



# Simplifying getting started with HPLC qualification using the HSQ Kit – How To

***A small investment to learn the system will save so much time and money should make it a good investment.***

As the first time, setting up an HPLC qualification method and using the HSQ Kit™ can seem a bit overwhelming. However, if you simply examine the data entry section of the Excel sheet provided in the HSQ Kit™, the items that need to be entered and getting started are in order of their injection.

There are 3 **basic methods** to be written, and 3 **sequential sections** of the injection run sequence.

## I. *System Suitability*

Method RTM. Inject one or more blanks, and the RTM. Check that the retention time and efficiencies meet specs.

## II. *Isocratic*

Method PQ. Inject blanks, the 10 **precision** samples of L3, the 6 **linearity** standards L1-L6 each in triplicate, then three blanks (to measure carryover). Continue with the injection volume **linearity** (assuming she has a suitable autosampler) injecting triplicates of L2 at each of 5 chosen injection volumes. Done. This is all there is to the isocratic testing.

## III. *Gradient*

If you have a gradient system to test, then set up method GRD, with the exact profiles as detailed in the Quick Start instructions. Spike about 500 mL of mp with the GVS solution (3 mL GVS per 500 mL mp), and flush the LC system, putting the spiked mp in the “B” position. Inject a sample of the actual spiked B mobile phase at the start. Run the gradient profile. At the conclusion, there will be a small void volume peak (Uracil) from the spiked B injection. That is the column void volume. Using an expanded scale, estimate the onset of the gradient. Those two values are entered into the spreadsheet, to calculate the Gradient Dwell Volume.

Use a ruler, or integration to measure the heights of the 10% and 90% gradient steps for A/B, C/B and D/B. Enter those values in the spreadsheet, for calculation of the gradient **accuracy**. Note that I recommend 2 injections of the gradient, using only the second one for the qualification data, since it is difficult to fully purge the system on the first try.

## **That's it.**

If you lay out the sequence and methods to match the Excel sheet, I think it will become more obvious and no overwhelming. Once you have been through it once, it will become much easier for future qualifications. It can seem a bit complex, but there are a lot of tests being run, as the qualification is very comprehensive.

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