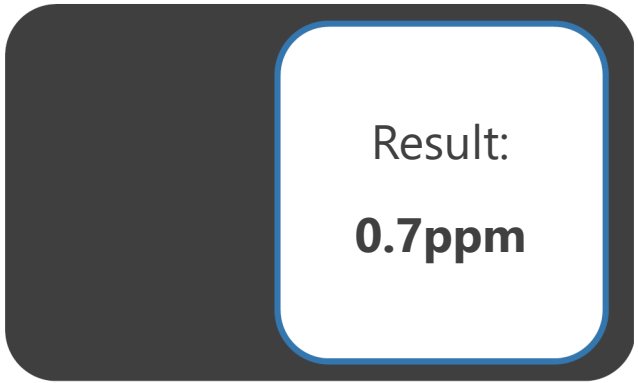
Recovery (with surrogate)



Surrogate Addition

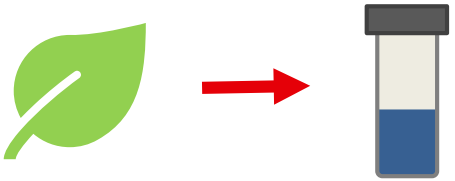
after all dilutions:

1ppm

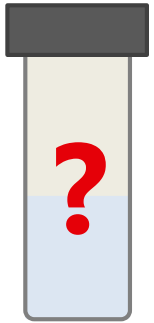


Recovery:

70%



Recovery (with surrogate)



$$\frac{\text{reported concentration}}{\text{recovery}} = \text{concentration in sample}$$

$$\frac{1.3\ \text{ppm}}{70\ \%} = 1.9\ \text{ppm of compound A in}$$

Surrogates

Advantages	Disadvantages

• Allows tracking of losses during sample preparation
• Can help account for sensitivity changes due to matrix
	• Requires further preparation of standards
	• Requires additional preparation
	• Typically requires ^{13}C, ^{2}D or ^{15}N isotopes
	  • Expensive
	  • Might not be readily available
	  • Requires MS

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Standard addition

Principles of headspace

In a **sealed vessel**, molecules of analytes exist in the **sample phase** or the **gas phase** (headspace).

A **partition coefficient** (**K**), is the distribution of analytes, at equilibrium, in the sealed vessel.

- **K is dependant on the analyte, the sample matrix & temperature.**

The **phase ratio** (**β**) relates to the relative volumes of sample and headspace in the vial.

Solvent	K Value
Ethanol	1355
Isopropanol	825
Ethyl acetate	62.4
Dichloromethane	5.65
Toluene	2.82
Cyclohexane	0.077

Air/water system at 40 °C

$$K = \frac{\text{Conc. (sample)}}{\text{Conc. (gas)}}$$

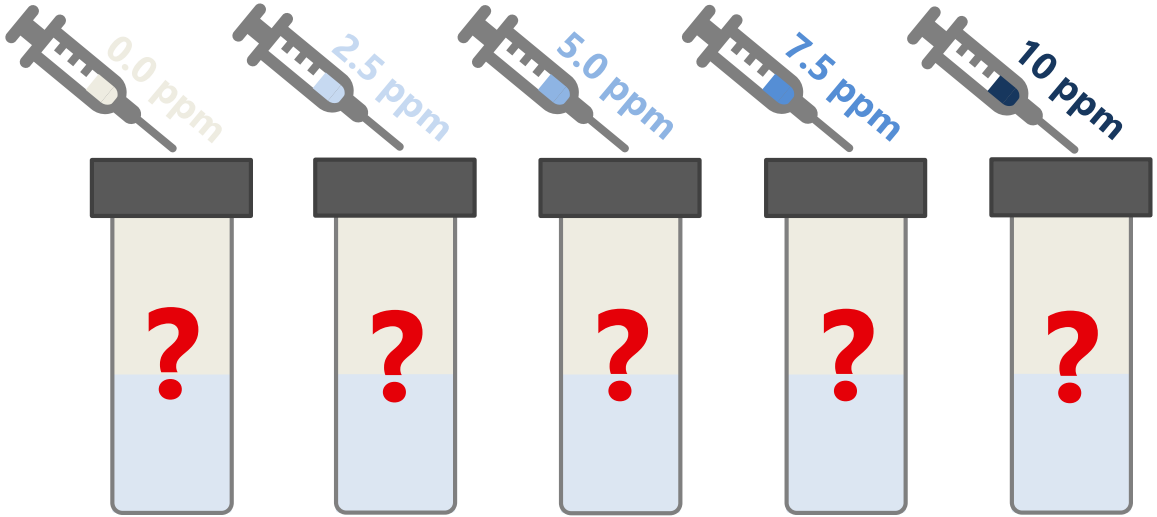
$$\beta = \frac{\text{Volume (gas)}}{\text{Volume (sample)}}$$

$$\text{HS conc.} = \frac{\text{Sample conc.}}{(K + \beta)}$$

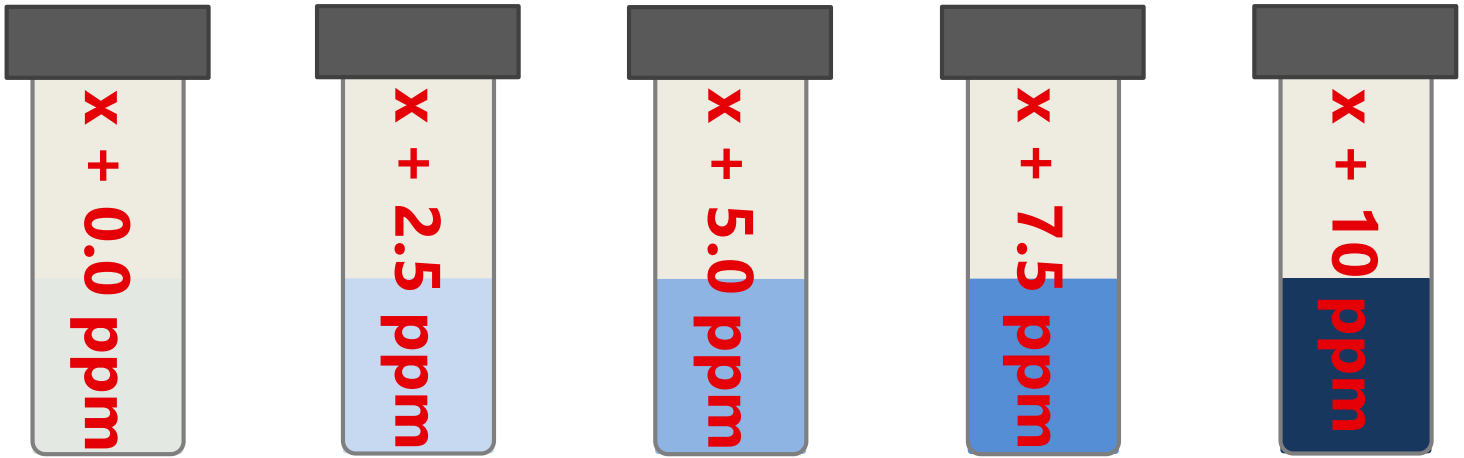
Smaller K = Higher sensitivity

Smaller β = Higher sensitivity

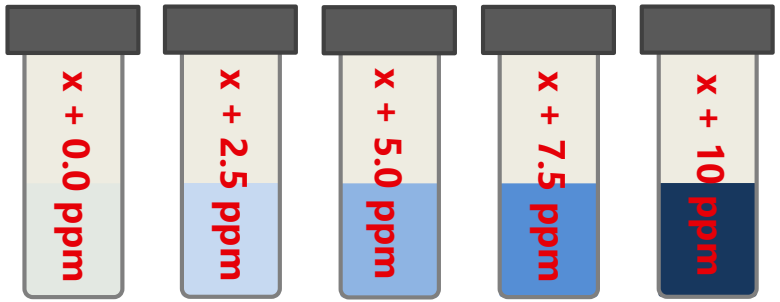
Standard addition



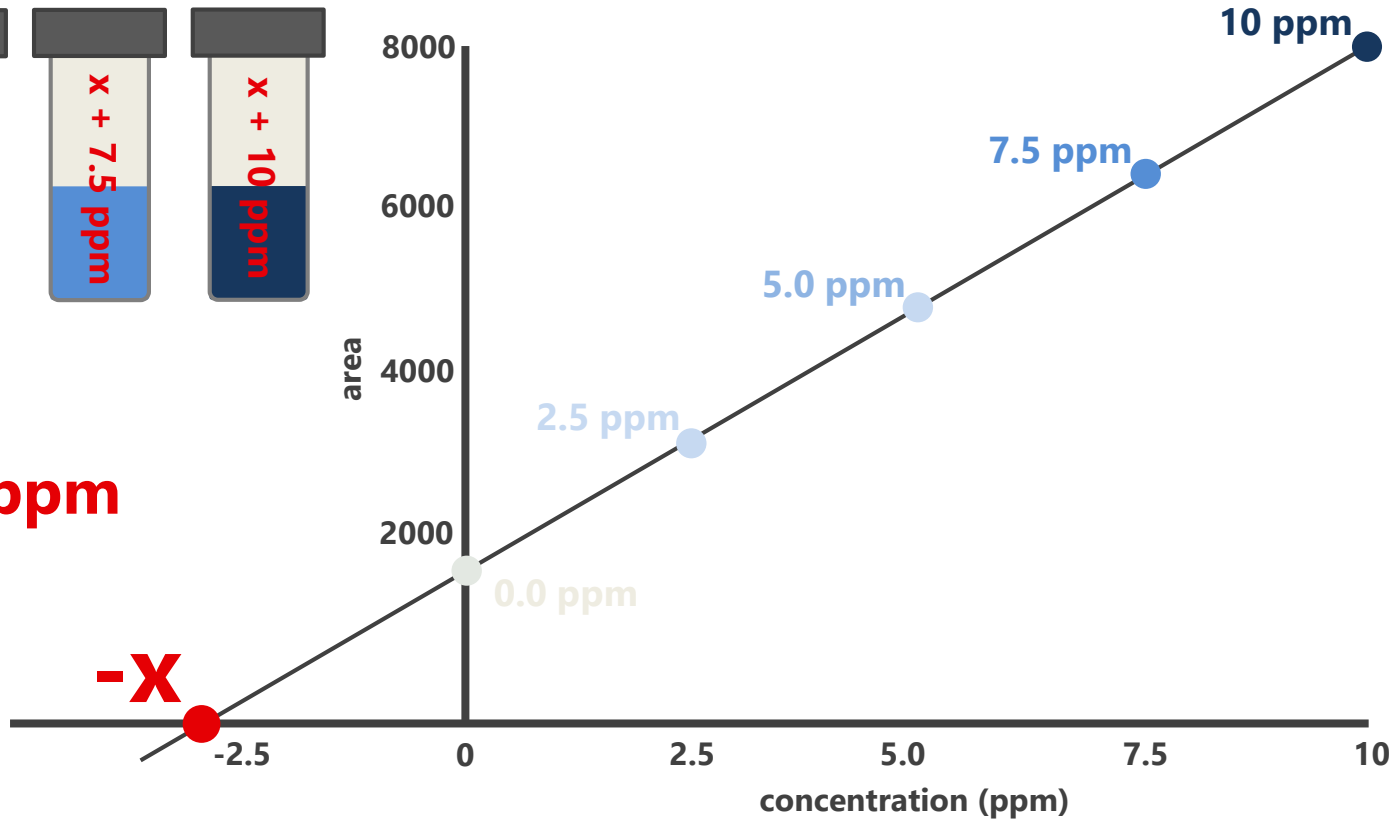
Standard addition



Standard addition



x = 2.6 ppm



Standard addition

Advantages	Disadvantages
• Accounts for matrix variations • Can be combined with ISTDs	• Very labour and instrument intensive • Requires a separate calibration for every sample!!

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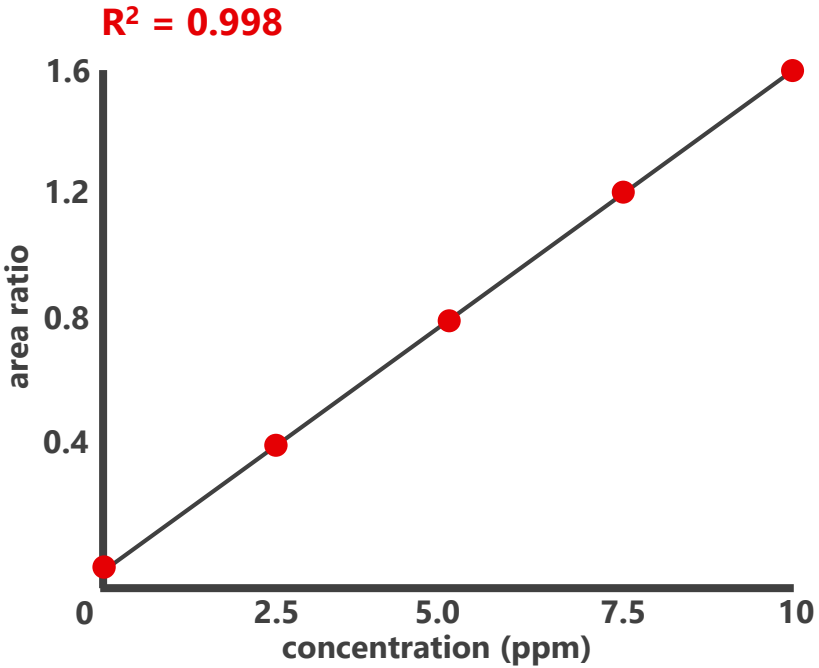
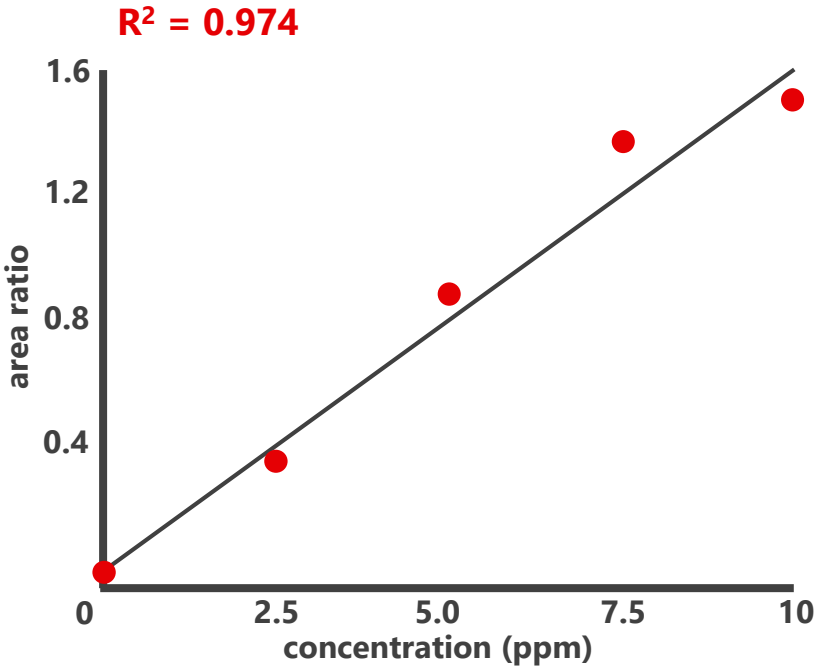
Calibration curve

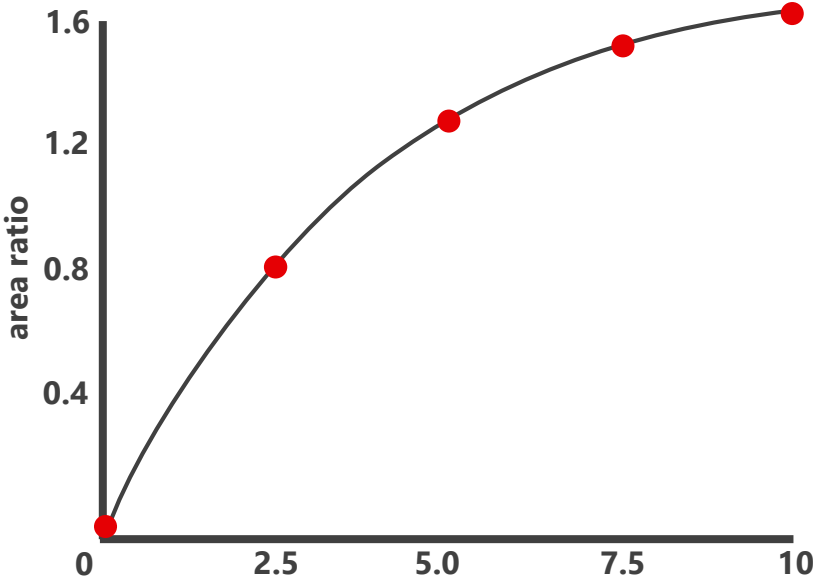
R^2 (coefficient of determination)



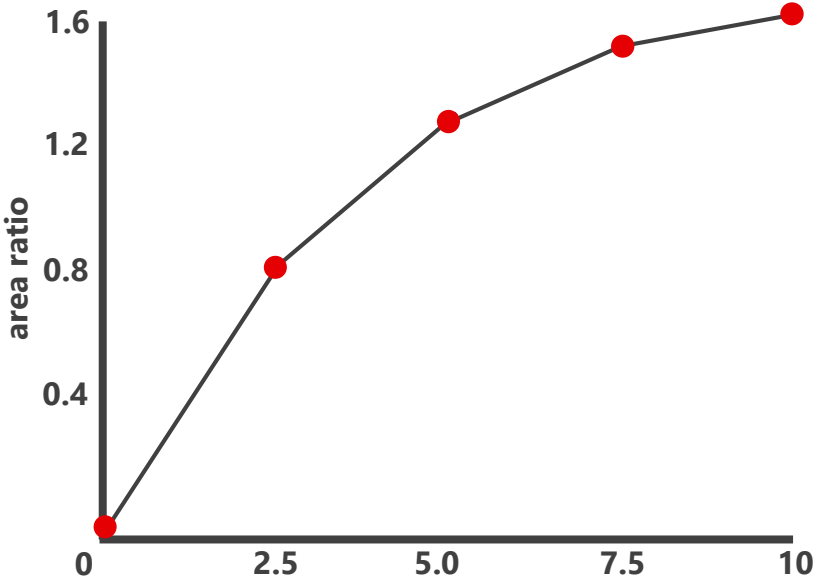


Curve fit

Quadratic

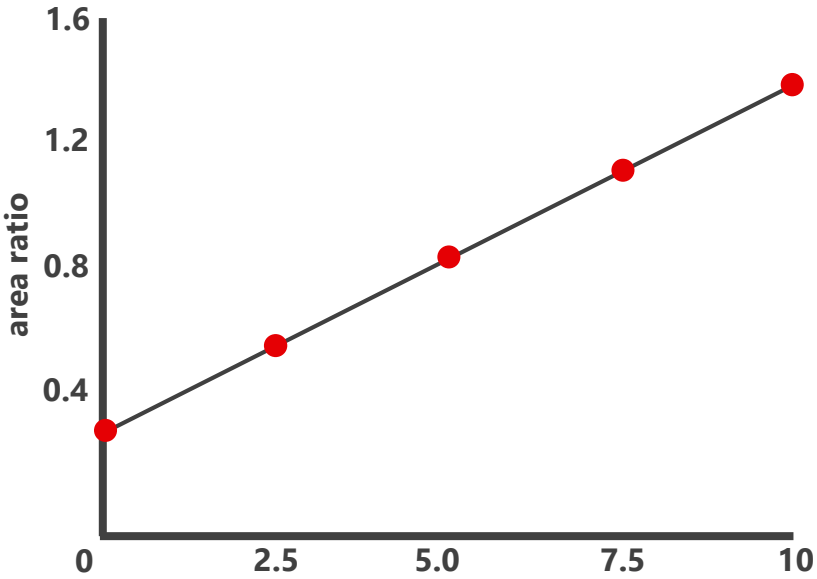


Point-to-point

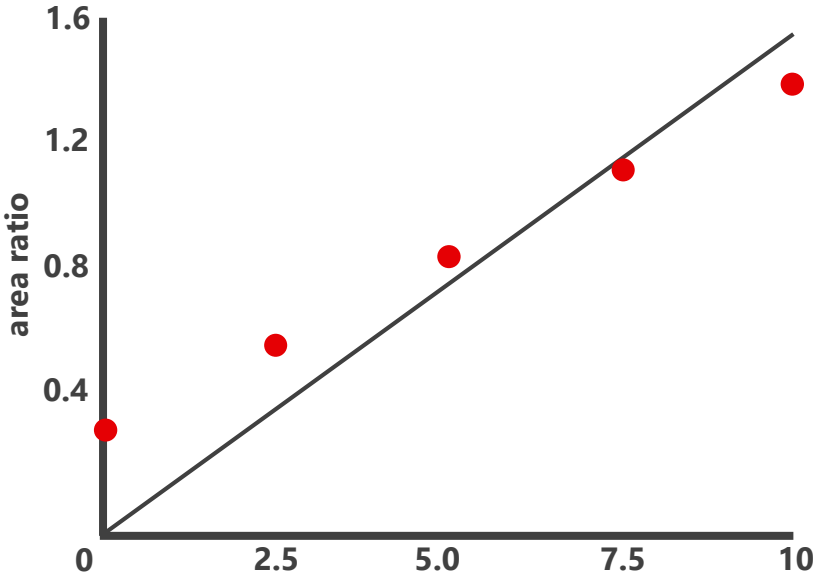


Origin

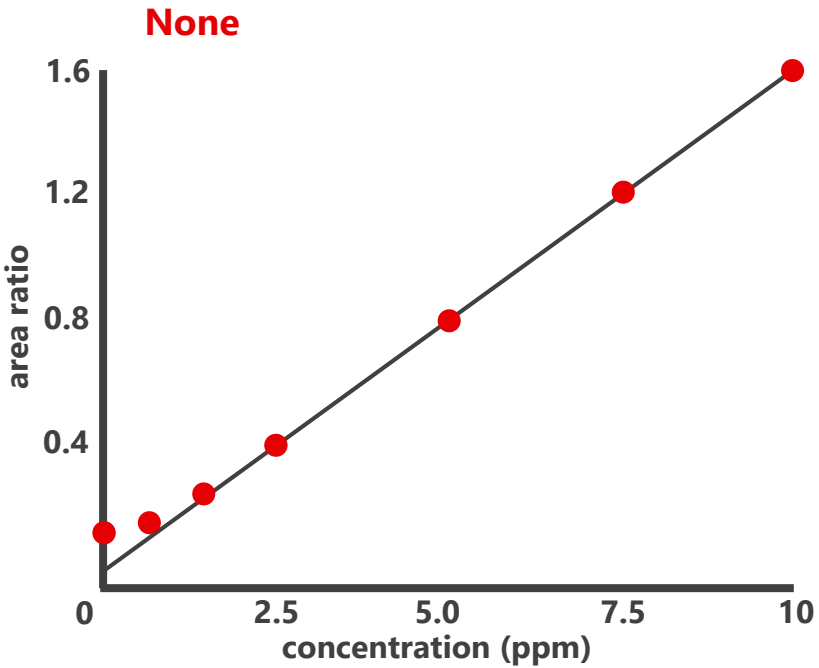
Ignore

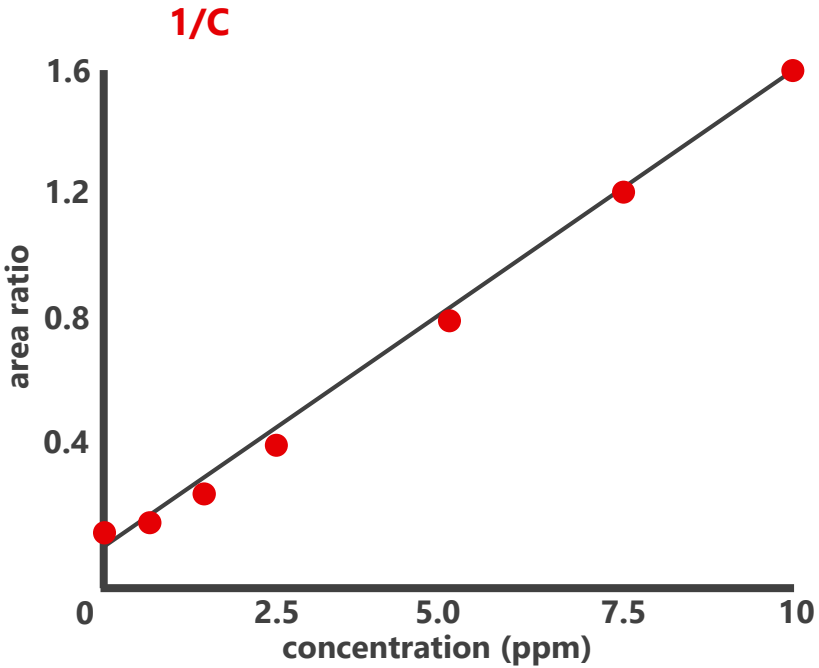


Force through



Weighting


None
1.6
1.2
0.8
0.4
area ratio
0
2.5
5.0
7.5
10
concentration (ppm)


1/C
1.6
1.2
0.8
0.4
area ratio
0
2.5
5.0
7.5
10
concentration (ppm)

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Summary

Summary

- **Sample types include samples (unknowns), standards and quality controls (QCs).**

- **Compound types include:**
  - **Targets** - compounds of interest
  - **Matrix** - compounds in sample but not of interest
  - **Internal standard (ISTD)** - added after sample preparation to correct for instrument variability
  - **Surrogates** - added before sample preparation to calculate recovery

- **Qualitative analysis** - what's there?

- **Quantitative analysis** - how much is there?

  - Area % (and corrected area %)
  - External standard
  - External standard with ISTD
  - Standard addition

- **Calibration curves**
  - R^2, origin, curve fit & weighting

Next time

The next session will be on…

Maintenance & Troubleshooting

This will cover:

- *Inlet maintenance*
- *Column installation & maintenance*
- *Detector maintenance*
- *Common troubleshooting*

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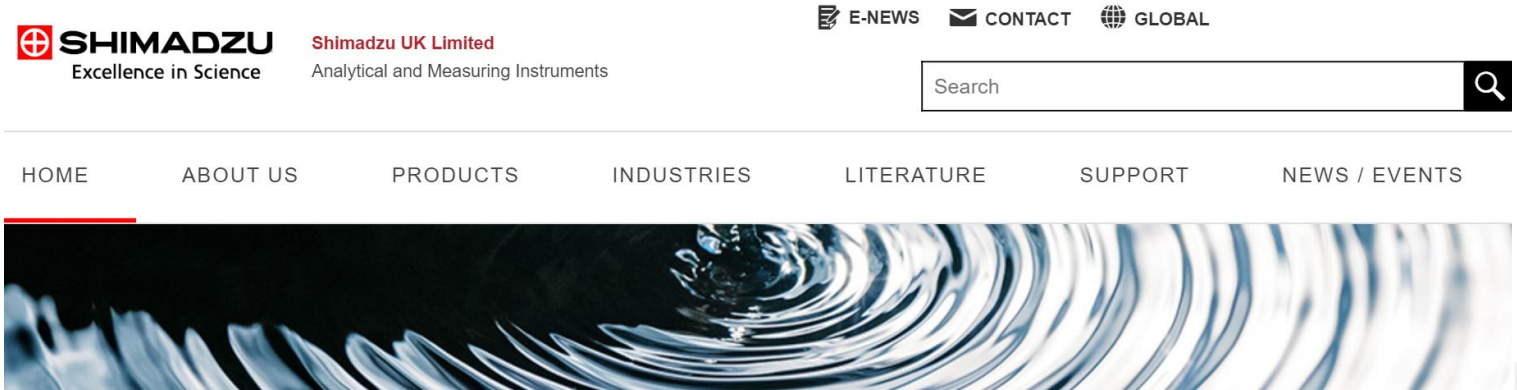

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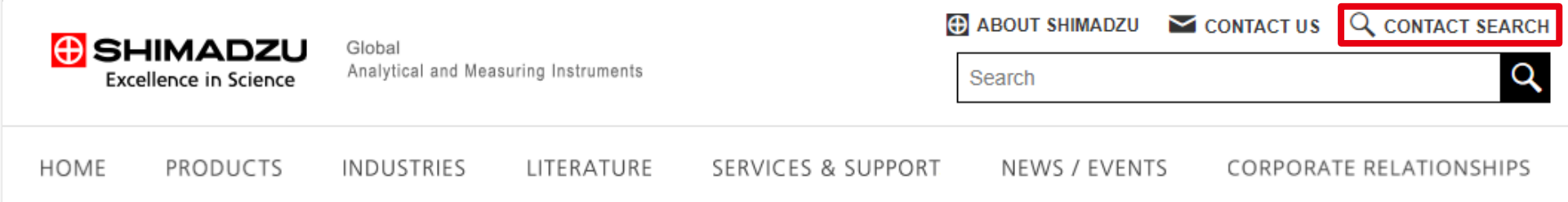

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