

Improving the Quantitation of Unknown Impurity Analysis Using Dual-Gradient HPLC with Charged Aerosol Detection

Christopher Crafts, Bruce Bailey, Marc Plante, John Waraska, and Ian Acworth
ESA—A Dionex Company, Chelmsford, MA USA



ABSTRACT

Quantification of substances such as drug impurities or library compounds when pure standards are unavailable is difficult yet often necessary. This is often accomplished by HPLC based on relative response using low wavelength UV detection. The dependence of response on the optical properties of each component can lead to large errors in estimated quantity. Charged aerosol detection is a mass sensitive detection technique with near uniform response for all nonvolatile analytes, provided that the eluent remains constant. However, response changes during gradient conditions are common with all nebulization-based detectors. The use of inverse gradient postcolumn addition can effectively normalize responses. Using a single platform capable of dual gradient HPLC or UHPLC combined with charged aerosol detection allowed a group of compounds ranging in chemical structure and properties, UV absorbance, HPLC retention, and application in the pharmaceutical industry to be studied. The response deviation was significantly decreased across the compound set to ~13% compared to the 46% without the inverse gradient applied, and >60% with UV-based detection. The work demonstrated very good correlation in the linear response curves over the range tested. This allows for a single calibrant to be used to calculate the mass concentration of unknown impurities independent of their optical properties. This fully integrated system can be used to improve accuracy for mass balance calculations, analysis of impurities and degradants, monitoring compound synthesis and quality of library compounds, and cleaning validations while providing significant cost and time savings with identification and individual standard approaches.

INTRODUCTION

Interest in metabolite or trace impurity analysis in pharmaceutical industries is intensifying due to concerns with mass balance studies, regulatory commitments in reporting API impurities, metabolite in safety testing (MIST), and cleaning validation of manufacturing equipment. Most often an analytical requirement for accurately reporting the level of metabolites or impurities is to obtain reference standards. Since many of these standards remain unavailable it makes exact quantification of impurities and metabolites difficult. The situation is further compounded since several types of HPLC detectors such as UV or evaporative light scattering detection (ELSD) either lack the sensitivity to detect these compounds or do not provide uniform response across the target analytes.

The development of cleaning validation methods is an area facing similar challenges. The need for a fast turn-around-time of the cleaned equipment to help maintain production schedules does not allow for identification of every peak present. So quantitation of impurities by UV detection is often done on a peak area bases. The difficulty that can be encountered when using a specific technique like HPLC-UV is how to quantify unknown peaks. UV detectors suffer from varying extinction coefficients for different structures and thus peak area percent calculations can result in significant errors in impurity calculations. Considering the major difference in UV response between an aromatic active ingredient and a non-aromatic surfactant such as dodecylsulfate, this can result in a potential source of significant underestimation of surfactant contamination. Another HPLC detection technique, ELSD often lacks sufficient sensitivity for trace analysis and due to the need to optimize methods for different compounds considerable response factor variation can occur even for compounds within a similar class structure.

The Corona[®] Charged Aerosol Detector (CAD[®]) 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 When running gradients from high aqueous to high organic content all nebulizer-based detectors tend to show increased response as the organic solvent proportion increases due to improved nebulization efficiency. Aerosol-based detection techniques using CAD are also sensitive to this phenomenon. Optimization of the detector response by delivering a second postcolumn solvent stream, which is inverted in composition relative to the elution gradient, enables a constant proportion of organic solvent to reach the detector and results in more uniform response factors for all compounds eluting from the column.^{2,3,4}

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

This work illustrates that the use of a single RSLC platform capable of dual-gradient HPLC or UHPLC combined with charged aerosol detection can be used to overcome gradient nebulization issues. To illustrate the power of this approach it's application for the low level quantification of a group of compounds ranging in diverse chemical structure and properties, UV absorbance, HPLC retention, and application in the pharmaceutical industry is presented.

METHODS

UHPLC System: UltiMate® 3000 RSLC system with dual gradient (see Figure 1).
 Columns: Acclaim® RSLC 120 C18 3 µm 120 Å, 3.0 × 33 mm
 Diode Array Detector: UV at 210 nm and 254 nm
 Corona *ultra*™ Detector: Nitrogen 35 psi; filter, high
 Mobile Phase A: 10 mM ammonium acetate, pH = 4.5
 Mobile Phase B: Acetonitrile
 Flow Rate: 1 mL/min from both gradient pumps (2 mL/min to all detectors)

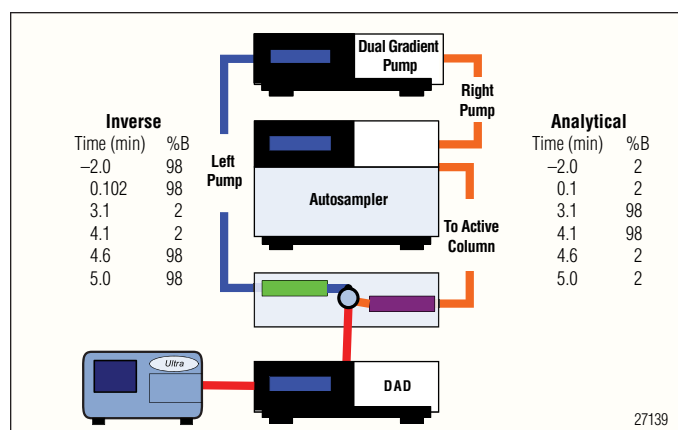


Figure 1. RSLC system flow path with primary column in purple and delay column in green.

METHOD DEVELOPMENT

The UltiMate 3000 dual-gradient pump allows a single system to be used for analytical method development. The implementation of an inverse gradient can be achieved by different approaches: 1) Column/Flow Restriction Approach: The delay times of both the primary gradient system with a column and second gradient system with an in-line filter (for pressure restriction) can be calculated. The delay time of the second gradient can then be used to determine the start of the inverse gradient so that it matches the primary gradient. 2) Two Identical Column Approach: Using two identical columns with similar tubing lengths (Figure 1) may be preferable as this removes the need to calculate the delay volume. Both techniques were found to offer similar results (data

not shown). The work described here was conducted using the second technique with two identical RSLC columns to test the feasibility of this approach for low level quantification.

Two sets of experiments were designed to test the effect of the inverse gradient first on response variability and then on low level quantification:

The first experiment used a group of five test standards which were all common APIs with similar retentive properties. These compounds were prepared at approximately equal mass quantities. These samples were injected individually with and without the inverse gradient (Figure 2).

The second experiment used a group of nine standard materials selected for their range in chemical composition, molecular weight, industrial use, and retention on a C18 column. These standards were then accurately weighed and individually dissolved in either 20% or 80% acetonitrile solutions (depending on solubility) at ~2 mg/mL. Aliquots of these solutions were then combined to give a mixture where each compound had a concentration of ~0.23 mg/mL. Five subsequent dilutions were then made creating six standard solutions from 7 to 230 µg/mL. The effect of the inverse gradient on nebulizer efficiency was measured by the comparison of multiple injections of the standard at 70 ng on column with and without the inverse gradient. The inverse gradient experiment was then used to analyze the standard mix at the six concentration levels.

RESULTS AND DISCUSSION

Improved Response Consistency with Inverse Gradient

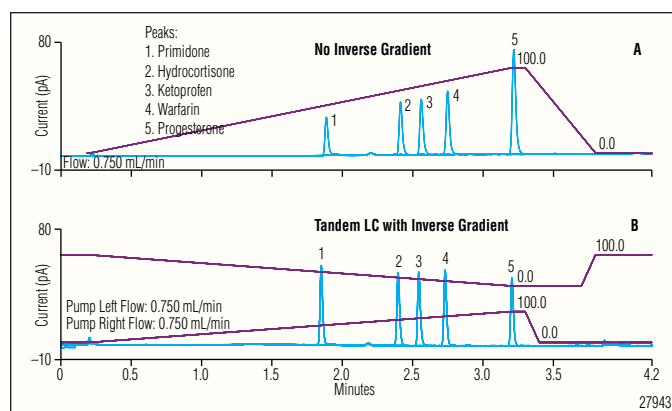


Figure 2. Data illustrating the effect of no inverse gradient vs. with inverse gradient on CAD response for five test compounds (gradients shown are slightly different from the final developed methods described in Figure 1).

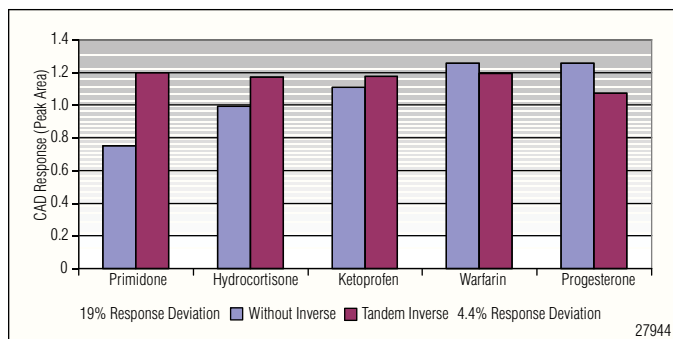


Figure 3. Comparison of the effect of inverse gradient on CAD response (peak area) deviation for five test compounds.

An initial study using five test compounds evaluated the gradient effects on response with and without using an inverse gradient (Figure 2). As expected, compound response improved significantly with the inverse gradient. Even though additional flow was going into the detector, no change in sensitivity for the test compounds was observed since the CAD is a mass sensitive detector and additional solvent does not influence response. Figure 3 illustrates that the CAD response deviation was reduced from 19% RSD to 4.4% RSD by employing 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 as the level of organic solvent going into the CAD was kept at a constant level during the inverse gradient.

Improved Quantification with Inverse Gradient

Additional experiments using nine different compounds were conducted to see if the charged aerosol detector, when operated with a postcolumn inverse gradient, could provide a sufficiently uniform response so that a single compound could be used as a calibrant. The method showed excellent resolution and reproducibility (Figure 4).

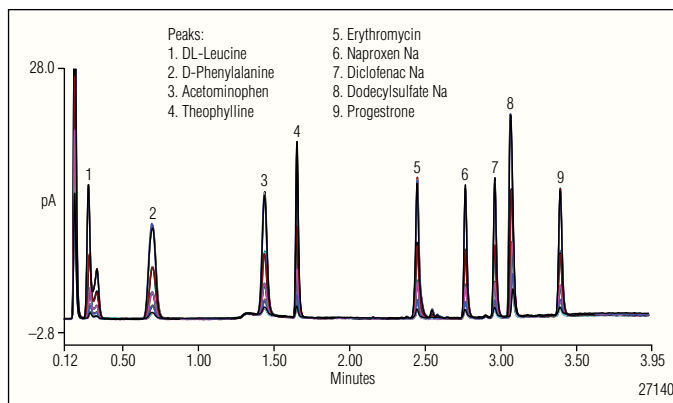


Figure 4. Overlay of five injections of standard mix at each of five concentration levels from 11 to 170 ng on column using Corona ultra detection with inverse-gradient (25 total injections).

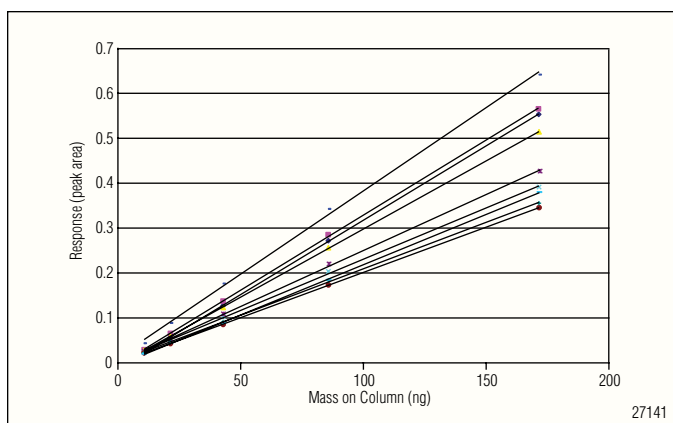


Figure 5. Response curves for data presented in Figure 4. Curve number correlates with the peak number (see Table 1). Identification from top to bottom: 8, 2, 1, 3, 5, 4, 9, 7, and 6.

Table 1A. % Recovery of ~20 ng on Column Each, Curves 1–4

Compound	Peak #	Curve 1	Curve 2	Curve 3	Curve 4
DL-Leucine	1	100%	88%	97%	112%
Phenylalanine	2	111%	99%	109%	128%
Acetaminophen	3	102%	89%	99%	115%
Theophylline	4	89%	77%	85%	96%
Erythromycin	5	93%	81%	89%	102%
Naproxen Na	6	79%	67%	74%	82%
Diclofenac Na	7	85%	73%	81%	90%
Dodecylsulfate Na	8	144%	131%	145%	176%
Progesterone	9	81%	69%	76%	84%

The response curves for each of the nine components are shown in Figure 5. The correlation coefficients for all nine linear fit curves were ≥ 0.999 . Each curve was used to not only back-calculate the recovery of the standard at 20 ng on column but also to calculate the recovery for the other eight components. The results are shown in Table 1 and color coded according to the deviation from the expected value of 100%. Sixty-six percent of the results had recoveries within 25% of the expected values and 87% were within 50%. The area result for sodium dodecylsulfate (peak 8) was higher than the rest of the values by ~50%. This peak was also observed in the solvent blank and indicates a potential carryover issue. When the results for sodium dodecylsulfate values were removed the recoveries improved significantly.

The data collected at two common UV wavelengths (210 nm and 254 nm) are presented in Figure 6. No response was detected at either wavelength for components 1, 5, and 8 due to the lack of suitable chromophores. Those area results were assigned a value of zero and the deviation in area calculations for the nine components was 101 and 125% for the UV at 210 and 254 nm, respectively. If only the compounds with UV chromophores are considered the CAD results still show twice as much consistency as the UV at 210 nm and three times as much as 254 nm.

Table 1B. Recovery of ~20 ng on Column Each, Curves 5–9

Compound	Peak #	Curve 5	Curve 6	Curve 7	Curve 8	Curve 9
DL-Leucine	1	106%	133%	124%	57%	131%
Phenylalanine	2	121%	152%	142%	67%	148%
Acetaminophen	3	108%	136%	127%	59%	134%
Theophylline	4	91%	115%	106%	47%	115%
Erythromycin	5	97%	122%	113%	51%	121%
Naproxen Na	6	79%	99%	91%	39%	100%
Diclofenac Na	7	86%	109%	100%	44%	109%
Dodecylsulfate Na	8	164%	205%	194%	96%	196%
Progesterone	9	81%	102%	94%	40%	103%

Table 1. Recovery calculated for each of the nine compounds using the nine different response curves. Results are colored according to deviation from expected value as follows: black <2%, purple <10%, blue <25%, green <50%, red >50%.

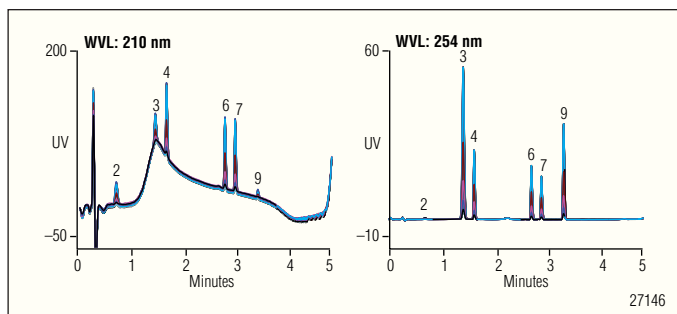


Figure 6. Data presented in Figure 2 for the UV at 210 nm (left) and the UV at 254 nm (right).

The UltiMate 3000-CAD HPLC system offers a new approach for the measurement of the active ingredients, potential degradants, by-products, and residual chemicals. Traditional approaches require several analytical techniques and often do not provide specific or quantifiable results. Consequently, long periods of time may be required for method development and validation. The approach discussed in this work uses a single HPLC platform and provides methods for quantification of known and unknown, nonvolatile residual materials overcoming many of the limitations found with common approaches. The use of the Corona *ultra*, with the inverse gradient, was shown to have very low response deviation across the mixture of nine compounds. When compared to the UV at either 210 or 254 nm with (101 and 125% RSD, respectively) the Corona CAD (23% RSD) offered a far superior approach. The estimation of unknown compounds by using response curves obtained from known compounds illustrates the power of this technique. By using one generic response curve of a non-volatile compound at known concentration (mass on column) the relative concentration of other material can be calculated.

CONCLUSION

The use of charged aerosol detection offers increased sensitivity in a more global mass sensitive approach. The LOD ($S/N > 3$) of the compounds used in this study was estimated between 1 to 5 nanograms on column, while the LOQ ($S/N > 10$) ranged from 6 to 11 ng on column for these test compounds.

The application of the inverse gradient with the UltiMate 3000 system overcomes nebulization efficiency issues and allows for quantification of nonvolatile components at trace levels without the need for compound specific standards. The data using CAD clearly illustrates that improved quantitation can be achieved with inverse gradient over UV detection. A low response deviation of only 23% RSD can be obtained using CAD with the inverse gradient to control changes related to nebulization efficiency. The UV detector for the same mixture of nine compounds showed that the deviation in response was $> 100\%$ RSD.

The combination of the dual gradient HPLC and Corona *ultra* technologies presents the opportunity for a manufacturer to implement significant cost savings over their current methods.

REFERENCES

1. Dependence of Response on Chemical Structure, ESA—A Dionex Company, Application Brief 70-8913 Rev B.
2. Matthews et al., Improving Quantitative Measurements for the ELSD, *Chromatographia* **2004**, *60*, 625–633;
3. Lane et al., Toward Single Calibrant Quantification in HPLC. *Anal. Chem.*, **2005**, *77*, 4354–4365.
4. T. Górecki, F. Lynen, R. Scucs, P. Sandra, Universal Response in Liquid Chromatography Using Charged Aerosol Detection, *Anal. Chem.*, **2006**, *78*, 3186–3192.

Acclaim, CAD, Corona, and UltiMate are registered trademarks and *ultra* is a trademark of Dionex Corporation.

Passion. Power. Productivity.



Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America

U.S. / Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com



LPN 2666-01 11/10
©2010 Dionex Corporation