QUESTIONS OF QUALITY

A Question of Balance?
Part 1: Principles

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Question: **What is not a chromatograph but can adversely impact your chromatographic analyses?**
Answer: **An analytical balance.**

Yes, we know there are many possible answers to this questions, but as we're focusing on balances in the next two "Questions of Quality" columns, our answer seemed appropriate. Being a little more serious, the question we want to pose you, the chromatographer, is "Do you understand the full impact an analytical balance has on the quality of your analysis?" Well, do you?

Before we delve into the detail, the terminology of balances, weight and masses should first be discussed briefly.

Balance or Scale? "Scale" is derived from old English and means plate or dish. Scales are used for weighing larger masses. In contrast, "balances" are used for precise weighing of substances and, as such, are typically used in most chromatography laboratories. We'll be looking at balances in these two articles.

Weight or Mass? Weight or mass — this is tricky because the two words are used interchangeably, even in the chromatography laboratory, which is unfortunate.

Strictly speaking we must consider the following facts before starting our journey

- The kilogramme is the unit of mass and is equal to the mass of the international prototype kilogramme.
- Weight is a force expressed in Newtons; the product of mass and the acceleration caused by gravity. Therefore, a weight may be defined as an object that embodies a mass or a mass-related property of interest!
- Note that a cubic decimetre (also known as a litre) is defined in terms of the volume occupied by 1 kg of pure water at its maximum density and at standard pressure. This isn't quite trivia, as it relates to the way we calibrate volumetric flasks via weights of water and density (we'll discuss buoyancy correction in Part 2!).
- For those readers that like more detail, find yourself a copy of the *Handbook of Mass Measurement* by F.E. Jones & R.M. Schoonover.[1] This is a definitive work — a particular favourite of ours is a figure that shows up to 95 years stability data on cleaning and washing platinum kilograms!! The mean loss is approximately 1 µg/year. NIST (National Institute of Science and Technology, Gaithersburg, Maryland, USA) concluded that before they sent their copies to be recalibrated at the BIPM (Bureau International des Poids et Mesures [International Bureau of Weights and Mesures]) in Paris they would be cleaned and washed twice. The bottom line is that the global standard is very stable and has been for almost a century. We thought you might be interested, but then again, perhaps not…

Impact of an Analytical Balance

As we begin our journey, let's look at what roles an analytical balance could play in a chromatographic analysis.

- Weighing of analytical reference standards prior to preparation of standard solutions
- Weighing mobile phase components, including buffers and salts
- Weighing the sample(s)
- Weighing blank matrix.

Let's face it, if the balance is not calibrated correctly, the whole chromatographic run could be rejected or, even worse, the error could remain undetected and an incorrect decision reached based on the wrong results.

The best chromatograph running the best chromatography data system with optimum chromatographic separation is worth nothing if the balance is incorrectly calibrated or simply faulty. In truth, everything depends on the analytical balance meaning that both weighing and the correct operation are fundamental to any quantitative chromatographic analysis.

There is also a subtle impact on technology transfer of chromatographic methods; for example, if there is a systematic balance error in one laboratory, will you ever reach analytical agreement with another laboratory? Here traceable standards are important and we'll look at how these bring consistency to measurement of mass.

Back to Basics: In the Old Days

Back in the days when men were men and they constructed arks in their spare time, Chris was taught to weigh materials on a twin pan equal arm balance that pivoted on a knife-edge. This is shown in Figure 1 (the balance not the ark!).

In essence, the knife-edge is the key and was made of either agate or diamond. Balanced above it was a horizontal beam or arm from which two balance pans were suspended, also on knife-edges. The middle of the balance arm was positioned over the pivotal knife-edge. There was a mechanism for raising the arm above the pivotal knife-edge and protect it when the balance was not in use; the mechanism released the balance arm when for weighting. At the bottom of the vertical column that houses the knife-edge was a graduated scale and a pointer on the balance arm that indicated whether the masses of the balance were equal or not. Known masses were added to the pan on the right and used to weigh the sample in the left-hand pan, on the other side of the balance arm.

Method of Swings

Weighing is a dynamic process. This is illustrated for dual-arm balances using the basic technique known as the "Method of Swings". The idea is to "balance" known masses on the right-hand pan in such a way as to equal the mass of the object being weighed on the left-hand pan. The pointer shown in the figure is ideally at zero.

However in this imperfect world, with mechanical balances the zero (or rest) point had to be established. There was no tare button with these balances and weighing was as much an art as a science.

This was how it was done: the observed number of divisions when oscillating was equal to the number of divisions on the graduated scale. The balance point was determined by the central pointer traversing the same number of divisions to the left and to the right (i.e., the method of swings). The key learning point was that weighing is a dynamic method not a static one as it is today with an electronic balance.

In this technique there is no correction for buoyancy, which is required for high accuracy work; however, the rationale for buoyancy correction will be discussed in the second part of this article (assuming of course you survive reading this part). In addition, for work of the highest accuracy the sample and masses were reversed and the average value for weight taken.

The correct use of the analytical balance is described by these quotations taken from a 1921 textbook[3] (which Chris probably used while he was training).

Determination of the Rest Point

"The beam and stirrups are first lowered upon their knife edges by slowly turning to the left the milled head at the front of the balance case; then the pans are released by gently pressing inwards the small button, also at the front of the case; and, with the beam swinging smoothly, a consecutive record is made of the number of scale divisions traversed by the pointer on either side of the centre."

"The swings to the left are recorded as negative numbers and those to the right as positive numbers; in the determination of the rest point, one more reading must be made on one side than on the other, and all of the readings must be consecutive."

"Upon dividing by 2 the algebraic sum of the averages for the two sides, the quotient is the rest point of the balance for the case under consideration (i.e., the position on the scale at which the pointer would finally come to rest.)"

Having determined this rest point we could now proceed to weigh our sample!

Weighing the Sample

"The object to be weighed is placed upon the left-hand pan of the balance and weights upon the right-hand pan, until, finally, the further addition of 5 mg (or 10 mg) more than counterbalances the object. This weight is then removed, the balance case closed, and the rider adjusted so that the pointer swings equal distances on either side of the rest point. This method of weighing is very common, and it is sufficiently accurate for ordinary analytical work. If necessary, the rest point of the unloaded balance should be determined before each weighing."

Weighing by Difference

Allied with a basic weighing of a sample is the variation of weighing by difference. This is because most samples (owing to their physical nature and/or their size) are not placed on the balance pans but in a vessel. Therefore, the analyst weighs the vessel alone first and then with the sample. The sample weight is calculated by difference. If this approach is used the total weight of the sample must be transferred to a volumetric by washing with a suitable solvent.

An alternative is where the vessel plus the sample is weighed, the sample is tipped into the volumetric and the vessel plus any residual sample is reweighed and the sample used is again calculated by difference.

Direct Comparison of Masses

Regardless of the weighing approach described above, we have a process where we are comparing masses directly via the knife edge. This is a key issue as the use of an electronic balance uses a completely different approach.

Electronic Balances

Let's get real — it is very rare if we have used or have even seen an equal arm balance apart from in a museum. Weighing is easy nowadays with a modern electronic balance: just plug in the lead, turn the balance on, put the vessel or the sample on the pan and read the display or print the result (no 21 CFR 11 issues here!)

We still use weighing by difference as described above in the majority of instances but the whole process is easier and simpler using the electronic balance (Figure 2).

However, electronic single pan balances are not balances in the traditional sense of balancing a sample against a known set of masses. They are based on load cells that operate on the basis of electromagnetic force compensation. This is a completely different principle of operation that must be understood in more detail.

Figure 3 is a schematic of an electronic balance principle of operation. When a "weight" is placed on the balance pan, the down force displaces the coil within the load cell from its original position. The load cell generates a current and a compensation circuit returns the balance pan to its original position. This compensation current is converted to a voltage and, via a suitable calibration function built in by the manufacturer, is displayed as the measured "weight".

You can even print the weights out from the balance; tare, final weight and weight of substance. The subtraction of the tare weight from the final weight always gives the same value as the weight of substance doesn't it? To answer this question do you want to go 50:50 or phone a friend?

Unfortunately the answer is "No". This is because electronic weighing is a dynamic process and the balance carries more significant figures within its internal memory than are displayed. Hence the value can be out by ±1 in the last place of decimals!! The software in the balance performs all sorts of data processing during the weighing operations but only prints out to the predetermined number of significant figures. This is a direct consequence for the dynamic process of weighing and rounding. It's not a technical issue because we should understand it but it could become a compliance issue. By analogy, this is just like Excel; but you knew that already didn't you?

Don't Do This at Home

The fact that you must calibrate your balance should be obvious to all readers of this article. However, there are some laboratories that have problems with this. In regulated industries the health authorities inspect laboratories and will issue non-compliances when they do activities incorrectly. As avid readers of the Food and Drug Administration (FDA) warning letters we came across this one which is pertinent to our discussion.

Earlham College in July 2002 received a warning letter from the FDA[4] and one of the non-compliances cited was

"3. Failure to comply with the General Requirements of Sub Part I. Laboratory Controls, as required by 21 CFR 211.160, in that there is:

g. (FORM FDA-483 Item #12) No certification to a recognized standard for the weights set used for checking the balance."

This point was also discussed in more depth in the FDA's Human Drug current Good Manufacturing Practice (cGMP) Notes[5] from the second quarter 2002 (the timing of the FDA note and issue of the warning letter may not be coincidental).

Question

Nowadays, many leading analytical balance manufacturers provide built-in "auto-calibration" features in their balances. Are such autocalibration procedures acceptable instead of external performance checks? If not, then what should be the schedule for calibration?

Answer

The autocalibration feature of a balance may not be relied upon to the exclusion of an external performance check. For a balance with a built-in autocalibrator, external performance checks should be performed on a periodic basis but less frequently when compared with a balance without this feature.

The frequency of performance checks depends on the frequency of use of the balance and the criticality and tolerance of the analytical step. Note that all batches of product manufactured between two successive verifications would be affected should the check of the autocalibrator reveal a problem.

Additionally, the calibration of an autocalibrator must be periodically verified — a common frequency is once a year using NIST traceable standards or NIST accredited standards in use in other countries.

A measurement uncertainty (random plus systematic errors) not exceeding 0.1% (0.001) of the reading is generally accepted for laboratory balances; (see the *US Pharmacopoeia*, Chapter 41, Weights and Balances for further information.)

There were also cross-references to the following sections of the cGMP regulations:

- 21 CFR 211.68: Automatic, mechanical and electronic equipment
- 21 CFR 211.160(b)(4): Laboratory Controls — General Requirements

So let's look at this further; summarizing the issues from the regulators we need external calibration masses for the balance, but they must also be to a recognized standard. So what sort of calibration standards can we have?

Classification of Calibration Masses

There are several classes of calibration masses that have been devised by the International Organization of Legal Metrology (IOLM) in its Report R111,[6] that is presented in Table 1.

You can see as you go from E1 to M3 that there is a cascade process, a mass calibrant in one range can be used for the same class and all lower ones. From a practical perspective, you must take great care of your master set of calibrated weights.

Regularly Calibrate the Masses

The calibration masses used in your laboratory must be checked on a regular basis to ensure that the masses themselves are still accurate. This is always done by comparison against higher class weight sets. Otherwise your calibration sets are useless.

What does this mean in practice? If you have an F2 weight set in your laboratory, then you'll need to have them calibrated against a higher mass set, such as F1, typically by an external calibration laboratory. The frequency of calibration should be regular. If standard mass sets are used frequently then an annual calibration may be suggested. If the only use of the mass set is for internal calibration of a working mass set, for example M1, then the length of calibration could be extended. Regardless of the actual frequency, it is important to review the actual masses over time to see if they have suffered any damage that adversely impact the accuracy of the set.

All calibration mass sets must be handled with care to avoid

- scratches
- dents
- dirt
- skin secretions from hands.

The most accurate standards will typically be handled with gloves. In a calibration laboratory, the individual masses will be examined visually and cleaned before the actual calibration. If they have to be adjusted this is a very precise and exacting task for the highest levels of standard. Typically, the means for mass adjustment is lead, which has a low melting point and any excess can be removed relatively easy by a scalpel or similar tool.

Calibrating the Balance

Therefore, in practice, you will use a master set of calibration weights to calibrate less expensive and more robust weights that you'll use on a day-to-day basis. The working range that these weights need to cover will depend on what you will use the balance for measuring. For a narrow range in the mg range you may just want a 1–500 mg range of calibration weights, but you'll need to decide this depending on how you use an individual balance.

It is interesting that German State law requires all balances to be regularly calibrated and checked by independent calibrators, who provide a sticker for the balance showing that it has been calibrated and when the recalibration is due.

Still Don't Do this at Home

Although you are calibrating the balance, is the work that you do adequate and "scientifically sound"? Not always as was noted in item 11 of a 483 observation at the end of an inspection at Forest Inwood Laboratories:[7]

- Reference standards are weighed on an analytical balance and the weights recorded to 4 decimal places (e.g., 0.0100g).

So far so good but now we slide down the slippery slope:

- Typically 10 mg of standard are weighed out; however, in some instances smaller quantities are weighed.
- Although management indicated that to avoid unacceptable error, a microbalance would be used to weigh quantities less than 10 mg, instances were noted in which smaller amounts of standards were weighed out on the analytical balance.
- These include the weighing of 8.8 mg and 1.1 mg of standard.

This is poor science, bad training and bad management to allow chromatographers to weigh out such small amounts on an analytical balance. Some people's only purpose in life is to serve as an example for others not to follow.

In the next thrilling episode we'll look at best weighing practices, practical traceability issues and performance monitoring, corrections for buoyancy and also using microbalances.

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