

Advanced UHPLC Setups to Overcome Limitations of Nebulizer-Based Detectors

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Keywords

Inverse Gradient, Dual-Gradient Pump, Charged Aerosol Detection, Corona Charged Aerosol Detector, Diuretics, Mass Spectrometry

Summary

Optical detectors such as UV detectors are often limited in their applications because many analytes lack a chromophore. Nebulizer-based detectors claim to be non-selective and to provide universal analyte response. The response of these detectors, however, highly depends on the current solvent composition which changes over time during a gradient elution. While mass spectrometry signals additionally depend on the analyte ionization properties leading to an analyte-depending detector signal, charged aerosol detection has the potential to give universal response. Here we describe the application of the inverse gradient concept and how it can be utilized with charged aerosol detection to overcome the limitation of nebulizer-based detectors. The suitability of that approach is highlighted for a variety of different applications.

Background

We as chromatographers are all challenged every day by measuring a broad range of analytes at low amounts in fast turnaround times. But in many cases, the performance of traditional detection technologies such as UV absorbance and fluorescence is not adequate to fulfill all those requirements, especially since these detectors lack a uniform analyte response. Even mass spectrometry (MS), which is considered by many as the new holy grail of detection, is no way out of this problem as signal response here relates to the individual gas phase proton affinity of the analytes. Consequently, new innovations in detection technology such as Evaporative Light Scattering Detection (ELSD) or Charged Aerosol Detection entered the stage, having principally a potential universal response for virtually any analyte.



What all these detectors—Mass Spectrometry, Evaporative Light Scattering and Charged Aerosol Detection—have in common is a nebulization process as a first step to form fine droplets of the eluent coming from the chromatographic column. The HPLC effluent is sprayed in a stream of inert drying gas, followed by the evaporation of the solvent. In both Charged Aerosol and Evaporative Light Scattering Detection, this nebulizing and evaporation process generates aerosol particles which are finally detected by the specific detection technique. While this last step depends on the detection principle, the evaporation of the solvent is governed by the same basic physical principles, no matter which detection principle is applied. The efficiency of aerosol particle formation strongly depends on the present solvent composition with higher amounts of organic solvent yielding higher efficiencies in particle formation. Thus, the better particle formation efficiency leads to a higher signal response during gradient elution—a clear no-go if you want to see a universal response for all your analytes despite a changing solvent composition.

While the response in MS additionally depends on chemical properties of the analyte such as proton affinity, Charged Aerosol Detection, which measures charged particles and not ions, should, by design, give the same response for all non-volatile analytes under isocratic elution conditions! Fortunately, for gradient elution, this limitation can be overcome by a second solvent stream which bypasses the analytical column and adds a mirrored gradient composition behind the column, resulting in a near-constant solvent composition entering the detector. This concept of an inverse gradient, firstly developed in an academic environment¹, compensates different analyte response due to the changing evaporation efficiencies during gradient elution. For a simple and convenient use in the lab, this promising approach needed then to be integrated in a single HPLC platform and supported by elaborated software tools.

Solution

The inverse gradient concept was commercialized in an easy-to-use way utilizing a unique dual-gradient pump (DGP) which in fact incorporates two independent ternary low-pressure gradient pumps in one single module. The first ternary pump enables the elution of the analytes from the column running the analytical gradient while the second ternary pump simultaneously delivers the mirrored gradient of the first pump. Both streams are merged behind the analytical column but before the Corona charged aerosol detector. As the Thermo Scientific™ Dionex™ Corona™ Charged Aerosol Detector is mass-sensitive, the sample dilution will not affect the detector sensitivity, in contrast to concentration-dependent detectors such as UV detectors. If you want to use an additional UV detector, simply put it in the flow stream directly behind the column and combine the solvent streams after the UV detector; this allows you to use such an Thermo Scientific™ Dionex™ UltiMate™ 3000 x2 Dual LC System with Inverse Gradient also for conventional, i.e. non-compensated LC runs. And don't be afraid of potentially complex calculations for the inverse gradient: An integrated software wizard does the math automatically. Even more, the calculation can be tuned in such a way that the organic solvent amount is maximized before infusing in the Charged Aerosol Detector, ensuring highest nebulization and evaporation efficiencies and leading to improved limits of detection and quantitation.

Two different setups open up your way to UHPLC separations with uniform response detection by Inverse Gradients and Charged Aerosol Detection. In the first configuration, the compensating solvent is bypassing the

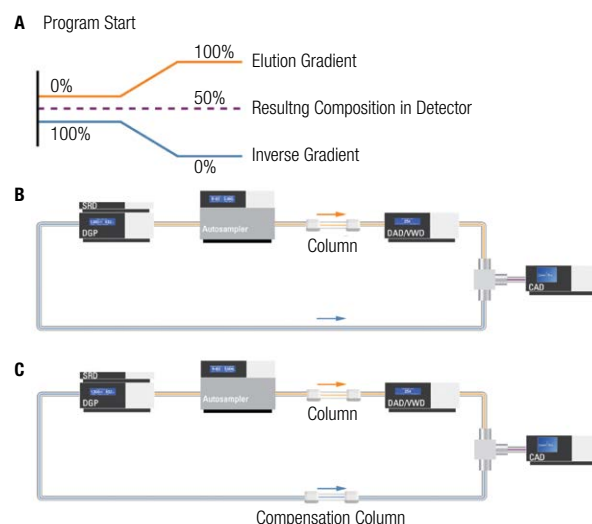


Figure 1. Configuration of the inverse gradient with (A) giving the effect of the inverse gradient on the final solvent composition, (B) the inverse gradient setup without an additional and (C) with an additional column.

analytical column only by capillary tubing, but doesn't run through a column. Thus, a delay time of the mirrored solvent stream must be considered in order to compensate for gradient delay times (Figure 1A). The second approach uses two similar LC columns with similar tubing. This way the calculation of a delay time becomes obsolete (Figure 1B).

The inverse gradient concept has been rigorously tested for both Charged Aerosol Detection and Mass Spectrometry. Let's start our discussion with mass spectrometry: six diuretics were separated on a C18 column using a gradient from low (15% ACN) to high (90% ACN) amounts of organic solvents and detected with an atmospheric pressure chemical ionization (APCI) based MS. When comparing conventional gradient with inverse gradient runs, we could show that the signal-to-noise ratio improves with the constant solvent composition.² Nevertheless, you can immediately see that the analyte signal intensity also improves over time even without the compensation, simply because both APCI and ESI profit from higher organic amounts during the gradient elution. Even when the mirrored gradient was applied, the response factors of these six diuretics still varied over more than one order of magnitude as the signal response in both APCI and ESI severely depend on the gas phase proton affinity of the individual analytes. Uniformity of response factors is something you simply cannot expect from APCI and ESI mass spectrometry.

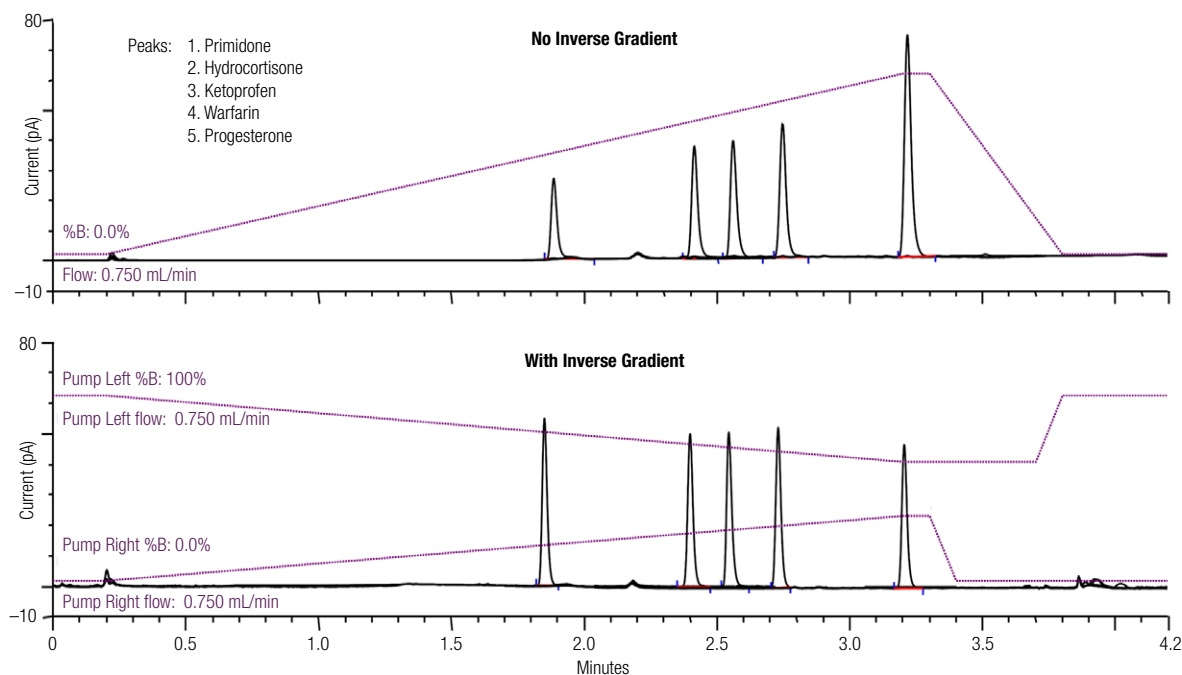


Figure 2. Chromatograms of five pharmaceutical compounds with and without the application of an inverse gradient and charged aerosol detection.

In contrast, applying the inverse gradient highly reliably provided uniform response for all diuretics when using charged aerosol detection. For example, the response deviation of 5 highly variable active pharmaceutical ingredients (API) was less than 5% compared to 19% in conventional gradient analysis, as shown in Figure 2.³ Do you want another proof? Sunflower oils have also been analyzed using the inverse gradient concept. Here, calibration curves of glycerol trilinoleate and glycerol

trioleate showed virtually identical slopes and correlation coefficients highlighting the potential to apply only one calibration curve to different analytes if a Charged Aerosol Detector is used.⁴ And we did a lot of more investigative research on that matter, impressively demonstrating response uniformity using 41 API, as outlined below. In this case the response varied only by an RSD of 18 % compared to 119 % for UV detection, despite the broad range of analytes (Figure 3).

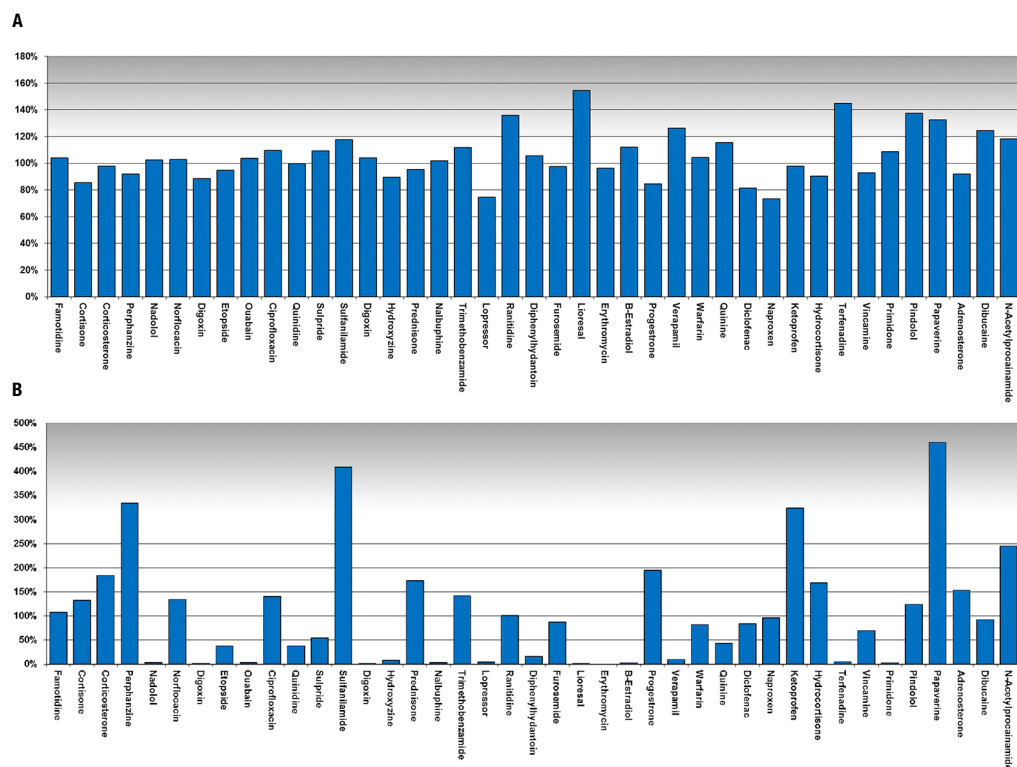


Figure 3. Recovery of 41 APIs using one single calibrant using (A) Charged Aerosol and (B) UV detection at 254 nm.

Call to Action

For questions contact the author directly using carsten.paul@thermofisher.com.

For more detailed literature as well as ordering information please visit

<http://www.dionex.com/en-us/products/liquid-chromatography/lc-solutions/dual-configuration/inverse-gradient/lp-89266.html>.

For further innovative solutions utilizing the Dual-Gradient Pump please have a look at

<http://www.dionex.com/en-us/products/liquid-chromatography/lc-solutions/dual-configuration/lp-72476.html>.

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