

### 8.1 Instrument Variations

If it is the instrument or the means by which the sample is introduced to the instrument that is causing a problem, then standard addition or matrix-matched standards will not help. More often than not, these problems are **intermittent**, changing with time or sample introduction. Examples are:

- injection volumes for GC & HPLC
- flame/plasma temperature for flame photometry and ICP
- nebuliser uptake rates for flame photometry and ICP

In these circumstances, we need to have a checking mechanism in the analysed solution that alerts us to when these transient variations occur. Most commonly, this will be an internal standard.

#### *Internal standards*

The principle of using an internal standard (IS) is that if the instrument (or sample introduction) affects the analyte response, it will affect the response of the IS by the same proportion.

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#### EXAMPLE 8.1

*A sample with an internal standard included is measured for both species twice and the responses are as below.*

	Analyte	IS
Run 1	100	50
Run 2	90	45

If these were two different samples, and the internal standard wasn't used, you would conclude that #2 was 10% lower in concentration than #1, when in fact, it is the same solution.

In real use, the IS does more than sound a warning; it is used in the calculations to correct for the variation.

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The correct use of internal standards is more complex than standard addition, where the only consideration was how much to add. This too must be considered, but other factors such "which species to choose" are also crucial.

#### CHOOSING A SUITABLE INTERNAL STANDARD

First and foremost, the internal standard is NOT the analyte, but a totally different species. It is not, however, a matter of simply grabbing whatever chemical happens to be at hand and throwing it in as the internal standard. The choice is critical to the success, as it will only work if the choice is based on the following criteria (1 & 2 are essential, 3 simply practical).

#### CHOOSING THE IS

1. The chosen compound cannot be present in the sample.
2. The chosen compound should have similar physical and chemical properties to the analytes.
3. The chosen compound should be readily available.

**EXERCISE 8.1**

Explain the reasons for each of these criteria.

- *can't be present in the sample*

- *similar properties*

- *readily available*

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The matter of matching similar properties is very much dependent on the technique. With emission spectroscopy, the similarity needs to be chemical, and generally means the same group in the periodic table. With chromatography, it is physical, and requires a similar (but not identical) retention time. There is more on this in Chapter 6.

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**EXERCISE 8.2**

How would you determine whether the chosen IS was present in the sample?

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***Adding the internal standard***

Once chosen and confirmed as the right choice, the internal standard (IS) is now ready to be included in the analysis. Again there are three basic rules, all essential.

**ADDING THE IS**

- 1. The IS is added to all solutions – standards, samples and recovery checks.**
- 2. The same concentration of IS is added to all solutions.**
- 3. The response of the IS should be similar to that of the main analyte.**

**EXERCISE 8.3**

Explain the reasons for each of these rules:

- *added to all solutions*
- *same concentration in all solutions*
- *similar response to analyte*

**Using the internal standard in the calculations**

The principle of the internal standard is that, since it is all solutions at the concentration, any variation in its response must mean something has happened in the process (eg low injection volume) and this will have affected the analyte response as well. If the IS response is down by 10%, then so presumably is the analyte's.

The simplest way to correct for these variations is not to scale up or down the analyte response based on the IS change, but simply to calculate **the ratio of the response of the two**.

$$\text{Ratio} = \frac{\text{Analyte response}}{\text{IS response}}$$

Note that the ratio must be this way, not IS ÷ Analyte.

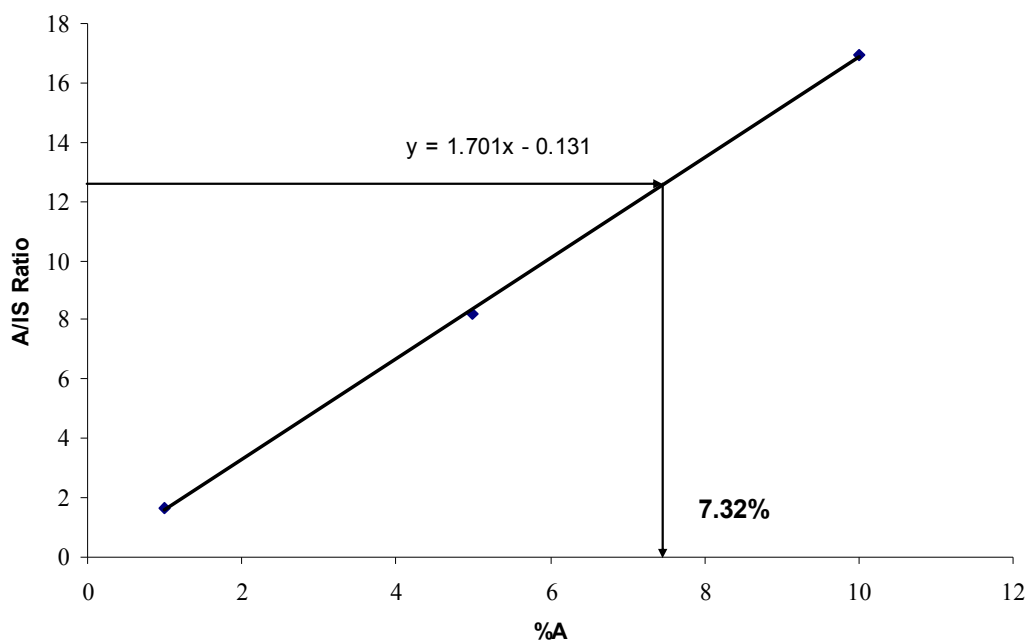
The **ratio becomes the number plotted on the y-axis in the calibration graph**. It then becomes exactly the same process as for a normal calibration graph.

**EXAMPLE 8.2**

Calculate the concentration of analyte A, give the following data.

Solution	A resp.	IS resp.	Ratio
1% std	125	75	$125 \div 75 = 1.67$
5% std	648	79	8.20
10% std	1238	73	16.96
Sample	703	57	12.33

See graph over page.



If the internal standard method had not been used, the answer for the sample would have been 5.6%.

#### EXERCISE 8

(a) Calculate the concentration of analyte in the sample, given the following data.

Solution	Analyte	IS	Ratio
0.05%w/v std	157	59	
0.2% std	753	72	
1 % std	3526	66	
Sample	1706	67	

Answer from graph:

- (b) If the analysed sample had been diluted from 10 mL to 50 mL, what would the concentration in the original solution have been?
- (c) If the original solution had been produced by dissolving 12.3456 g of sample in 100 mL (before dilution and analysis), what mass of analyte was in the solid sample?
- (d) What was the %w/w in the solid sample?

### Summary

At this point, it is probably worthwhile making a clear distinction between the techniques of standard addition and internal standards. Table 8.1 sets out the important features of each.

**TABLE 8.1** *Features of standard addition and internal standards*

<i>Feature</i>	<i>Standard Addition</i>	<i>Internal Standards</i>
Errors compensated	Matrix interference	Instrumental or technique variations
Species added	Analyte	Chemically/physically similar to analyte, but not present in sample
Method of addition	Increasing concentrations to fixed amount of sample	Fixed amount to all standards and samples
Graphical method	Response vs concentration added, extrapolate to horizontal axis	Ratio of analyte to internal standard responses vs concentration
Reason for use	Perfectly duplicates matrix	Response of internal standard changes in same proportion as analyte

#### What You Need To Be Able To Do

- define important terminology
- explain aspects relating to use of internal standards
- perform internal standard calculations

#### Revision Questions

1. (a) Why would potassium be a poor choice as internal standard for sodium analyses in food products?  
(b) Why is lithium a better choice?
2. A sample of tap water was analysed for its potassium content, using lithium as an internal standard. The sample was filtered, but otherwise unaltered. Given the following data, calculate the concentration of potassium in the water as mg/L.

<i>Conc. K (mg/L)</i>	<i>Int. K</i>	<i>Int. Li</i>
5	28	18
10	50	17
15	77	18
20	100	18
Sample	64	16

3. A beer sample, diluted by a factor of 10, was analysed for potassium by the internal standard method. Determine the concentration of K in the original sample.

<b>Conc. Na (mg/L)</b>	<b>Int. Na</b>	<b>Int. Li</b>
1	12	11
2	25	10
5	66	9
10	99	12
Sample	60	13

4. Calculate the concentration of ethanol in a sample of low-alcohol beer, given the following data.

<b>Solution</b>	<b>Peak Area EtOH</b>	<b>Peak Area IS</b>
0.5% ethanol	157	59
2% ethanol	753	72
5% ethanol	1723	66
Sample	706	58

5. The sodium content of chips was analysed by dissolving the salt from 1.0456 g of sample into 500 mL. This was diluted 5 to 100 mL with lithium IS added. Calculate the concentration of sodium in %w/w.

<b>Conc. Na (mg/L)</b>	<b>Na int.</b>	<b>Li int.</b>
5	31	45
10	68	46
20	110	41
Sample	96	48

Answers to these questions on following page.

Answers to class exercises can be found in the Powerpoint file provided on the website.

**Answers to Revision Questions**

1. (a) Because K is present in most natural materials  
(b) Because Li is similar to K, and not present to any extent in natural materials
2. Answer from graph = 14.1 mg/L
3. Answer from graph = 4.28 mg/L, with x10 diln factor 42.8 mg/L
4. Answer from graph = 2.33%
5. Answer from graph = 14.6 mg/L; with x20 diln factor is 292 mg/L, in 500 mL is 146 mg; %w/w is 14.0.