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PRODUCT SPOTLIGHT

Achieving standard-free quantitation:

Thermo Scientific Charged Aerosol Detectors

Uniform response for every analyte

Chromatographic methods rely on the availability of individual standards for quantitation. But what happens when no reference standards are available, for example during drug discovery, and the purity of the drug candidate is also unknown? UV absorbance and mass spectrometry (MS) detectors are commonly used to quantify analytes, but the response of those detectors highly depends on their chromophoric properties or ability to form gas phase ions, respectively, making a quantitation difficult. How can compounds be measured that do not respond to these approaches?

The answer is the charged aerosol detector (CAD). The CAD is a universal detector that gives uniform response independent of a compound's physico-chemical properties for any non-volatile and many semi-volatile compounds (Figure 1A). This makes CAD ideal if quantitation is needed and no reference standards are available. With a single calibrant the quantification of multiple analytes is therefore possible, even in the absence of individual standards (Figure 1B).

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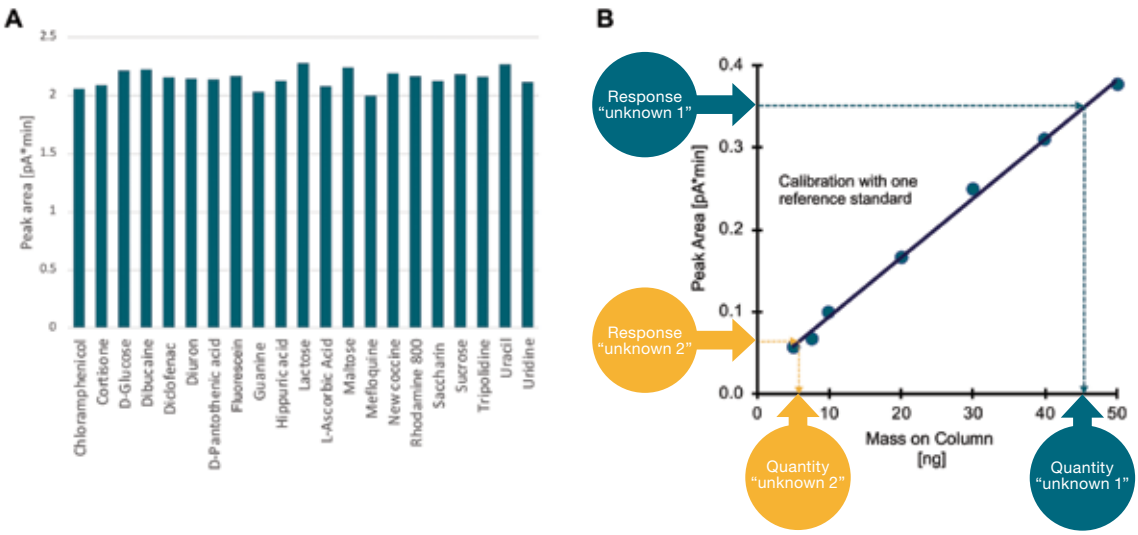

Figure 1. Because of CAD's uniform response (A) the quantification of unknowns with a calibration curve of a single reference standard is possible (B).

Uniform response with every method

Although the response of the CAD is independent of an analyte's chemical structure, it is dependent upon the composition of the mobile phase entering the detector. The efficiency of pneumatic nebulization increases as the amount of organic solvent in the mobile phase is increased during gradient elution. Therefore, analyte response for a given mass appears to increase throughout the gradient (Figure 2). This can cause quantitation errors when using a single calibrant.

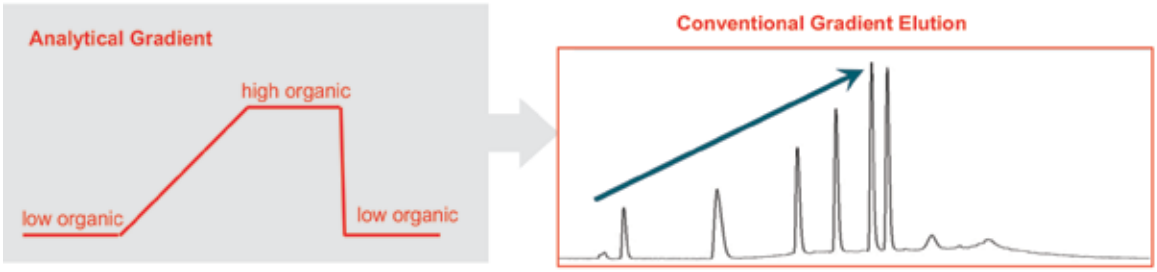

Figure 2. Detector response is dependent on mobile phase composition and will vary during the gradient.

Single calibrant quantitation without limitation

In order to overcome the effect of the gradient and to once again regain response uniformity, a second gradient, the inverse of the analytical gradient, is applied post column so that the detector always experiences mobile phase of constant composition. In this way, response uniformity is maintained (Figure 3).

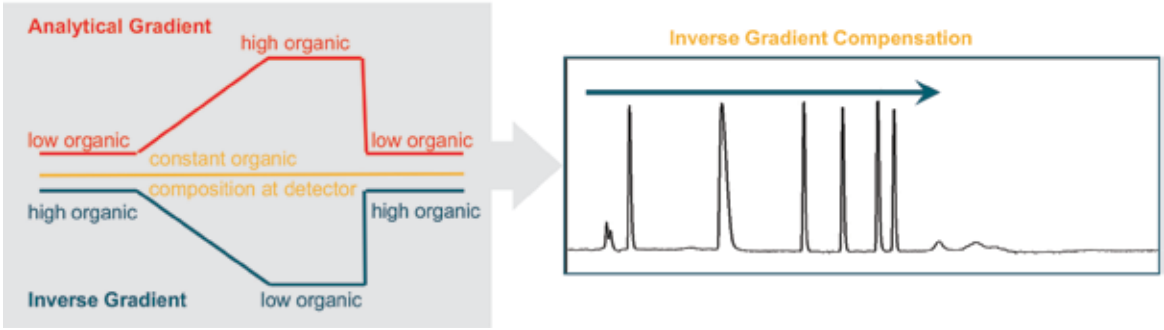

Figure 3. Maintain analyte response uniformity by using inverse gradient compensation.

The solution: Thermo Scientific™ Vanquish™ Duo UHPLC system for Inverse Gradient

The method setup of an inverse gradient workflow is easily implemented with the Thermo Scientific Vanquish Duo UHPLC system (Figure 4) and complemented by advanced software tools. One pump delivers the analytical gradient for the separation of analytes on the column, while the second pump generates the inverse gradient flow. A T-piece combines both flow paths before entering the CAD inlet, achieving a constant eluent composition.

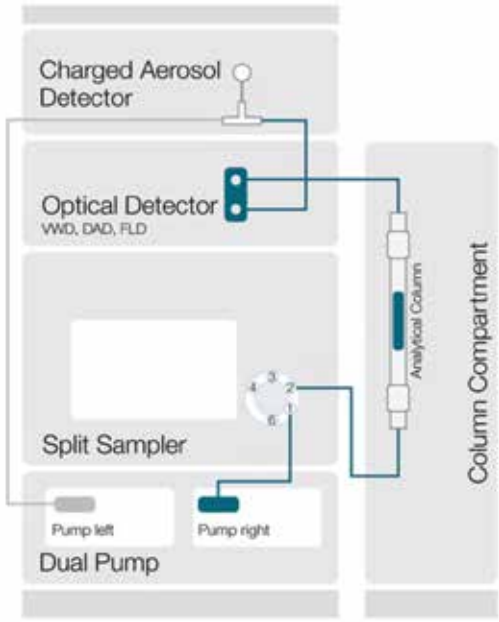

Figure 4. An inverse gradient flow delivered by the left pump ensures that the composition of the mobile phase entering the CAD remains constant thereby maintaining the response uniformity throughout gradient elution.

Fine tuning the inverse gradient

There are three options available to address gradient compensation, ensuring that mobile phase of constant composition is delivered to the CAD. Selection of the best option will depend upon required sensitivity and other factors.

Option 1: Constant solvent composition:

- What it is: The ramp of the compensation gradient is an exact inverse of the analytical gradient (Figure 5). The flow rate is the sum of both flow paths, so the flow rate to the detector is double that for just the analytical gradient.
- When to choose: Easy to program and the preferred option if the resulting sensitivity is sufficient and if the resulting flow rate is within the supported flow rate range of the detector.

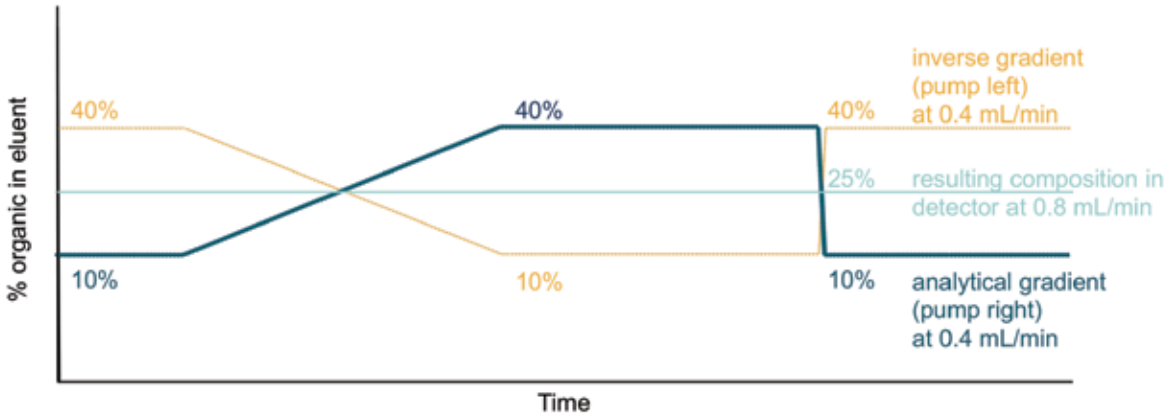

Figure 5. Option 1: Constant Solvent Composition.

Note: For programming the exact gradient dwell time differences between analytical and make-up flow paths needs to be considered.
For visualization Figures 5 to 7 show the composition at the detector inlet and not the actual programmed gradients

Option 2: Maximize organic composition:

- What it is: The make-up flow is shifted to higher organic content while the gradient shape of the inverse gradient is kept constant (Figure 6). The flow rate is the sum of both flow paths, so the flow rate to the detector is double that for just the analytical gradient.
- When to choose: Preferred option if high sensitivity is the focus. The higher organic composition allows for higher nebulizer efficiency resulting in increased analyte response.

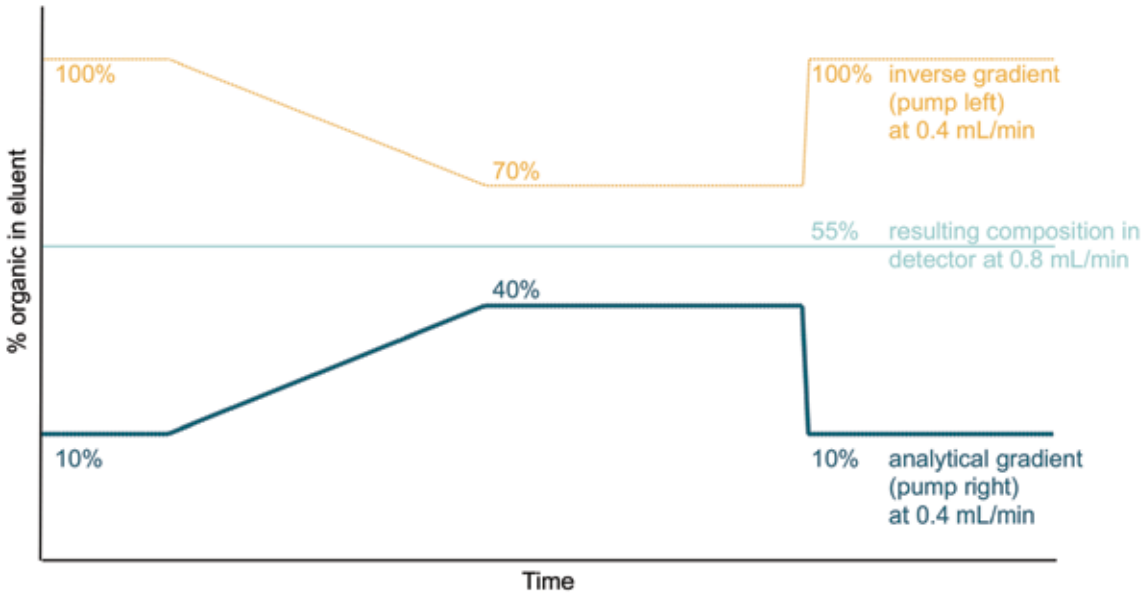

Figure 6. Option 2: Maximize Organic Composition.

Option 3: Minimize flow:

- What it is: The make-up flow of the second pump is reduced to a minimum, while the full range of organic portion for the pump is applied from 100% to 0% (Figure 7).
- When to choose: This option allows for uniform response with gradient separations in cases where option 1 and 2 cannot be applied due to flow rate limitations. Combined flow of the analytical and inverse gradient must not exceed the limit of 2 mL/min. With option 3 the flow rate is significantly lower than with option 1 and 2.

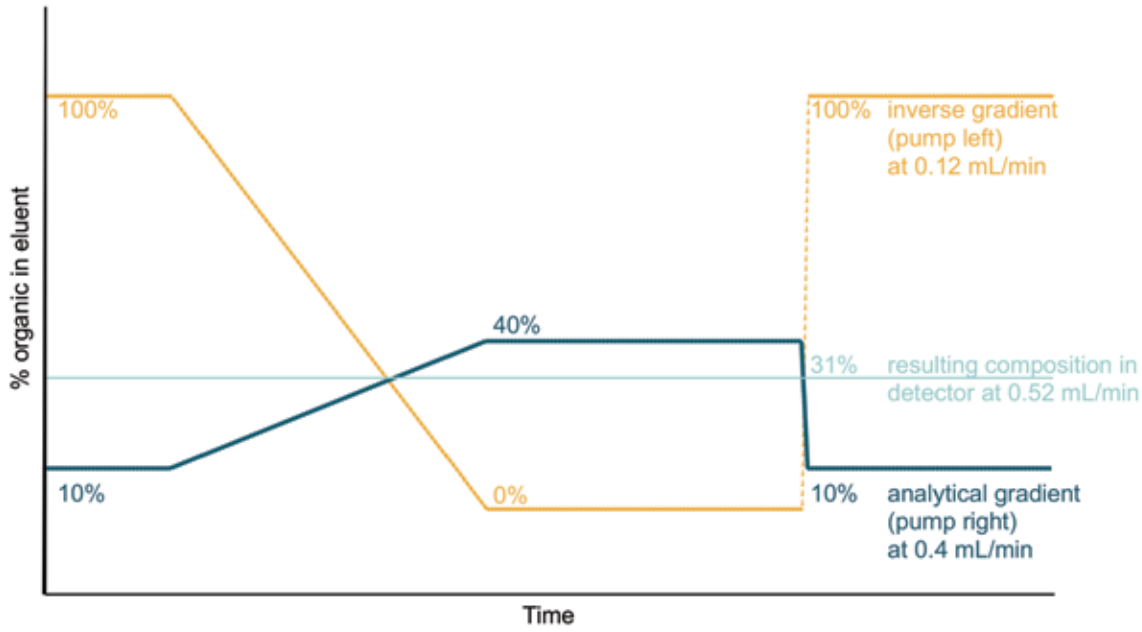

Figure 7. Option 3: Minimize Flow.

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Inverse gradient applied: Example of a pharmaceutical separation

Four pharmaceuticals were separated using a reversed phase gradient. Figure 8A shows the chromatogram and the calibration curves with analytical gradient only. The early eluting and late eluting compound show different response curves and drifting baseline. The inverse gradient serves to normalize peak height and area relative to the analytical gradient and to reduce the baseline drift. All substances in the sample can be quantified by a single calibration curve (Figure 8B).

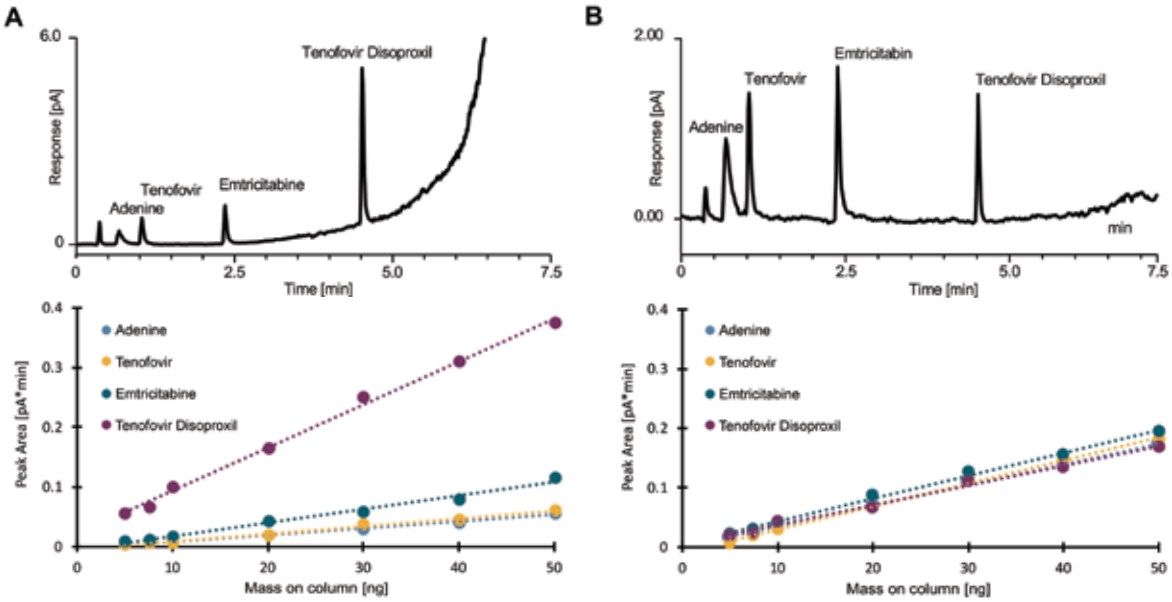

Figure 8. Chromatograms and calibration curves of four pharmaceuticals without (A) and with (B) inverse gradient compensation (option 1 applied). Similar analyte response curves were only obtained when using inverse gradient compensation.

The Thermo Scientific™ Vanquish™ Duo UHPLC system for inverse gradient provides

- Confident uniform response with gradient elution using dual pump technology
- Reliable single calibrant quantitation of knowns and unknowns independent of gradient composition
- Easy method implementation with defined instrument setups
- Flexibility with inverse gradient options depending on the need for sensitivity or flow rate.

References

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