# Quantitative analysis

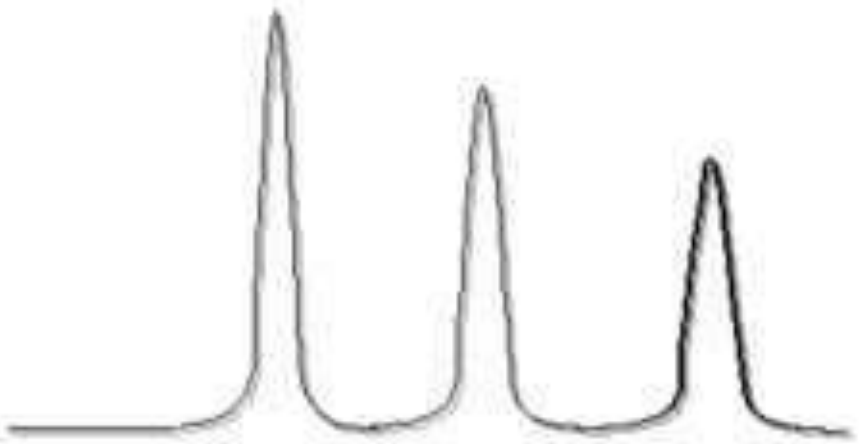
All chromatographic detectors produce a signal that drives a meter, recorder, integrator or A/D converter.

While the detectors used for GC and LC are not the same, quantitative methods are identical.

Each detector will produce a response/unit concentration. This is substance dependent so standards must always be used.

# Peaks

Each quantitative method assumes that you have one or more reasonably resolved peaks.



You must be able to find the beginning and end of each peak as well as it's maximum.

# Peak height

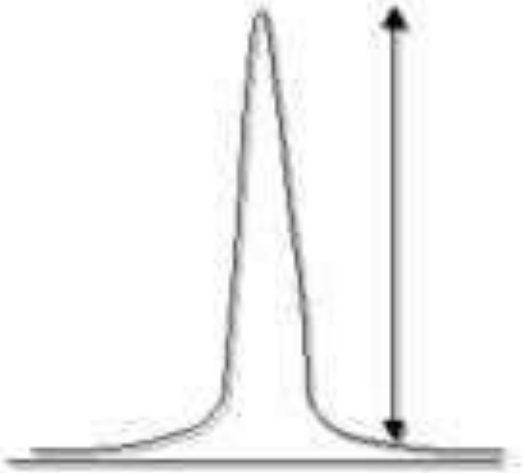
In some cases, you can assume that peak height is proportional to concentration.

## Advantages

- Simplicity
- Rapid calculations

## Disadvantages

- Height is more variable than area



Typically used only with capillary columns

# Peak area

This is the major approach for establishing a relationship between peaks and concentration.

$$\text{area} \propto \text{concentration}$$

Area is determined from a large number of measurements and detectors usually have very large dynamic ranges. This results in a very reliable measurement.

# Peak area

## Major problem

If the peak is approximately Gaussian, how do we accurately measure its area?

### Manual

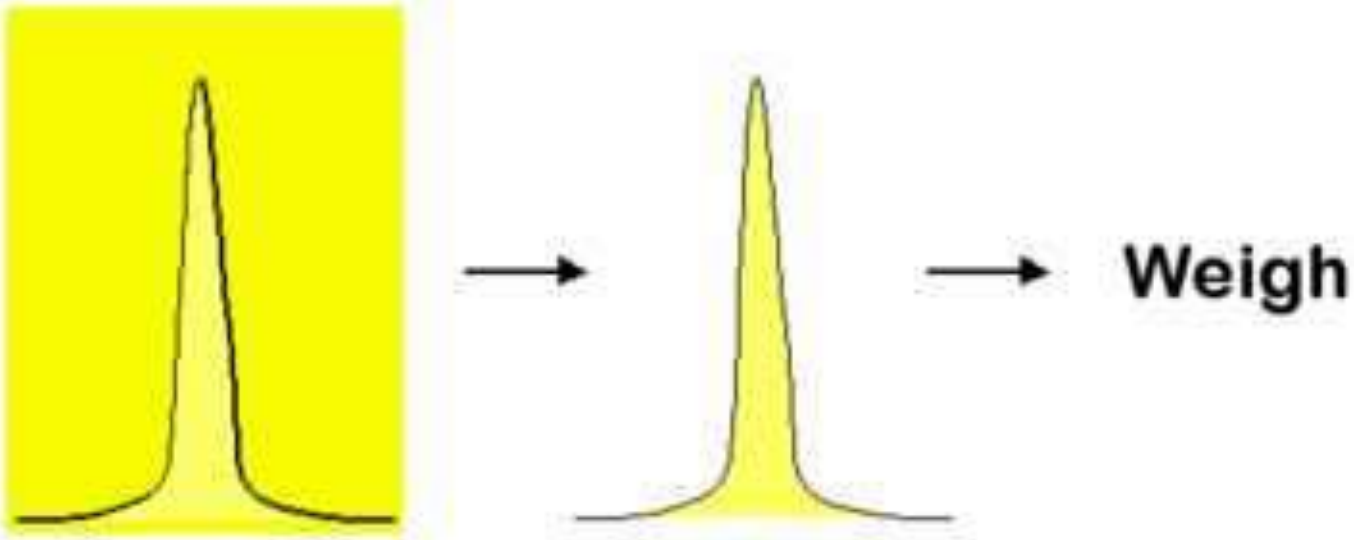
- Cut & Weigh
- Planimeter
- Triangulation

### Automated

- Integrating recorder
- Digital integrators
- Computer systems

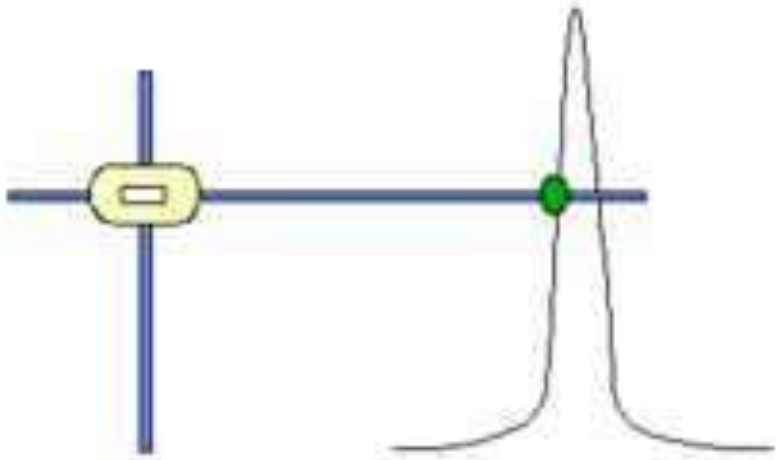
# Cut and weigh

With this approach, each peak is cut from the recording paper and weighted. Weight is then considered proportional to area.



# Planimeter

A device used to trace the peak. It produces a number that is proportional to peak area.



# Triangulation

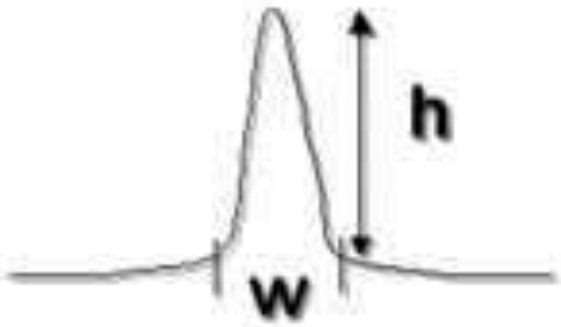
## Main manual method.

Assumes that each peak approximates a triangle.
Area can be determined by

$$\text{area} = \text{peak height} \times \text{width}$$

or

$$\text{area} = \text{peak height} \times 2\ W_{1/2}$$



# Integrating recorders

A special two pen recorder.

The first pen tracks the chromatographic signal. The second traces a series of zigzags.

# Integrating recorders

The larger the peak response gets, the more rapidly the second pen sweeps back and forth.

The total number of zigs and zags can then be related to the peak area.

If the peak gets to large, the second pen stops moving. You must keep the peak with in range.

# Digital integrators

Relies on A/D conversion of detector response.

# Digital integrators

## Caution!

Peaks are typically process for area on the fly.

This includes not only peak detection but may also handle other methods for dealing with poorly shaped or resolved components.

If a peak is missed, the run must be repeated.

# Computer systems

Include the same methods of peak detection and integration as integrators.

Major advantage is that the entire chromatographic run is stored prior to analysis.

This allows you to test out various methods of integration on a single run and to reanalyze data if a peak is missed.

# Summary

| Method | Time, min | Precision, % |
|---|---|---|
| Planimeter | 15 | 4.1 |
| Triangulation | 10 | 2.5 - 4 |
| Cut & weigh | 20 | 1.7 |
| Int. Recorder | 5 | 1.3 |
| Integrator | N/A | 0.44 |
| Computer | N/A | 0.44 |

# Quantitative interpretation

OK, now you have all of your peak areas.

Lets assume you knew what you were doing and all the areas were measured properly.

**Big deal!**

A relationship between concentration and area must be established or we're just spinning our wheels.

# Determining concentration

Several approaches can be used. Use the one that is most appropriate for your method.

Methods we'll cover

- Internal normalization
- External standard method
- Use of detector response factors
- Internal standard method

# Internal normalization

Calculate the total area of all peaks in a sample and assume:

Each component is producing a peak

Detector response is not concentration dependent

The solvent peak, if any, is typically ignored.

# Internal normalization

With these assumptions:

$$\%C_i \;-\; \%\text{Area}_i \;=\; 100\frac{\text{Area}_i}{\text{Area}_{total}}$$

This method is commonly reported as the default for integrators.

Since most detectors give responses that are both concentration and substance dependent, the method only serves to give a 'ballpark' estimate of relative concentrations.

# External standard method

## Requirements for proper use:

Standard solution containing all eluents to be quantified.

Standard eluents should be of similar concentration as unknowns.

The standard and sample matrix should be as similar as possible

Analysis conditions must be identical - stable instrument, same sample size ...

# External standard method

You either assume that response is linear over the entire concentration range or actually measure it. Then:

$$conc_{unknown} = \frac{Area_{unknown}}{Area_{known}} \quad conc_{known}$$

This is assuming that the same injection volume was used for both the unknown and standard.

# External standard method

Example - determination of X in $MeCl_2$

Prepare a standard of X

( 20.0 mg in 100 ml $MeCl_2$) - 0.200 µg/µl

Use an injection volume of 5 µl for both the standard and the unknown.

Measure the areas produced by both the sample and the unknown.

$$\text{Area } X_{std} = 2000 \text{ units}$$

$$\text{Area } X_{unk} = 3830 \text{ units}$$

# External standard method

Now, determine the concentration of X in you unknown.

$$\text{conc}_{\text{unknown}} = \frac{\text{Area}_{\text{unknown}}}{\text{Area}_{\text{known}}} \text{conc}_{\text{known}}$$

$$\text{conc}_{\text{unknown}} = \frac{3830}{2000} \; 0.200 \; \mu g / \mu l$$

$$= 0.384 \; \mu g / \mu l$$

You can now convert to a more appropriate concentration if required.

# Detector response factors

Determines the actual response per unit concentration for a set of sample components.

detector response, f = concentration/area

Let's work with a set of three species.

We'll call them 1, 2 and 3

# Detector response factors

Using a standard where each component is present at the same concentration, we obtain three peaks with areas $A_1$, $A_2$, $A_3$.

$$C_1 = f_1 A_1$$

$$C_2 = f_2 A_2$$

$$C_3 = f_3 A_3$$

# Detector response factors

Since $C_1 = C_2 = C_3$

$$f_1 A_1 = f_2 A_2 = f_3 A_3$$

and

$$\frac{f_1}{f_3} = \frac{A_3}{A_1} \quad \& \quad \frac{f_2}{f_3} = \frac{A_3}{A_2}$$

# Detector response factors

We can now establish one of the components as the "NORM" - assigning its response value as 1.000 .
We use $f_3 = 1.000$

Relative response factors can now be calculated.

$$f_1 = \frac{A_3}{A_1} \qquad f_2 = \frac{A_3}{A_2}$$

# Detector response factors

Now, when assaying a sample containing these three components, the corresponding concentrations (%C) are calculated using these factors.

$$A'_1 f_1 + A'_2 f_2 + A'_3 f_3 = \sum_{i=1}^{n} A'_i f_i$$

and

$$\%C_x = 100 \frac{A'_x f_x}{\sum A'_i f_i}$$

# Detector response factors

OK, how about we use some numbers!

Example

A sample consists of three component:
X, Y and Z.

The standard contains 200 mg of each component in 100 ml of an appropriate solvent.

# Detector response factors

Injection of 5 µl of the standard produces the following peak areas:

| Component | Area |
|---|---|
| X | 238 |
| Y | 660 |
| Z | 1190 |

We'll make Z the NORM for this example.

# Detector response factors

Determine the relative response factors for the other components.

| | | | | |
|---|---|---|---|---|
| X | $f_X$ | = | 1190 / 238 | = 5.0 |
| Y | $f_Y$ | = | 1190 / 660 | = 1.8 |

Now run your actual sample.

| Component | Area |
|---|---|
| X | 90 |
| Y | 265 |
| Z | 460 |

# Detector response factors

Multiply each peak area by the appropriate response factor and calculate the total corrected area.

| | | | |
|---|---|---|---|
| X | 90 x 5.0 | = | 450 |
| Y | 265 x 1.8 | = | 477 |
| Z | 460 x 1.0 | = | 460 |
| | Total area | = | 1387 |

# Detector response factors

Finally, calculate the corrected % by weight for each of the species:

$$\%\,X = 100\,(\,450 / 1387\,) = 32.4$$

$$\%\,Y = 100\,(\,477 / 1387\,) = 34.4$$

$$\%\,Z = 100\,(\,460 / 1387\,) = 33.2$$

# Detector response factors

So why bother?

- First, Z must be present in each sample assayed. It is actually serving as a type of internal standard.
- This method corrects for variations in the amount of sample injected.
- Is there a better way? Sure - the internal standard method.

# Internal standard method

Overall, the most reliable approach.

Basis

A known substance is added at a constant concentration to all standards and samples - internal standard.

Since the internal standard is always present at a constant amount, it can be used to account for variations such as injection volume during an analysis.

# Internal standard method

## Requirements for an internal standard.

- Must be present at a constant concentration in all samples and standards.
- Must be stable and measurable under the analysis conditions.
- Must not interfere with the analysis or co-elute with sample components.

# Internal standard method

## Three common approaches are used

Classical method - weighed portions of the standard and sample are combined

Stock solution - a known volume of the sample is 'spiked' with a known volume of the standard

Calibration plot - a series of standards are run and a curve plotted based on corrected peak areas.

# Internal standard method

Regardless of the method for introducing the standard or calibrating, the calculations are the same.

They are the same as with the detector response factor method.

Our NORM substance is now predetermined and a fix value.

# Internal standard method

$$C_{ISTD} = f_{ISTD} A_{ISTD}$$

$$C_{unk} = f_{unk} A_{unk}$$

Since the internal standard is assigned an f of 1.00 and is held constant, we can correct for run to run variations by:

$$C_{unk} = \frac{A_{ISTD1}}{A_{ISTD2}} \frac{A_{unk}}{A_{known}} C_{known}$$

known & ISTD1 are obtain from the standard, unk & ISTD2 from the unknown

# Internal standard method

It is assumed that variations in the internal standard area are representative of the whole analysis.

Accounts for factors such as:

Sample injection errors or changes

Slow detector variations

Slow column changes

# Internal standard method

## Example

Prepare a standard that contains 11.3 mg of X and 12.00 mg of ISTD.

Make several 2 µl injections and calculate an average response for each component.

| Component | Average area |
|---|---|
| X | 635 |
| ISTD | 1009 |

# Internal standard method

Now, inject your unknown.

$$\text{Area}_X = 990$$

$$\text{Area}_{ISTD} = 1031$$

$$C_X = (1009/1031)\,(990/635) \times 11.3 \text{ mg}$$

$$= 17.24 \text{ mg X in the unknown.}$$

# Internal standard plot method

- Hold the ISTD constant but vary the amount of the target species in a series of standards.
- Create a calibration curve using the corrected areas.
- Useful when the linearity of the detector is in question.