Novel Analytical Methods to Verify Effectiveness of Cleaning Processes

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Overview

Purpose: A complete HPLC/UHPLC system equipped with charged aerosol detection and a unique tri-mode column was evaluated for its application to the pharmaceutical cleaning process. This work focuses on the figures of merit for the analytical method and not on performance of the sampling process, as this is manufacturer dependent.

Methods: Standards, along with cleaning solutions from a pharmaceutical company, were analyzed to determine limits of detection and quantification. Two analytical approaches were evaluated: chromatographic separation and flow injection analysis (FIA).

Results: The system was sensitive with limits in the sub ppm range for some analytes and low ppm range for all other non-volatile compounds. The flow injection analysis results showed good correlation with limits detection typically in the low ppm range. The sensitivity for the components in the cleaning solution was several orders of magnitude better by charged aerosol detection than low-wavelength UV detection.

Introduction

Cleaning processes are essential in order to minimize human exposure to potentially toxic compounds and to insure batch-to-batch reproducibility of products. The FDA guidance on cleaning validation takes into account the diverse nature of the pharmaceutical industry and the impossibility to mandate one specific method or specification. The burden of proof is therefore with individual firms to ensure that any residual materials are detected and are below the acceptable dosage level in the final product. This process is not static as the FDA continuously challenges manufacturers to improve their analytical methodologies to meet these requirements. The major challenge for manufacturers is that the chemical structures of residuals and contaminants are highly diverse and their chemical nature is often unspecified and highly variable (e.g., impurities, detergents). Consequently, the need to achieve the required quantitative accuracy and sensitivity to ensure product safety is a major challenge for any analytical method.

When deciding on a cleaning verification method one of the major decisions is whether to use a non-selective technique such as total organic carbon (TOC) or to use a more selective technique such HPLC-UV or MS. This decision can be complicated depending on what the potential risks are in the process and the needed turnaround time. HPLC with low-wavelength UV detection is a well-defined technique offering more specificity than TOC for active ingredients. However, the shortfall of this technique is that many of the ingredients in cleaning products, along with associated excipients, 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 production schedule does not allow for identification of every peak present. Therefore, quantitation by UV detection is often based solely on peak area. Since 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. Presented here is a novel approach using charged aerosol detection with solvent compensation that overcomes all the problems mentioned above. Its application to the measurement of active ingredients, detergents, and some acid residues is described.

Charged aerosol detection is a highly sensitive universal technique that can deliver near uniform response for non-volatile analytes.[1] One issue found with all nebulization-based techniques is that detector response will change during gradient elution due to changes in nebulization efficiency. This can easily be overcome by applying solvent compensation or an inverse gradient after the analytical column but before the detector.[2,3,4] The combination of the Thermo Scientific Dionex Corona *ultra* Charged Aerosol Detector (CAD™) with the Thermo Scientific Dionex UltiMate 3000 RSLC system equipped with a dual gradient pump (DGP) forms a single analytical platform capable of delivering highly sensitive and near uniform response data for the majority of the compounds encountered in the cleaning process. Using a simple six-port two-position switching valve the system can be adapted to conduct both fast non-specific screening of samples (FIA) along with specific full chromatography for high sensitivity measurement. The Corona™ *ultra*™ Charged Aerosol Detector with its low detection limit and uniformity of response is ideal for the semi-quantitative analysis of unknown residues found in the cleaning process.

Methods

Sample Preparation

All dilutions were made in 18 MΩ-cm deionized water in polypropylene containers. 600 µL polypropylene vials were used for all samples and standards in order to reduce interference from sodium borosilicate leaching from glass vials.

All standards (citric acid, PEG 400, diclofenac sodium, sodium dodecylsulfate, naproxen sodium, sodium acetate, acetaminophen, polysorbate 80, ammonium chloride and phosphoric acid) were dissolved in DI water at ~4 mg/mL. The samples were then diluted 1/10 to form the working solution for each standard. That working solution was serially diluted to ~ 200 ppb to 200 ppm for calibration studies.

Stock solutions of 5 cleaning products used by a pharmaceutical company were also analyzed. The stock solutions were at an unknown concentration. The CIP 100, Adept®, Citrisurf® and Sentol were each diluted 1/10 followed by serial dilution until they were in a workable dilution for the detector. Two solid samples of cleaning products (Monarch® Sepko ®, Sprex) were dissolved at ~2 mg/mL in deionized water. These were diluted and analyzed from 200 ppb to 200 ppm.

Liquid Chromatography

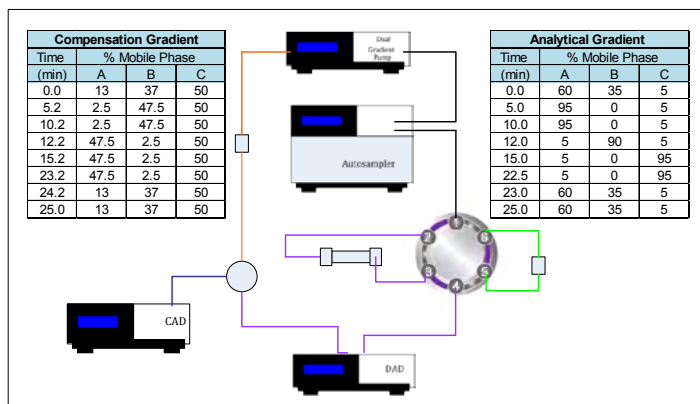|	
System:	UltiMate™ 3000 x2 Dual RSLC
	DGP-3600RS, WPS-3000TRS, TCC-3000RS,
	DAD-3000RS, Corona *ultra*
Column:	Thermo Scientific Acclaim Trinity P1 3 µm 3.0× 50 mm
Mobile Phases Analytical:	A) acetonitrile; B) deionized water ;
	C) 200 mM ammonium acetate pH 4.5
Mobile Phases Compensation:	A) acetonitrile B) deionized water C) 20% methanol
Flow Rate Right Pump:	0.5 mL/min
Flow Rate Left Pump:	1.0 mL/min
Gradients:	See Figure 1
Injection Volume:	50 µL
Corona *ultra* Settings:	Gas 35 psi
	Nebulizer Temp: 25 ˚C
	Filter: High
FIA Conditions	
Flow Rate Right Pump:	1.0 mL/min (20%A, 78%B, 2%C)
Flow Rate Left Pump:	0.5 mL min (100 %C)
Injection Volume:	50 µL
Run Time:	0.65 min

FIGURE 1. Schematic of the HPLC UltiMate 3000 with charged aerosol detection platform with gradient compensation and screening FIA approach. The gradient tables for the analytical pump and compensation gradient are also shown.

Compensation Gradient			
Time	% Mobile Phase		
(min)	A	B	C
0.0	13	37	50
5.2	2.5	47.5	50
10.2	2.5	47.5	50
12.2	47.5	2.5	50
15.2	47.5	2.5	50
23.2	47.5	2.5	50
24.2	13	37	50
25.0	13	37	50

Analytical Gradient			
Time	% Mobile Phase		
(min)	A	B	C
0.0	60	35	5
5.0	95	0	5
10.0	95	0	5
12.0	5	90	5
15.0	5	0	95
22.5	5	0	95
23.0	60	35	5
25.0	60	35	5

Data Analysis

Thermo Scientific Dionex Chromeleon 6.8 SR 11 Chromatography Data System (CDS) was used for all data collection and processing. All standards analyzed were subjected to baseline subtraction of a solvent blank.

Results

Analysis of Standards

The standards were analyzed individually at multiple levels. Figure 2 is an overlay of the 9 individual standards at low ppm concentrations for both the CAD and UV detector at 210 nm. The two APIs, naproxen and diclofenac sodium, peaks 3 and 6 respectively, have similar sensitivity by both techniques. The remaining 7 peaks, primary ionic materials, are not visible in the UV but can be analyzed at a high sensitivity by the charged aerosol detection. Table 1 lists the peak identity, retention time, and both limits of quantification (LOQ) and detection (LOD) for all components analyzed. Data for Tween® 80 were not included in the analysis as its major component failed to elute under these chromatographic conditions. The versatility of this technique permits other chemistries to be chosen if materials of interest are non-polar leading to shorter analysis times.

The LOQ was measured at > 10/1 signal-to-noise. Components 2-6 on the CAD had LOQ values ≤ 0.5 ppm. The analytes listed as ND by UV where those analytes not detected even at the highest level analyzed (~200 ppm). This sensitive and selective technique can deliver accurate low level results for both the inorganic and organic components simultaneously.

FIGURE 2. Overlay of 9 standards at ~3 ppm for peaks 1-6 and ~15 ppm for peaks 7-8 (top) charged aerosol detection with blank subtraction (bottom) UV at 210 nm. Peak identity in Table 1.
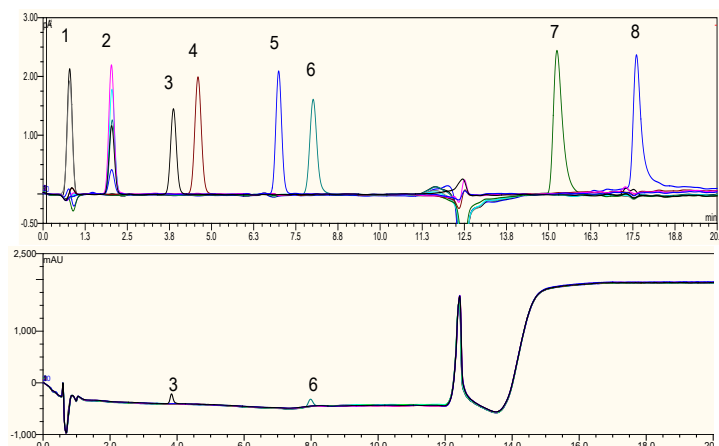

Table 1. Peak identity, retention time and limits of quantification and detection by both charged aerosol detection and UV at 210 nm.

	Peak Number	Retention Time	CAD		UV @ 210	
			LOQ	**LOD**	**LOQ**	**LOD**
Acetaminophen	1	0.6 (Void)	2.20	0.80	50.00	20.00
PEG 400	1	0.6 (Void)	1.00	0.40	ND	
Sodium	2	2.03	0.20	0.05	ND	
Naproxen	3	3.87	0.30	0.15	0.35	0.20
Chloride	4	4.60	0.15	0.05	ND	
Dodecylsulfate	5	7.00	0.10	0.05	ND	
Diclofenac	6	8.01	0.50	0.20	5.00	2.00
Phosphoric Acid	7	15.25	1.20	0.50	ND	
Citric Acid	8	17.61	3.00	1.00	ND	
Polysorbate 80	Did not elute					

Limits of Quantification and Detection (μg/ml) — table header spanning above.

Quantification

The CAD is a universal detector that provides near uniform response for all non-volatile analytes. The method addresses the change in response resulting from gradient conditions by introducing a compensation solvent flow post column. The limits of quantification for all 9 components were reasonably close for such a highly diverse group of analytes, being within one order of magnitude. If we now focus on analytes 2-6 the consistency of detection is now much more prominent. This provides a much greater degree of confidence if a simple area count calculation of an unknown is being used. The use of a single calibrant response curve for calculations of unknowns in cleaning samples was discussed in length in the previous work.[4] This technique was shown to consistently offer accurate semi-quantitative results and provided confirmation to UV area results removing the large unknown when using UV technique alone.

Cleaning Products

The HPLC-charged aerosol analysis of several of the cleaning products that are used by a major pharmaceutical company are presented in Figure 3. While several peaks did correlate to some of standards analyzed, the identity of the components was not confirmed for this work. The top three chromatograms are at ~ 1/5000 of the original concentration and major peaks are still observed in all. The Sep-KO® sample was analyzed at ~200 ppm and the Citrisurf sample is at 1/10 of the original concentration. The CIP, Adept, and Sep-KO are basic and contain large quantities of either sodium or potassium. The Sentol and Citrusurf are acidic and the Citrusurf's major component is citric acid. Other unidentified components at lower levels were observed in all samples. The major components in each of these samples were detected at levels three orders of magnitude below those shown in Figure 3. The UV at 210 nm data collected for these samples did not yield the same level of data quality. The same high concentration points with several quantifiable peaks by charged aerosol detection had no integratable peaks in the UV at 210 nm (Figure 4). The only peak observed in any of the five samples was a large, not quantifiable spike in the void of the Citrusurf sample. The additional of large quantities of volatile buffer at the end of the analysis results in a significant baseline disturbance by in the UV with only slight impact in the CAD.

FIGURE 3. Analysis of five cleaning solutions used in the pharmaceutical industry by charged aerosol detection.
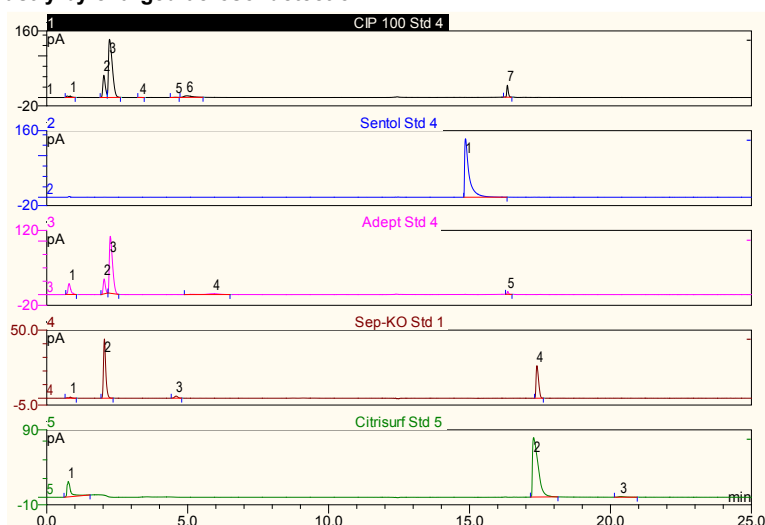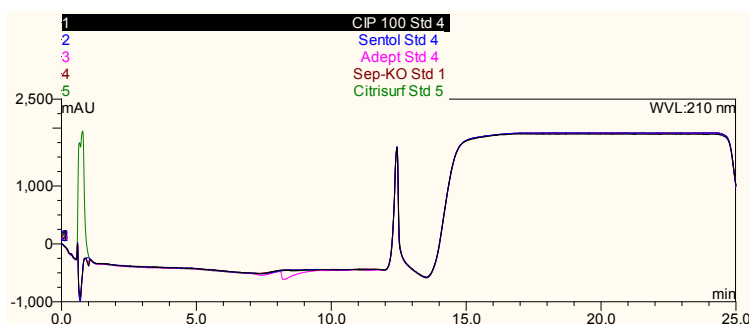

FIGURE 4. Overlay of the analysis of the same five samples as Figure 3 using UV at 210 nm for detection.


The FIA Rapid Screening Approach

One advantage of a mass sensitive detector is that the injection of volatile solvent has minimal impact on detector response. This is in contrast to UV where solvent may present as either a large positive or negative spike in the injection void. This non-response of the charged aerosol detector offers the analyst the potential to make semi-quantitative decisions based on injections of samples without a column. This non-chromatographic FIA approach is the second configuration of the UltiMate 3000 system presented in Figure 1(green flow path). While FIA will give no selective information as to the composition of a sample, it does have the ability to give an approximate amount of material. Because the detector has a near universal response, a single calibrant could be used to set a lower response threshold. Anything with a response greater than that low concentration can be flagged for full HPLC analysis according to the chromatographic method discussed above.

The FIA technique and the analysis of sodium dodecylsulfate at 7 concentration points overlaid with a blank is shown in Figure 5. The response curve covered 0.42 to 146 ppm with 50 µL injection volume at each concentration. The correlation coefficient was 0.9993 for the curve fit with a 2nd order polynomial function. The UV results for both 210 and 254 nm over the same concentration range yielded no correlation. The same analysis was repeated for all of the analytes analyzed previously and the ~ lowest threshold for this screening approach was < 10 ppm. Depending on the specification needed in the cleaning process this may be sufficient to screen all samples first and only analyze those at or above the bottom threshold. This would represent a significant savings in time and cost.

FIGURE 5. Flow injection analysis results for sodium dodecylsulfate from 0.42 to 146 ppm and solvent blank injection. Inlet shows response curve for 7 points.
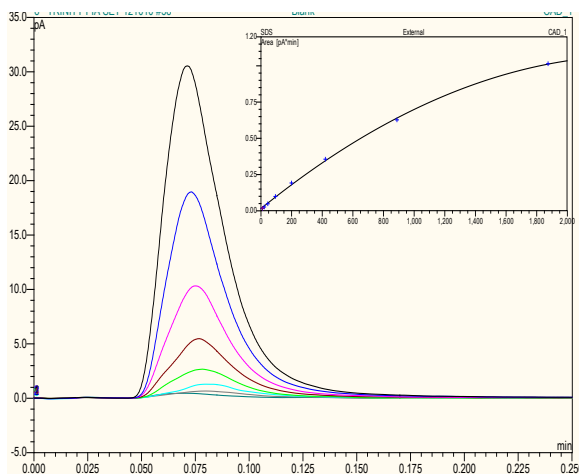
Conclusion

The use of the UltiMate 3000 with Corona *ultra* Charged Aerosol Detector platform for verification of cleaning process can provide significant benefits over current techniques including:

- Sensitivity for all non-volatile organic and in-organic analytes in a single analysis.
- Uniform response leading to semi-quantitative results without individual standards.
- Flexibility to run both selective analysis and fast screening in a single platform.

References

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