# HPLC gradient validation using non-invasive flowmeters

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Gradient HPLC serves as a crucial tool for analysing samples containing compounds with varying polarity. Its effectiveness stems from the ability to tailor both gradient time and composition, enabling the manipulation of analyte retention times. However, challenges arise from deviations in the programmed gradient profiles produced by HPLC systems, impacting retention time and peak shape. Several factors contribute to this variability in gradient HPLC, such as solvent quality, pump performance, changes in system pressure, and the condition of the columns. Addressing these challenges requires precise control and monitoring of gradient profiles.

The traditional and generally accepted method for validation of gradients in an HPLC system currently relies heavily on the assistance of a UV detector. Typically, for a binary gradient, pure water and water containing 0.1% acetone is utilised. Determination of absorbance of acetone as function of time, represents very accurately the gradient performed by the system. However, although this method is accurate, it is not representative for the gradient used in an actual chromatographic separation as several important factors, including viscosity and density of the utilised solvents, are not considered. Furthermore, peculiarities of the HPLC system solenoid valves used, such as different switching times for the on and off phases, do not really come into play when the used solvents are very similar, which is the case in the traditional validation method.

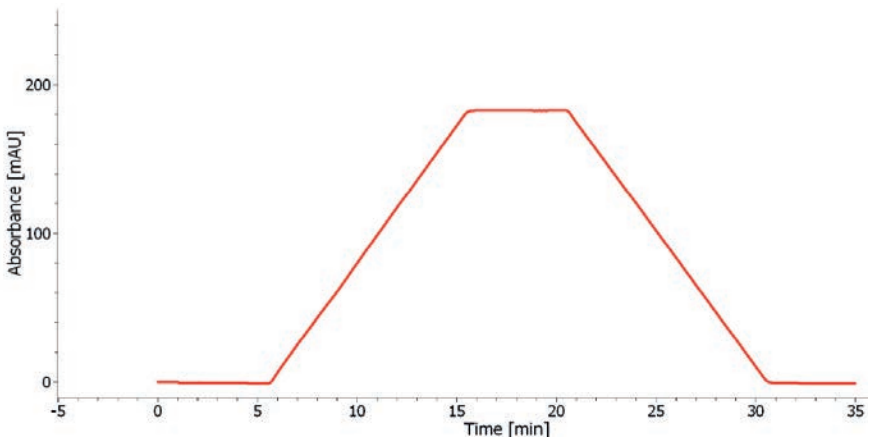


*Figure 1. UV absorption chromatogram at 265 nm of a standard gradient test.*

Considering, however, the importance of reproducible gradients for any HPLC application, a reproducibly accurate method of continuous monitoring of the solvent mixture created by the system should be of paramount interest for the many critical gradient HPLC applications used in the pharmaceutical, cosmetics, food, and similar strictly regulated industries.

Modern non-invasive flowmeters, based on a thermal principle, are well known for being a fast and accurate tool for determination of flow rate in HPLC systems. This has been demonstrated in several articles and publications [1]. An interesting feature of these devices, in the context of gradient composition monitoring, is that the reported flow rate values are strictly related to the solvent the device has been calibrated for. In other words, these flowmeters, with no specific calibration, deliver an apparent flow rate which is dependent on the composition of the measured eluent. As such, they are, at least in theory, the perfect tool to continuously monitor the composition of the eluent in a gradient HPLC system, aiming to determine the reproducibility of the mixture.

Proof of the concept described above was performed by simultaneous determination of eluent composition using the generally recognised method of UV absorbance while measuring the apparent flow rate reported by a flowmeter connected at the very end of the HPLC system. This setup assures that the measurement of the apparent flow rate does not interfere in any way with the HPLC separation. In contrary to the traditional method, it is a fully non-invasive and therefore ideal as a method to be used for monitoring of eluent composition independently from a particular HPLC application. In detail, our aim of monitoring was not to determine the absolute composition of the mixed eluent, but to check whether reproducibility of the mixture can be determined by this method thus confirming the validity of the assumed fitness of this approach for the task of continuous monitoring of reproducibility.

*Figure 3* shows results of the two methods run at the same time. The experiment was repeated twice, traces of the two experiments are overlapped. It is clearly visible that both, the traditional and the ‘apparent flow’ methods (using the LC Flowmeter) reproducibly report on the composition of the eluent mixture.

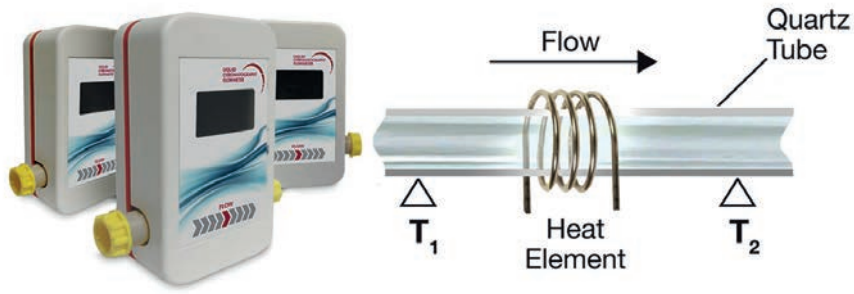


*Figure 2. Testa Analytical LC Flowmeter.*

While the ‘apparent flow’ method, does not provide an absolute determination of eluent composition, it does however offer the real advantage of not interfering with the separation, thus allowing monitoring of any required gradient shape and composition, which is not possible with the traditional validation method.

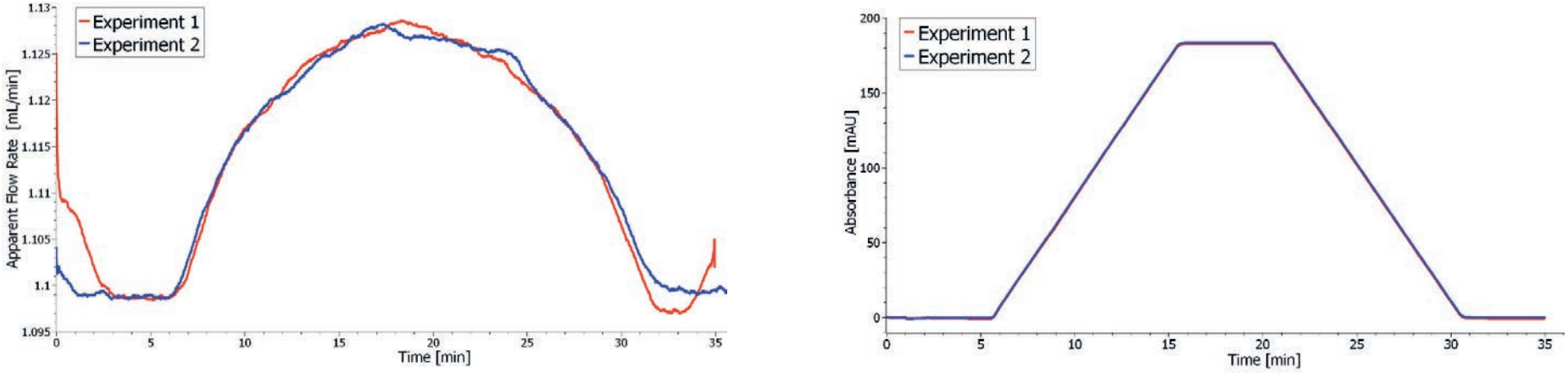


*Figure 3. Reproducibility of the flow rate profile in comparison to the UV Absorbance.*

## Conclusion

This experimental study demonstrates the ability of modern non-invasive flowmeters to be valuable tools for validating and monitoring gradient in HPLC systems in a way which was impossible until now. By accurately monitoring apparent flow rates in real-time and enabling data logging, these flowmeters ensure that programmed gradient is faithfully reproduced, facilitating precise and consistent separation in HPLC. This enables the use of gradient HPLC with a higher degree of confidence.

Testa Analytical LC Flowmeters are the perfect tool for labs where higher confidence in HPLC results is desired or necessary. The flexibility and advantages of these non-invasive flowmeters as monitoring device for eluent composition in gradient HPLC systems, has been confirmed in comparison to the traditional UV detection techniques.

## Reference

*1 See https://www.testa-analytical.com/papers/paper45.html*