Simple, Sensitive, and Semiquantitative Analytical Approach for Cleaning Validation Studies

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Abstract

Cleaning validation is a critical consideration in the pharmaceutical industry. Inadequate cleaning can result in the contamination of drug products with active pharmaceuticals from previous batch runs and cleaning solution residues. Such contaminants must be reduced to safe levels, both for regulatory approval and to ensure patients' safety. Residuals and contaminants are comprised of many chemical substances whose nature is often unspecified and highly variable (e.g., impurities, detergents). This poses a significant challenge for analytical methods to achieve the required quantitative accuracy and sensitivity. This study evaluated the use of charged aerosol detection with both ultrahigh-performance liquid chromatography (UHPLC) and flow injection analysis (FIA) as complementary approaches for the measurement of low levels of residuals from a variety of sources. Quantitative values for a disparate group of compounds determined using the response data of a single substance (i.e., single calibrant) demonstrated that the Thermo Scientific Dionex Corona™ Charged Aerosol Detector (CAD™) provided results at the 20 ng on column (o.c.) level which were ± 25% of target. Results obtained by FIA were in agreement with those obtained by HPLC. These data illustrate that the CAD detector can provide sensitive detection of residual substances with more universal and uniform response than low-wavelength UV. This enables the use of a single calibrant for quantitation of residuals and contaminants from various sources. The CAD detector may be used with FIA for high-throughput, nonspecific analysis, while UHPLC-charged aerosol detection can be used to provide higher specificity where needed.

Introduction

The U.S. FDA requires that a cleaning process be documented for all critical equipment used in the development of pharmaceutical products. This process involves defining objectives for the cleaning procedures, determining effectiveness of cleaning techniques, defining sampling techniques, qualifying analytical equipment, establishing acceptable limits, and testing controls.[1]

One analytical technique currently in use as a cleaning method uses a total organic carbon (TOC) analyzer. While this is a powerful and sensitive tool, it lacks specificity. A response can result from residual active ingredients or from a trace amount of surfactant or other cleaning agents. The FDA tolerance for a residual active ingredient such as penicillin will be far less than for a residual of a nonactive excipient or a surfactant from the cleaning process.[2] With nonspecific analytical techniques, any residual measured must be assumed to be the active ingredient and, therefore, be held to the lowest tolerance level.

The second common technique is HPLC with low-wavelength UV detection, offering more specificity than TOC for active ingredients. However, the shortfall of this technique is that many of the ingredients in cleaning products contain very weak chromophores. This leads to little or no sensitivity for this group of potential contaminants.

Another difficulty often encountered using a specific technique like HPLC-UV is the quantification of unknown peaks. The need for fast turnaround time of the cleaned equipment to maintain a production schedule does not allow for identification of every peak present. Therefore, quantitation by UV detection is often based solely on peak area. Because the UV response of an aromatic active ingredient would be different than a nonaromatic surfactant such as dodecylsulfate, this presents a potential source of error.

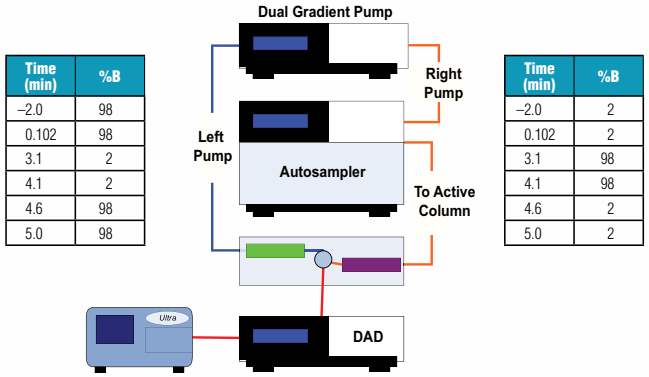
The CAD detector is mass sensitive and can be added to the traditional HPLC-UV platform. This detector provides the most consistent response, across nonvolatile and some semivolatile analytes, of all HPLC detection techniques.[3]

With all aerosol-based detectors, nebulization efficiency is often increased as the organic solvent proportion increases. When running gradients from high aqueous to high organic, charged aerosol detection response increases. The delivery of a second postcolumn solvent stream that 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.

Method

UHPLC System:	Thermo Scientific Dionex UltiMate™ 3000 RSLC rapid separation LC system with Dual-Gradient Pump (see Figure 1)
Columns:	Thermo Scientific Acclaim™ RSLC 120 C18, 3 µm, 120 Å, 3.0 × 33 mm
Detectors:	Diode Array Detector at 210 nm and 254 nm Thermo Scientific Dionex Corona *ultra*™ detector, nitrogen: 35 psi; filter: high
Mobile Phase:	A) 10 mM Ammonium acetate, pH = 4.5 B) Acetonitrile
Flow Rate:	1 mL/min from both gradient pumps (2 mL/min to all detectors)
Gradients:	See tables in Figure 1

FIGURE 1. RSLC system flow path with analytical column in purple and delay column in green.



Time (min)	%B
–2.0	98
0.102	98
3.1	2
4.1	2
4.6	98
5.0	98

Time (min)	%B
–2.0	2
0.102	2
3.1	98
4.1	98
4.6	2
5.0	2

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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. The delay times of both the primary gradient system with column and second gradient system with an inline filter for pressure restriction were calculated. This delay time is then adjusted for the start of the inverse gradient so that it matches the primary gradient.
2. Using two identical columns with similar tubing lengths on both pumps (Figure 1) removes the need to calculate the delay volume.

Both techniques produced similar results (data not shown). The work in this study was conducted using the second technique with two identical Acclaim RSLC columns.

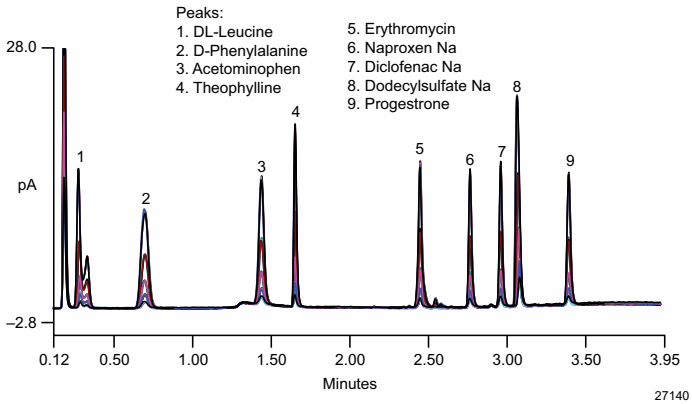
A group of nine standard materials was selected ranging in chemical composition, molecular weight, industrial use, and retention on a C18 column. These standards were then accurately weighed and individually dissolved in either 20% or 80% acetonitrile solutions (depending on solubility) at ~2 mg/mL. Aliquots of these solutions were then combined to provide 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 170 ng o.c. with and without the inverse gradient. The inverse gradient was then used to analyze the standard mix at the six concentration levels.

A second experiment of FIA investigated the replacement of the primary column with an inline filter. The six concentration levels of the standard mix were then analyzed again running 1 mL/min isocratically at 50% mobile phase B.

Results and Discussion

Improved Quantification with Inverse Gradient

FIGURE 2. Overlay of five injections of standard mix at each of the five concentration levels from 11 to 170 ng o.c. using Corona *ultra* detection with inverse gradient.



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The variation in peak areas among the nine components in Standard 1 (170 ng o.c. each) was found to be 23% relative standard deviation (RSD) using the inverse gradient approach and 76% RSD without the inverse gradient. The method showed good reproducibility for these individual components as shown by the overlaid chromatograms in Figure 2. The variation in peak area for the individual components in the standard at 170 ng o.c. was < 2% RSD. The limits of quantification (LOQ) and detection (LOD) were similar for all nine components. The LOQ, defined as signal-to-noise (S/N) > 10, showed values ranging from 6 to 11 ng o.c., while the LOD, defined as S/N > 3, showed values estimated between 1 and 5 ng o.c.

FIGURE 3. Response curves for data presented in Figure 2. 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.

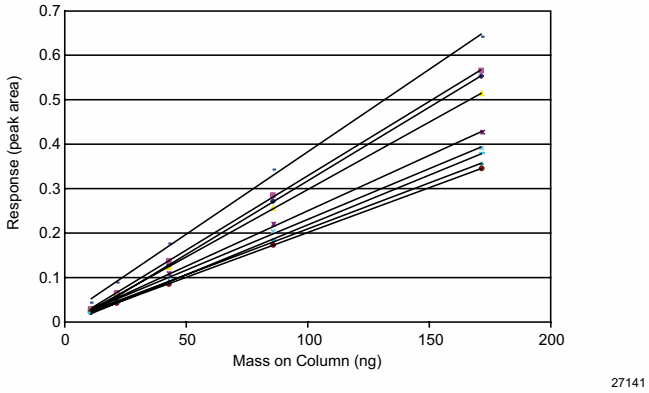


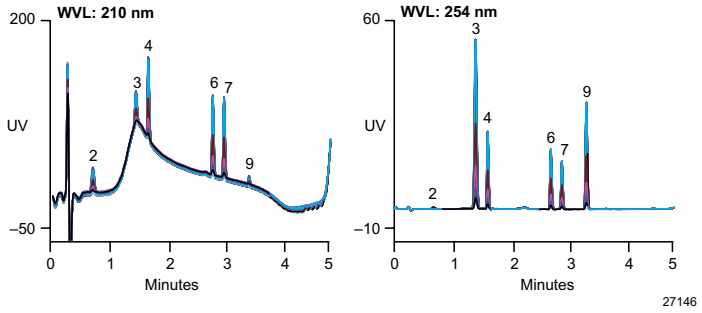
TABLE 1. Recovery calculated for each of the nine compounds using the nine different response curves. Results are colored according to deviation from expected value as follows: black < 2%, purple < 10%, blue < 25%, green < 50%, red > 50%.

Table 1A. % Recovery of ~20 ng O.C. 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%
Acetominophen	3	102%	89%	99%	115%
Theophylline	4	89%	77%	85%	96%
Eryhromycin	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%
Progestrone	9	81%	69%	76%	84%

Table 1B. Recovery of ~20 ng O.C. 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%
Acetominophen	3	108%	136%	127%	59%	134%
Theophylline	4	91%	115%	106%	47%	115%
Eryhromycin	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%
Progestrone	9	81%	102%	94%	40%	103%

The response curves for each of the nine components are shown in Figure 3. The correlation coefficients for all nine linear fit curves were ≥ 0.999. Each curve was used to calculate the recovery of the standard at 20 ng o.c. and 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%. 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. Sixty-six percent of the results showed recoveries within 25% of the expected values and 87% were within 50%. When the results for sodium dodecylsulfate values were removed, the recoveries improved significantly.

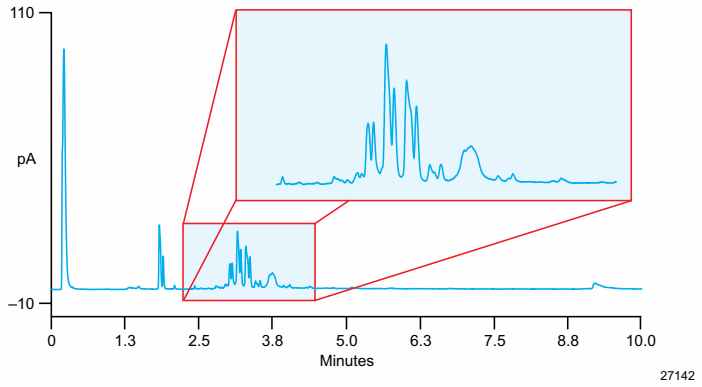
FIGURE 4. Data collected at two common UV wavelengths (210 nm and 254 nm).



The data collected at two common UV wavelengths (210 nm and 254 nm) are presented in Figure 4. 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.

Cleaning Agent

FIGURE 5. Injection of Liqui-Nox® cleaning solution (50 µL) at 0.01% of the concentrated solution (1% of recommended concentration for use). At least 26 analytes were resolved using this approach.



Cleaning agents, such as Liqui-Nox, contain an array of ionic and nonionic surfactants as well as acids, such as citric acid and inorganic ions. The analysis of this material using charged aerosol detection resulted in 26 identifiable peaks with the citric acid and ionic material present in peak 1 at the column void (Figure 5). The UV at 210 nm (data not shown) had far fewer identifiable peaks and the column void peak was negative.

Flow Injection Analysis

A nonspecific FIA approach was also evaluated with charged aerosol detection. This approach provides less detailed information than traditional HPLC-charged aerosol detection analysis, but can be used for high-speed screening analysis. When compared to HPLC data, it can also be used as a confirmation that all material has eluted from the HPLC column. Standard solutions at the six different concentrations levels and the solvent blank were analyzed using FIA (Figure 6, each point in triplicate). This nonspecific approach easily distinguished each of the six concentrations and the solvent blank. The total area response from the chromatographic data (Figure 2) and the response using FIA (Figure 6) correlate well to the total mass o.c. (Figure 7). The FIA results follow a second-order polynomial fit as shown in Figure 7 due to the detection properties of the CAD detector.

FIGURE 6. Overlay of 21 FIAs using the CAD detector. Results represent the six concentrations of the mixed standard and a solvent blank, each injected in triplicate.

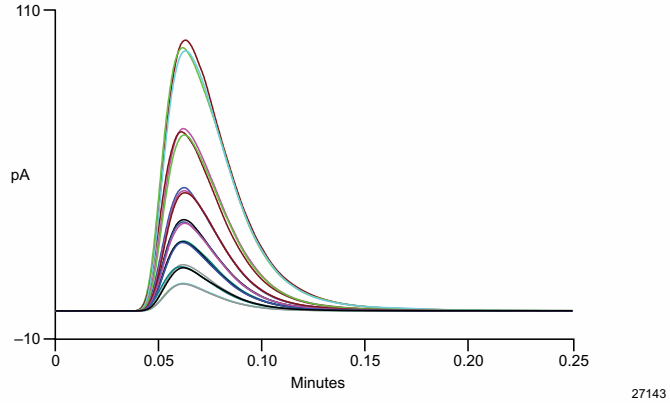
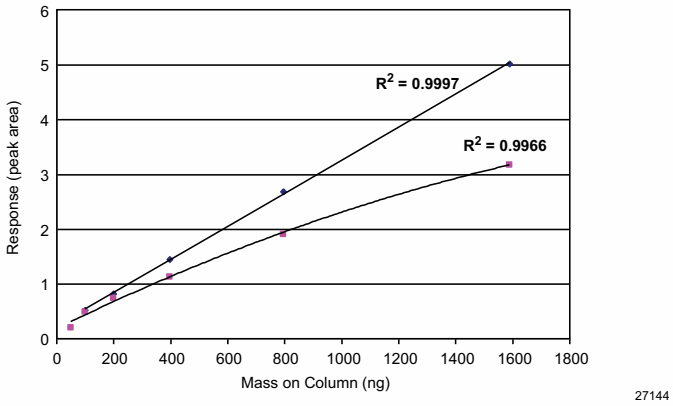
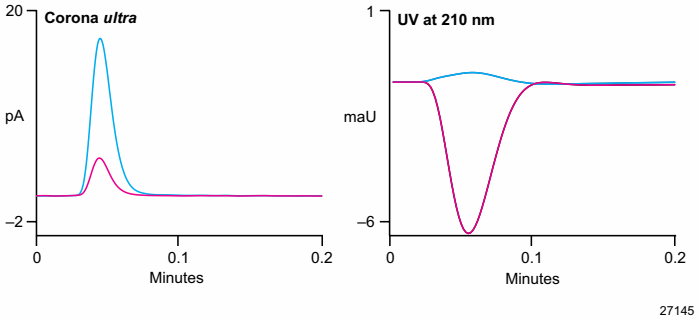


FIGURE 7. HPLC-charged-aerosol-detection response curves for total area vs total mass o.c. for both the chromatographic results (blue points) from Figure 3 (fit with linear regression) and the flow injection results (pink points) from Figure 5 fit with a second-order polynomial fit.



The approach using FIA offers a mass-dependent response with very good reproducibility and high correlation coefficients. The UV detector shows only a very small positive increase for the Liqui-Nox sample (Figure 8). However, a large negative area in the blank makes accurate quantification very difficult. Similar evaluation of this cleaning product at low concentration using the Corona CAD detector illustrates a higher response than UV at 210 nm. This area could then be converted into a mass-on-column using a simple response curve of any nonvolatile standard material.

FIGURE 8. Overlay of flow injection results for 1 µL injection of Liqui-Nox at 0.01% of the concentrated solution (blue trace) and solvent blank (magenta trace) for both the Corona *ultra* detector (left) and the UV detector at 210 nm (right).



The UltiMate 3000 HPLC-charged aerosol detection system offers a new approach for the evaluation of cleaning methods for the measurement of active ingredients, potential degradants and byproducts, as well as residual chemicals for the cleaning process. Traditional approaches require several analytical techniques and possibly 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* detector with the inverse gradient was shown to have a very low response deviation across the mixture of nine compounds. When compared to the UV at either 210 nm or 254 nm (with 101 and 125% RSD, respectively), the Corona CAD detector (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 nonvolatile compound at known concentration (µg/mL), the relative concentration of the other material can be calculated.

Comparing curves for HPLC vs FIA (seen in Figure 7) can confirm that all material present in the sample has been accounted for. Both curves correlated well to the total mass o.c. This can be extremely useful when a) large nonionic surfactants, such as Tween® 80 are present and may not fully elute from the column, and b) a screening approach is desired to determine if the total mass present requires a full HPLC run.

Evaluation procedures, as described here, can speed up cleaning validations by helping the analyst use only the analytical methods required, thus saving both time and money. The ability to measure all the major ingredients in cleaning products—e.g., citric acid and surfactants—along with full compatibility with traditional HPLC and UHPLC approaches generates faster and more accurate results.

Conclusion

- The use of the CAD detector for cleaning validation methods offers increased sensitivity in a more global mass-sensitive approach.
- The application of an inverse gradient with the UltiMate 3000 system overcomes nebulization efficiency issues and provides quantification of nonvolatile components at trace levels without requiring compound specific standards.
- The combination of the dual-gradient HPLC and Corona *ultra* technologies presents the opportunity for manufacturers to implement significant cost savings over their current methods.

References

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