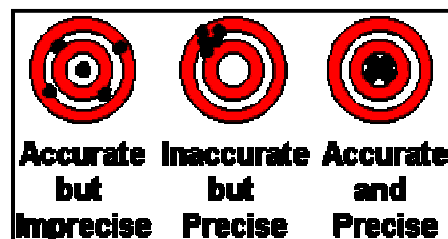


# Getting Started in HPLC

## Section 4D. Precision and Accuracy

People often confuse "[precision](#)" with "[accuracy](#)". Both words suggest that we are doing careful work and getting the right answers in quantitative analysis. But precision is not the same as accuracy, and it is important to know what we are talking about. Accuracy means getting an answer that is correct. Precision means being able to get the same answer for a particular sample every time, when we repeat an analysis on that sample.



Let's use an example from the LC lab. Suppose we weigh out 500 mg of aspirin and dissolve it in a 100 mL flask. The concentration of aspirin in our sample will then be :

$$\begin{aligned} (\text{quantity}) / (\text{volume}) &= (500 \text{ mg}) / (100 \text{ mL}) \\ &= 5.00 \text{ mg/mL} \end{aligned}$$

of aspirin.

Now let's send 25 mL of this solution to three different laboratories: lab A, lab B and lab C. Each lab then analyzes the sample for aspirin (by means of HPLC) 6 times and reports the results to us as shown below right (Correct Concentration = 5.00 mg/mL)

The results for lab A all fall quite close to each other: 5.40-5.45 mg/mL aspirin. When replicate analyses on a sample agree closely, as in this example, we say that the assay is precise. That is, a precise analysis is a reproducible analysis. However we also see that these values (5.40-5.45) are not very close to the true value of 5.00 mg/mL. The average value (5.42 mg/mL) is about 8% too high.

Now consider the results for lab B. These range from 4.80 to 5.18 mg/mL. When we see values that scatter this much, we say that the analysis is not very precise or is imprecise. However if we average these values for lab B, we see that the average value (5.06 mg/mL) is pretty close to the true value of 5.00 mg/mL. So even though lab B does not report precise values, the

LAB A	LAB B	LAB C
5.45	5.18	5.03
5.40	4.80	4.98
5.42	5.20	5.00
5.43	5.06	5.03
5.40	5.15	4.98
5.41	4.98	5.03
<b>PRECISE</b>	<b>IMPRECISE</b>	<b>PRECISE</b>
<b>INACCURATE</b>	<b>ACCURATE</b>	<b>ACCURATE</b>

values reported are closer to the true value than for lab A. We say that lab B is accurate - even if it is imprecise.

Finally for lab C in the above example, we see that the values reported (4.98-5.03 mg/mL) agree with each other quite well, and the average value (5.01 mg/mL) is also close to the correct value of 5.00. So lab C can be said to be both precise and accurate.

Both accuracy and precision are important in HPLC analysis. However it is much easier to measure precision than it is to measure accuracy. We can easily rerun a sample several times and show that the results are reproducible or precise. It is often more difficult to know the exact concentration of some compound in a given sample - particularly a "real" sample that comes to us in some strange mixture. This often results in laboratories reporting answers that appear precise but are actually wrong (inaccurate). It is actually much more important that our answers be accurate than precise, although good accuracy also requires good precision. The bottom line is: if you have shown that your analysis is precise, don't assume that it is also accurate. Accuracy has to be demonstrated in a different way.

While we are talking about precision - which is essential to good HPLC results - it is important to mention a common error in quantitative analysis. This is the practice of using too few decimals in recording results or carrying out calculations. Be sure to retain enough SIGNIFICANT FIGURES in all weights, volumes and calculations. Generally in LC analysis we want to have at least 4 significant figures in every number, and sometimes more. For example, if weighing out a sample, make sure that the sample weight after subtracting off the tare weight has at least 4 significant figures as shown at the right.

	<b>CORRECT</b>	<b>INCORRECT</b>
<b>flask</b>	124.3433 g	124.34 g
<b>flask+sample</b>	123.8877 g	123.89 g
<b>sample</b>	0.4556 g	0.45 g

The most common measures of precision in chromatographic measurements are the standard deviation, the relative standard deviation, and the coefficient of variation. Detailed definition of these measures is outside the scope of this course; it can be found in any textbook on quantitative analysis or statistics. In practice, the values are computed automatically by the data system or a computer spreadsheet.

Very briefly, the standard deviation is a measure of the amount of possible random error in a series of replicate measurements. For truly random errors, two-thirds of the values will lie within  $\pm 1$  standard deviation of the mean, 95% of the values will lie within  $\pm 2$  standard deviations of the mean, and 99% of the the values will lie within  $\pm 3$  standard deviations of the mean. The estimated standard deviation for a quantity is symbolized by a lower-case sigma ( $\sigma$ ).

The relative standard deviation is the standard deviation as a fraction of the mean value. Thus, if we measure a concentration of 9.52 mg/mL with a standard deviation of 0.110 mg/mL, the relative standard deviation is:

$$\text{RSD} = 0.110 / 9.52 = 0.0115$$

In practice, this is often expressed as the coefficient of variation (CV), sometimes also called "percent relative standard deviation" (%RSD). This is simply the relative standard deviation expressed as a percentage instead of as a decimal fraction. The CV for the example above is 1.15% (the percentage equivalent to the fraction 0.0115). To convert from RSD to CV, multiply the RSD by 100.

We can now re-examine the results of the aspirin analysis at three different laboratories that we discussed near the top of the page. The mean, standard deviation, and CV give us a more meaningful picture of the laboratories' performance than the terms "precise" or "imprecise".

For most purposes, HPLC methods are expected to have CV values on the order of 1%. Less precision may be acceptable in the case of extremely low-level samples or where a simple yes/no decision is required. The expected precision will usually be stated as part of the method specification.

	LAB A	LAB B	LAB C
	5.45	5.18	5.03
	5.40	4.80	4.98
	5.42	5.20	5.00
	5.43	5.06	5.03
	5.40	5.15	4.98
	5.41	4.98	5.03
<b>MEAN</b>	<b>5.42</b>	<b>5.06</b>	<b>5.01</b>
<b>STD. DEV.</b>	<b>0.019</b>	<b>0.15</b>	<b>0.025</b>
<b>CV</b>	<b>0.35%</b>	<b>4.6%</b>	<b>0.36%</b>

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