Validating Analytical Methods with Charged Aerosol Detection

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Abstract

This work evaluates data reported in several peer-reviewed publications as well as detailing the validation of an HPLC-UV-CAD method developed for the simultaneous analysis of an active pharmaceutical ingredient (API) and its counterion recovered from a final dose tablet.

Naproxen tablets were treated with a dissolution matrix of Tween® 80/formic acid/DI water for 4 h with periodic sampling. The HPLC method used a dual-gradient pumping system with two C18 columns in tandem for sample clean up. A mixed-mode column was then used for the retention and analysis of both the API (naproxen) and the counterion (sodium). The final method took 15 min per injection and allowed for complete flush and reconditioning of the analytical column.

The method was able to resolve the sodium and naproxen in less than 5 min. The quantification of the sodium counterion was measured using Thermo Scientific Dionex Corona™ CAD™ Charged Aerosol Detector, and the naproxen was measured using UV at 254 nm. A bracketed standard with multiple injection sequence was used to improve accuracy and intermediate precision. The observed limits of detection (LOD) for analytes were ~2 ng on column for both detection techniques. The acceptance criteria for accuracy of 100±1% for the API by UV and 100±3% for the counterion by charged aerosol detection were met for the suitability standards. The reproducibility specification for multiple injections of < 3% RSD was met for all samples. The intermediate precision over the seven-day study was also within the acceptance criteria set.

As the need for universal detectors increases in regulated environments, the design of experiments specific to nebulizer-based detector platforms becomes more important. As shown in this work, a more complex analytical system was able to remove matrix effects, significantly decrease run time, and maintain the needed reproducibility. This savings in time enabled the replicate injections needed to create a robust and accurate method. The use of mixed detection platforms should be encouraged if a multicomponent analysis is required, where universal detection is needed for some but not all of the analytes.

Introduction

There are currently over 60 peer-reviewed publications discussing the application of charged aerosol detection in various areas, including pharmaceutical, foods and beverages, and academic research. A closer examination of these papers reveals that at least 13 discuss procedures that were used to qualify or validate analytical methods according to the standard criteria of specificity, linearity, reproducibility, accuracy, and LODs. In all the articles, the authors discuss the success of the Corona CAD Charged Aerosol Detector in meeting the acceptance criteria required. However, not every validation attempt was successful. The goal of the material shown here is to highlight what worked, to correctly set expectations, and to help guide researchers to make their validation protocols successful.

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The first step to any successful validation is the development of a strong analytical method. Before validation is begun, steps must be taken to ensure that there is specificity to the compounds of interest, especially in the presence of matrix. The Corona CAD Charged Aerosol Detector is a universal detector and, as such, is more sensitive to matrix effects, which may not always present a problem for UV detection. The use of both sample preparation and sound analytical method development (e.g., correct mobile phase and column combination) improves resolution of target analytes. These techniques, using the Corona CAD Charged Aerosol Detector in complex mixtures, are discussed in several publications.[1,2,3,4]

Once a strong analytical method is developed and preliminary data have been generated and processed, a validation document with specific criteria can be created. In this step it is very important to take into consideration not only the preliminary results, but also the vendor's specifications for the instrument in use. The expectation set during validation, and which is used in release criteria, should not go beyond vendor recommendations unless significant research has been done to ensure long-term reproducibility of those results.

Like all nebulization-based detectors, the specification for reproducibility for charged aerosol detection is higher than for that of UV. The current specification for charged aerosol detection reproducibility is 2% RSD under factory testing.[5] From this 2% value, an appropriate % RSD range can be selected based on the analytical results. Previous works have shown success when setting the upper limits between 3 and 5% with sample amounts ≥ 100 ng on column.[1,6,7] The reproducibility or precision of the analysis is crucial to the overall quality of the results. It is recommended that several steps be taken to ensure that these values are understood and factored into any validation and release testing to ensure that quality control test results are meaningful and meet their intended goals and specifications.

The precision of results is based on the reproducibility of the method and the slope of the calibration curve used. The Corona CAD Charged Aerosol Detector is a nonlinear detector, which fits to a second order polynomial or log-log function over its four orders of magnitude range. Later work has determined that the truest fit to the curve can be obtained using a second-order polynomial function with the x and y axis inverted (i.e., amount on y and area on x).[8] However, the detector typically shows a linear response from the LOD 1–10 ng up to 250–500 ng on column. The slope of the line is largest in this range, thereby making it the best range to bring the precision results in line with the reproducibility data. The best way to factor the precision into a validation method is to run multiple analyses of each sample with bracketed standards, using the average for all results.

In this work, the method development, optimization, and validation of a system looking at the simultaneous analysis of an API and counterion in a complex dissolution media are discussed.

Methods
Instrument Parameters

Instrument:	Thermo Scientific Dionex UltiMate™ 3000 RSLC with Thermo Scientific Dionex Corona *ultra*™ Charged Aerosol Detector
Components:	DGP-3600RS Dual Ternary RS Pump
	WPS-3000TRS Well Plate Sampler
	TCC-3000RS Column Compartment
	DAD-3000RS Diode Array Detector
	10-port 2-position valve
Column 1 and 2:	Thermo Scientific Acclaim™ RSLC 120 C13.0 × 33 mm
Analytical Column:	Thermo Scientific Acclaim Trinity™ P1, 3 µm, 3.0 × 50 mm
Pump Right Mobile Phase:	A) Acetonitrile
	B) DI Water
	C) 200 mM Ammonium acetate, pH 4.5/methanol (95:5)
Pump Left Mobile Phase:	A) Acetonitrile
	B) Isopropyl alcohol
	C) DI Water
Injection Volume:	10 µL
Diode Array:	UV at 254 nm
Corona *ultra*:	Filter at high; Nebulizer temperature at 25 °C

Standard Preparation

Naproxen sodium (Sigma-Aldrich >98%) was dissolved at ~30 mg/mL in a solvent of methanol/DMSO mixture (1:1). This solution was diluted once in mobile phase A and subsequent dilutions were made in 50/50 CH_3CN/DI. Two curves were prepared for this study: a five-point targeted curve around the expected concentration of ~31 to 54 µg/mL; and a 12-point range curve of ~0.2 to 220 µg/mL (run at a separate point).

Preparation of Dissolution Solution

50 mL volume of Polysorbate 80 (Tween 80) was added to 950 mL DI water with an additional 10 mL of formic acid. The solution was sonicated and degassed prior to use.

Tablet Dissolution and Sample Preparation

One name brand naproxen sodium pain-reliever caplet (capsule shaped tablet) was added to 100 mL of the dissolution solution in a polypropylene mixing vessel. A magnetic stir bar was added and the tablet was allowed to stir for 4 h. A 200 μL sample was removed at 1, 2, and 4 h. Each 200 μL aliquot was added to 1.8 mL mobile phase A.

A 500 μL volume of the diluted sample was then added to a Spin-X® centrifuge tube filter (0.22 μm nylon) and these tubes were centrifuged for 5 min at 10,000 RPM. A 10x dilution was then made in 50/50 acetonitrile/water to provide the analytical samples.

This procedure was followed for a generic brand naproxen sodium tablet shaken on an autovortexer set at low. Two recovery samples were also prepared in the same fashion by adding ~210 and 230 mg of the naproxen sodium standard in the 100 mL of dissolution solution for the low and high recovery values, respectively.

Discussion

The Acclaim Trinity column was chosen based on its unique mixed-mode chemistry, which enables the simultaneous analysis of polar and nonpolar species. The column uses a Nanopolymer Silica Hybrid (NSH) technology that provides multiple retention mechanisms, including reversed-phase, anion-exchange, and cation-exchange. With its ion-exchange functionality, the elution mechanism for polar material is affected by increases in buffer strength, while that for the nonpolar material retained on the reversed phase is affected by increases in solvent strength.

Tween 80 is a large nonionic surfactant that is strongly retained by the C18 reversed-phase functionality and is eluted from the column only with strong organic solvents. In this method, a C18 column was used as a trap column to prevent the Tween 80 from reaching the Acclaim Trinity column and the Corona CAD Charged Aerosol Detector. The system schematic in Figure 1 utilized two small C18 columns in tandem, allowing the Acclaim Trinity column and the active trap C18 column to be properly flushed and re-equilibrated between each injection. The C18 columns were switched in and out of the analytical stream using the 10-port, two-position valve and alternating injection methods to decrease the time between injections. This optimization is important as it accelerates the method from a duration of one hour (to elute the Tween 80 without affecting the buffer solubility and recondition the column) to only 15 min. It also prevents the overloading of both the column and the detector.

FIGURE 1. Schematic of tandem SPE system.
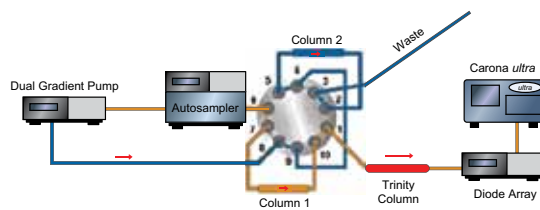
The 10-tablet samples along with the high and low standard recovery samples comprised the 12 samples for analysis. The first injection group was analyzed in triplicate, with an additional block of standards at the end of the sequence. Prerun conditioning was accomplished by having three zero-volume runs, followed by a blank injection of DI water. Consequently, the total number of injections made per day was 76. The 19 h run time for the sequence using the SPE tandem system was shorter than the 24 h period required by a single-sample injection sequence bracketed by standards.

As described in the methods section, a five-point standard curve targeting the expected concentration was evaluated. The additional dilution step was added to the method to reduce the amount of matrix being injected, and to bring the amount of sodium within the linear range of the Corona CAD Charged Aerosol Detector (Figure 2A). The correlation coefficients for that targeted linear range, as well as the wider polynomial range, were all equal to 0.999 (Figure 2B). Naproxen has a strong UV chromophore at 254 nm. Therefore, the UV calibration curve was used for all calculations for naproxen.

FIGURE 2. A) Linear response curve for sodium with the Corona CAD Charged Aerosol Detector from ~100 to 175 ng on column. B) Response curves for naproxen and sodium with Corona CAD Charged Aerosol Detector fit with a second-order polynomial fit.
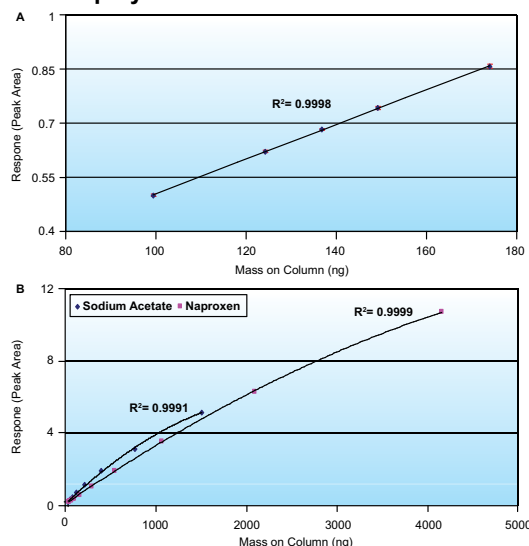
The accuracy results for the calculation of both sodium by the Corona CAD Charged Aerosol Detector and naproxen by UV were within the acceptance criteria of 97–103% for the counterion and 99–101% for the API (Table 1). These acceptance criteria will require adjustment if the charged aerosol detector is to be used to also measure the API. The LOD for the Corona CAD Charged Aerosol Detector were in the subnanogram level for the sodium and low nanogram level for naproxen, which is only slightly above that of the UV detector (Table 2).

Table 1. Accuracy Results for System Suitability Samples by Charged Aerosol Detection and UV				
	Corona CAD Detector		**UV at 254 nm**	
	Sodium	Naxproxen	Sodium	Naproxen
Mean	99.85%	101.61%	ND	99.88%
% RSD	0.82%	2.10%	ND	0.46%

Accuracy

Table 2. Limits by Charged Aerosol Detection and UV				
	Corona CAD Detector		**UV at 254 nm**	
	Sodium	Naxproxen	Sodium	Naproxen
LOQ	2 ng	12 ng	ND	4 ng
LOD	0.75 ng	5 ng	ND	1.5 ng

Quantification and Detection Limits

The reproducibility of the method was investigated for both raw peak area results and precision results over the three-day study. The peak area reproducibility specification for all standard and sample replicate injections was set to 3% within each day. The intermediate precision results for the samples were also set to 3% over the course of the three-day study. The UV criterion was 1% for each. All of these criteria were met for both charged aerosol detection and UV. Tables 3 and 4 show the raw peak area reproducibility for the system suitability samples within each day and throughout the duration of the study. The raw area reproducibility over three days was used for information only and subsequently did not have any acceptance criteria.

Reproducibility and Intermediate Precision—Charged Aerosol Detection

| Table 3. Inter- and Intraday Reproducibility with Dionex Corona CAD Detection								
	Sodium				**Naproxen**			
	R.T. (min)	RSD	Peak Area	RSD	R.T. (min)	RSD	Peak Area	RSD
Day 1	1.34	0.17%	0.487	0.97%	4.73	0.11%	0.798	2.12%
Day 2	1.34	0.16%	0.501	0.78%	4.73	0.10%	0.809	2.24%
Day 3	1.34	0.13%	0.503	0.42%	4.73	0.10%	0.805	1.01%
All Points	1.34	0.21%	0.500	0.86%	4.73	0.13%	0.804	1.86%

Reproducibility and Intermediate Precision—UV

| Table 4. Inter- and Intraday Reproducibility with UV Detection at 254 nm				
	Naproxen			
	R.T. (min)	RSD	Peak Area	RSD
Day 1	4.68	0.08%	4.749	0.50%
Day 2	4.69	0.13%	4.768	0.45%
Day 3	4.68	0.10%	4.802	0.49%
All Points	4.68	0.13%	4.773	0.65%

The matrix blank contained a small sodium peak, which was not present in the DI water blank. The average of that peak area found in the matrix blank was subtracted from the average peak area results for each sample before any calculations were completed. In the Tween 80 dissolution medium, there were low-molecular-weight impurities, which are not strongly retained on the C18 column. This material can be seen in the void volume of the Corona CAD Charged Aerosol Detector chromatogram (Figure 3) along with another matrix peak, which elutes after the naproxen peak. If the objective of this analysis is to measure both the sodium and naproxen by the Corona CAD Charged Aerosol Detector, the method will require further optimization to remove this matrix effect. However, the UV detector showed a clear naproxen peak at 254 nm signal, which was not affected by the matrix, making this orthogonal technique the best approach without requiring further optimization.

FIGURE 3. A) Overlay of three injections of the naproxen-sodium system suitability sample with Corona CAD Charged Aerosol Detector (top A) and UV (bottom A) at 254 nm. B) Chromatographs of naproxen sodium from a name-brand, pain-relieving drug after 4 h of mixing in dissolution solution with Corona CAD Charged Aerosol Detector (top B) and UV at 254 nm (bottom B).
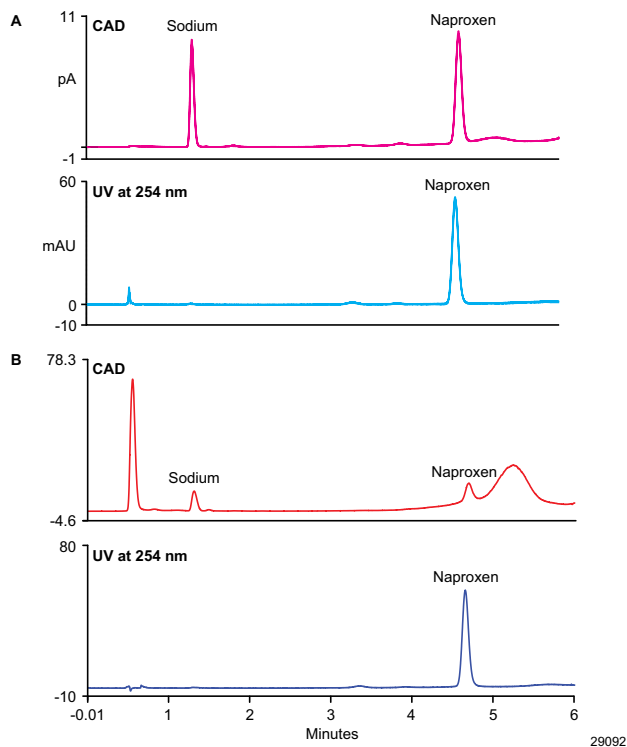
FIGURE 4. Recovery results of sodium and naproxen from tablets after hours in dissolution solution.
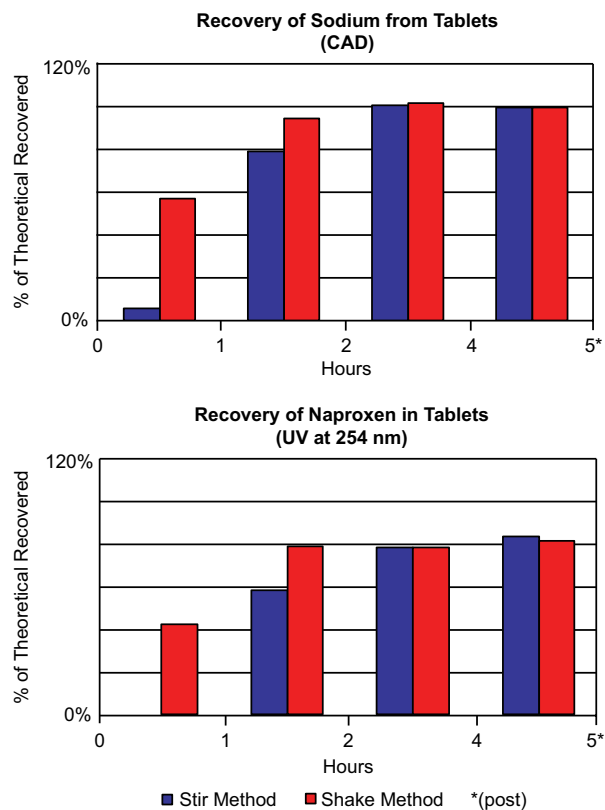
The recovery results for the sodium in the tablets after 4 h, as well as the prepared high- and low-recovery samples, were within the 97–103% acceptance criteria (Figure 4). The naproxen results, however, did not meet specification for the tablets or for the prepared recovery samples. Solubility issues with the parent compound and the matrix may be responsible for this result. In future studies, the tablets will be dissolved in larger volumes for longer periods of time.

Conclusion

The use of universal detectors in a regulated environment can be accomplished and become routine. The development of robust methods and setting of acceptance criteria that reflect those methods and manufacturer's recommendations are extremely important steps in that process. Adequate testing in the development and validation stages should be performed to determine the reproducibility and intermediate precision of the method. This information should then be used to determine the proper number of replicates required to ensure accuracy. As shown here, a more complex analytical system significantly decreased run time while maintaining the needed reproducibility. This time savings permitted the development of a more robust and accurate method because sufficient replicate injections would not be possible with the one-hour method. The use of mixed detection platforms should be encouraged if a multicomponent analysis is required where universal detection is needed for some—but not all—of the analytes. As is the case with all validated analytical methods in a regulatory setting, it is more cost effective to take the time to develop a robust, accurate method using the correct tools in the early stages rather than to investigate the method and attempt to optimize parameters after it has been approved.

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