Semiquantitative Analysis for High-Throughput Screening of Compound Libraries

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Abstract

High throughput compound screening has been used by pharmaceutical companies for over 20 years. Management of these large libraries to ensure that the purity of each candidate has not been compromised during storage is time-consuming, but necessary. The challenge is how to estimate the quantity of so many different compounds without preparing specific standard solutions. Here we explore the development of a novel approach for increasing the throughput and accuracy of screening. A group of > 40 APIs was measured using dual-gradient UHPLC and a Thermo Scientific Dionex Corona™ *ultra*™ Charged Aerosol Detector. Charged aerosol detectors can detect any non-volatile compound with low ng sensitivity while maintaining similar response for all compounds (independent of chemical structure). The tandem system configuration used here enabled both the active and re-equilibration column eluents to be combined prior to the detectors, providing a constant solvent composition to the charged aerosol detector, thus maintaining nebulization efficiency. A response curve from one compound was used to estimate w/w% recovery of itself and the rest of the APIs. The mass results obtained compared to theoretical had a mean result of 105% and 4-fold improvement over UV. The UHPLC system with a Corona *ultra* detector provides a suitable means of calculating the semiquantitative recovery of APIs without the need for individual calibration curves. As the mobile phases used are compatible with both UV and MS, a multidetector platform can be used to generate a vast amount of important information on a large number of APIs in a single injection.

Introduction and Theory

Monitoring purity of components in large compound libraries can present a difficult analytical challenge. No single analytical method or detector can effectively analyze all pharmaceutical compounds. The goal of most companies is to develop automated systems that combine several detection techniques to obtain the most information possible and in a timely manner.

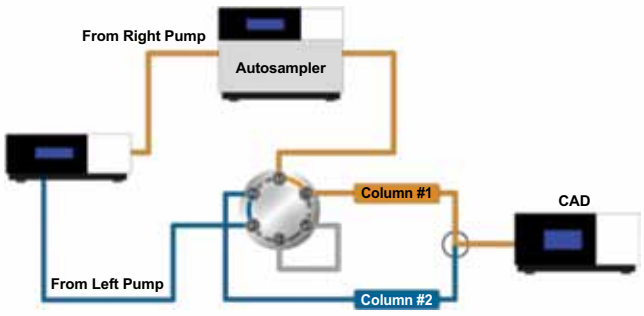
The Corona *ultra* detector is mass sensitive and can be added to the traditional HPLC-UV platform. This detector provides the most consistent response across all nonvolatile and some semivolatile analytes of all HPLC detection techniques.[1] With all nebulizer-based detection techniques like charged aerosol detection, the nebulization efficiency is usually increased as the organic solvent proportion increases. Thus, when running gradients from highly aqueous to highly organic, detector response increases. Delivering a second post-column solvent stream that is inverted in composition relative to the elution gradient ensures that a constant proportion of organic solvent reaches the detector and results in more uniform response factors.[2,3,4]

Development of Methodology

RSLC System

Column:	Thermo Scientific Acclaim™ RSLC Polar Advantage II, 2.1 × 50 mm
Column Temp.:	45 °C
Mobile Phase:	A) 20 mM Ammonium acetate, pH 4.5/acetonitrile (98:2)
	B) Acetonitrile/isopropanol/20 mM ammonium acetate pH 4.5 (94:4:2)
Gradient (Right Pump):	0 to 100% B in 3 min
Gradient (Left Pump):	100 to 0% B in 3 min
Flow Rate:	1 mL/min each pump

FIGURE 1. Schematic of flow path used for the tandem column inverse gradient approach.



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For the dual-column, tandem charged aerosol detector analyses, two identical separation columns are switched between two flow paths—an analysis flow path and a regeneration/gradient compensation flow path (blue, Figure 1). The analysis flow path includes the autosampler and the separation column, which is used for the separation of the analytes in the sample. The regeneration/gradient compensation flow path allows column washing and re-equilibration offline and gradient compensation at the same time. While one column is washed and equilibrated, the sample is separated on the other and the separation gradient is compensated for universal charged aerosol detector response. A two-position, six-port switching valve is used to alternate the columns. This tandem approach provides higher sample throughput capacity using a single UHPLC system.

The traditional tandem approach using the Thermo Scientific Dionex UltiMate™ 3000 RSLC tandem configuration has the second column equilibrating for the entire first column's analytical run (Figure 2). In this approach, because the second column is used to deliver the inverse, a new gradient profile was created (Figure 3). The timing of this setup was tested under gradient and inverse gradient using water and 0.5% acetone and UV detection. This ensured that the gradients matched, that the column was equilibrated, and that the lines were ready for the switching and the next run.

FIGURE 2. Illustration of potential throughput increases using the tandem LC approach.

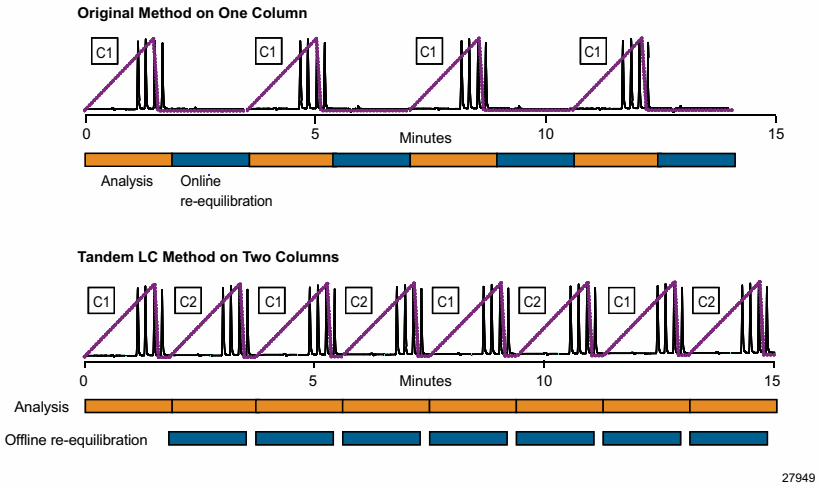
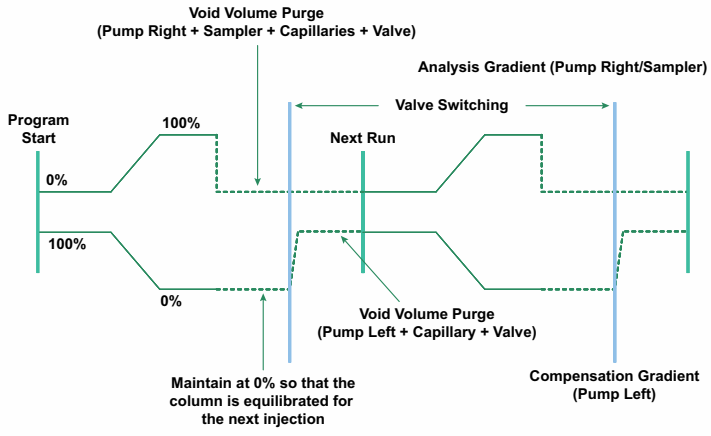


FIGURE 3. Timing for the valve switching with the tandem column inverse gradient approach.



Results and Discussion

Evaluation of Inverse Gradient

An initial study using five test compounds evaluated the gradient effects on response with and without using an inverse gradient (Figure 4). As expected, compound response improved significantly as the percent of organic solvent increased during linear gradient elution of the five test compounds (Figure 4A). The gradient area for the first peak (primidone) was approximately one half of the last eluting peak (progesterone). The change of response was minimized by using an inverse gradient post separation (Figure 4B). Even though additional flow was going into the detector, no change in sensitivity was observed for the test compounds because the Corona *ultra* is a mass-sensitive detector so additional solvent does not influence response. Figure 5 illustrates that the Corona *ultra* detector response deviation was reduced from 19% RSD to 4.4% RSD by using the inverse gradient. The response for early eluting compounds (primidone, hydrocortisone, and ketoprofen) was enhanced due to the addition of organic solvent during the inverse gradient. The responses for later eluting compounds (warfarin and progesterone) were decreased because the percent of organic solvent is lower than in the original, gradient-only elution. The reproducibility of both retention and peak area was then tested for each compound with four injections on each column (Table 1).

FIGURE 4. Analysis of five common pharmaceutical compounds by charged aerosol detection with and without the inverse gradient.

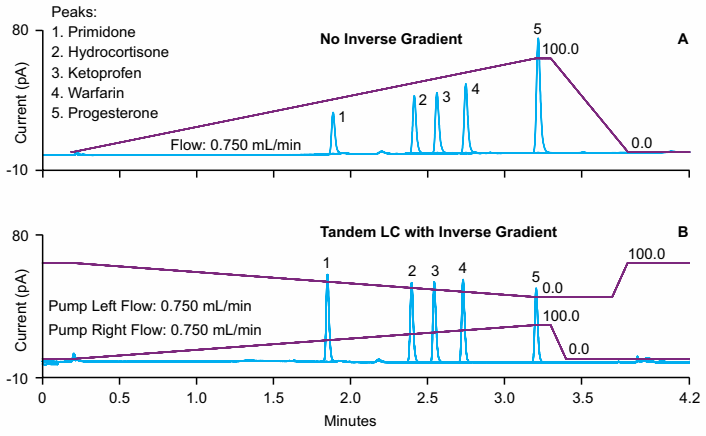


FIGURE 5. Comparison of peak area results with and without inverse gradient.

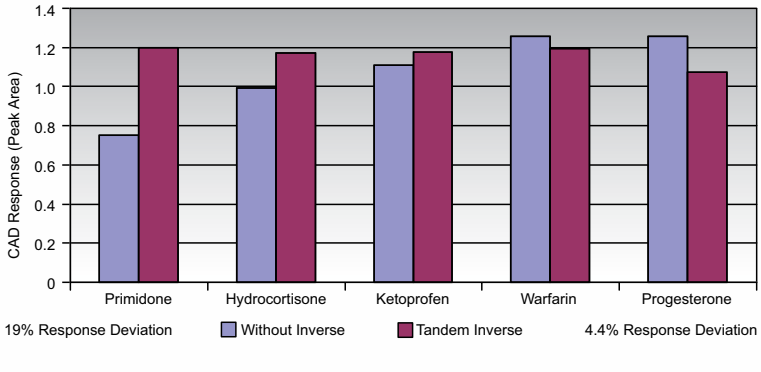


Table 1. Figures of Merit for 8 Interlaced Injections Using the Tandem LC Inverse Approach (n=4 for Each Column)

Peak #	Sample	Retention Time (min)	RSD	
			R.T.	Area
1	Primidone	1.9	0.08%	2.50%
2	Hydrocortisone	2.4	0.07%	1.19%
3	Ketoprofen	2.5	0.07%	1.27%
4	Warfarin	2.7	0.06%	1.10%
5	Progesterone	3.2	0.03%	1.68%

Compound Library Study

The second study examined a larger group of more than 60 common pharmaceutical compounds. Individual solutions of each compound were prepared in DMSO then analyzed using the UlitMate 3000 RSLC system method (Figure 6). The concentration of these solutions ranged from 13 to 120 µM with mass on column ranging from ~250 to 750 ng. Four-point response curves of three compounds chosen for their elution time during the analysis (i.e., beginning, middle, and end) were also evaluated. The effect of the inverse gradient on these response curves is shown in Figure 7. The correlation of the curves with respect to both slope and response become almost identical after the introduction of the inverse gradient stream. The curve for famotidine was then used to back-calculate the recovery of all other compounds analyzed. This recovery value would be 100% with no deviation in response or other analytical errors. The deviation from this value was then used to study uniformity of response for the Corona *ultra* detector with the inverse method.

FIGURE 6. Overlay of inverse-gradient, tandem LC analysis of 44 APIs using the Corona *ultra* detector.

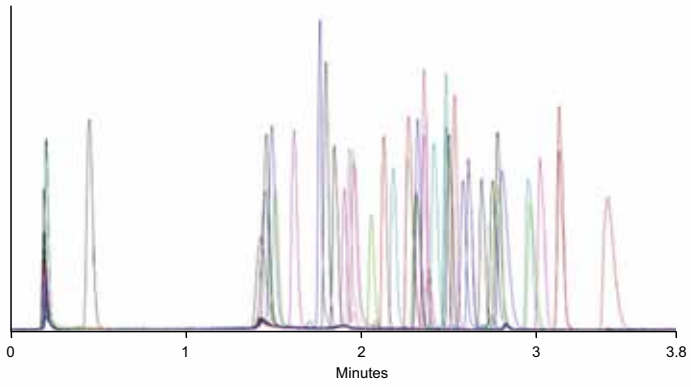
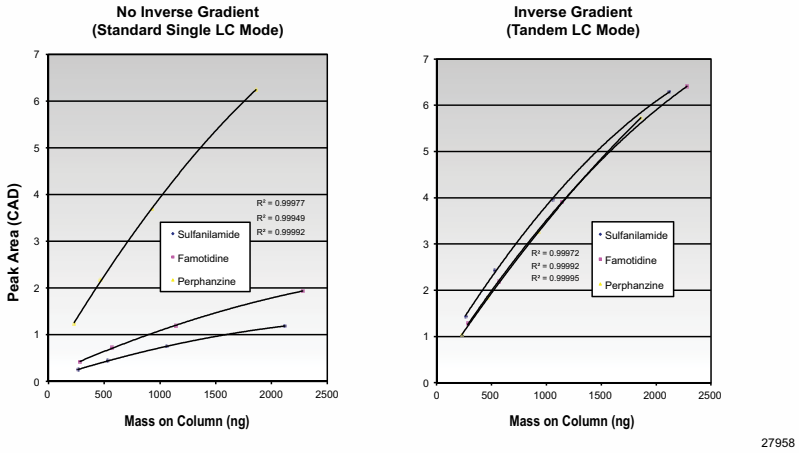


FIGURE 7. Response curves for three compounds analyzed in Figure 6 with and without the inverse gradient.

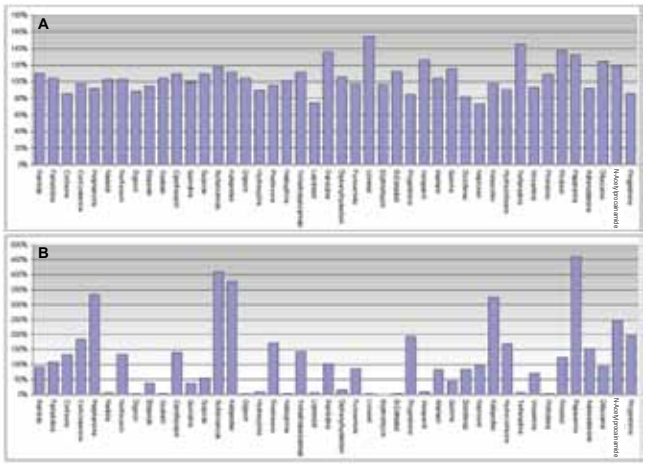


Of the compounds initially chosen for this study, several where eliminated for one of the following reasons: 1) the compound eluted at or near the void volume, 2) the vapor pressure was > 1 × 10^{-7} mm Hg at 25 °C, which put the compound in a semivolatile class, or 3) the compound showed poor peak shape (noticed with several basic APIs). Neostigmine bromide data were also removed. Although it did not fit any of the three criteria above, this compound exhibited more than twice the expected response.

The recovery results for the 44 compounds using charged aerosol detection showed a mean of 105% with an RSD of 18%, with a high recovery of 155% and a low recovery of 73%. The UV results at 254 nm had a mean of 112% with an RSD of 119%. The highest recovery was 461% and one compound was not detected. The Corona *ultra*™ detector results showed a 10-fold increase in response consistency. It also highlighted the potential for large over- or under-estimation by UV detection (Figure 8).

The tandem approach was initially explored to increase throughput. However, the slope of the gradient in this approach did not result in a significant increase in throughput, with only two minutes saved per sample. Optimizing chemistry and using smaller volume mixers may improve throughput. The response uniformity resulting from the tandem inverse gradient approach can be duplicated with the simpler inverse gradient approaches if minimum run time is not a factor.[5]

Figure 8. Calculated recovery for 44 APIs samples using a single calibrant A) Corona *ultra* Charged Aerosol Detector, B) UV at 254 nm.



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Conclusion

The introduction of the inverse gradient to the analytical stream provided a dramatic improvement in response uniformity with the Corona *ultra* Charged Aerosol Detector. This was demonstrated with both a small group of compounds injected with an equal mass-on-column, and with a large group of 44 APIs injected at different concentrations then back-calculated using a single calibrant. In both cases the charged aerosol detector provided a significant improvement over the UV detector for consistency of response.

The combination of this response consistency, hardware, and automation of the UltiMate 3000 dual-gradient system enabled the development of a fast tandem column approach for compound screening. This approach may be supplemented with a technique such as mass spectrometry to provide a significant amount of information for a large group of drug candidates on a fast, automated platform. The semiquantitative results form the single-calibrant, charged aerosol detection approach can be used to examine compound stability issues or other sample preparation problems for large groups of library compounds prior to proceeding with additional testing.

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