

# Improving the Quantitation of Unknown Trace Impurity Analysis of Active Pharmaceutical Ingredients Using HPLC with Charged Aerosol Detection

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## ABSTRACT

Accurate quantitation of unknown trace impurities of active pharmaceutical ingredients (APIs) is a significant issue related to mass balance calculations. A review of the literature indicates that various analytical approaches have experienced limited success. A model system which employs the analysis of several test compounds is used to investigate the quality of data obtained for trace-impurity analysis. This study uses an HPLC analytical system and focuses on the comparison of data quality collected from two universal detectors—evaporative light scattering detection (ELSD) and charged aerosol detection. A known technical issue that influences data quality on both these nebulizer-based detectors is related to increased response of compounds during gradient elution when greater solvent composition is employed. This issue can be partially resolved by employing simple postcolumn addition of organic solvent before the detectors. Further mitigation can be found when a known compound with a similar retention time to those of unknown components is used as a calibrant. The enhanced sensitivity of the Charged Aerosol Detector (CAD<sup>®</sup>), along with uniform response characteristics, offers a clear advantage over ELSD for mass balance calculations. This can be further improved by postcolumn addition of organic solvents to mitigate any response changes observed using gradient elution.

## INTRODUCTION

- Impurities are defined by the ICH as starting materials, byproducts, intermediates, degradation products, reagents, ligands, catalysts, inorganic salts, heavy metals, other residual metals, or residual solvents found in the drug substance.
- There are several HPLC detectors that are employed to analyze trace impurities from APIs. Once an impurity has been detected, it becomes necessary to estimate its quantity. The ability to detect an impurity generally means that a given component provides a signal at least twice that of the background noise. Initially, quantity is estimated based on response relative to the parent compound because in most cases a pure sample of the impurity is often not available. It is important to use a known standard compound for quantitation, if it is available. If the determined quantity of a given impurity is > 0.1% then it must be further characterized as per the FDA requirements.
- The results of assay, counterion, and moisture content must add up to 100% (with a tolerance of  $\pm 5\%$ ) in order to ensure that no significant quantities of inorganic salts or other unaccounted impurities from the purification process remain in the API.

- The CAD is a sensitive, mass-based detector, especially well suited for the determination of nonvolatile analytes. CAD has the additional advantage of providing nearly constant response, regardless of the molecular structure of the analyte. This study presents an example for evaluating mass balance utilizing CAD technology with the enhancement of inverse gradient technology, easily implemented with the use of a dual-gradient HPLC pump, to further reduce differences of inter-analyte response factors.

## CAD Function (See Figure 1)

- The liquid eluent from the HPLC column enters the Corona<sup>®</sup> detector (1) where it undergoes pneumatic nebulization by nitrogen or air (2).
- Small droplets enter the drying tube (3) and form particles while large drops exit the drain (4) to waste.
- Dried particles enter the mixing chamber (5). Another gas stream passes over a charged corona needle (6). Charged gas then mixes with the dried particles forming charged particles (7).
- High-mobility species are removed by an ion trap (8) while the remaining charged particles pass to a collector where the charge is measured with a very sensitive electrometer (9). Signal is transferred to chromatographic data software (10).

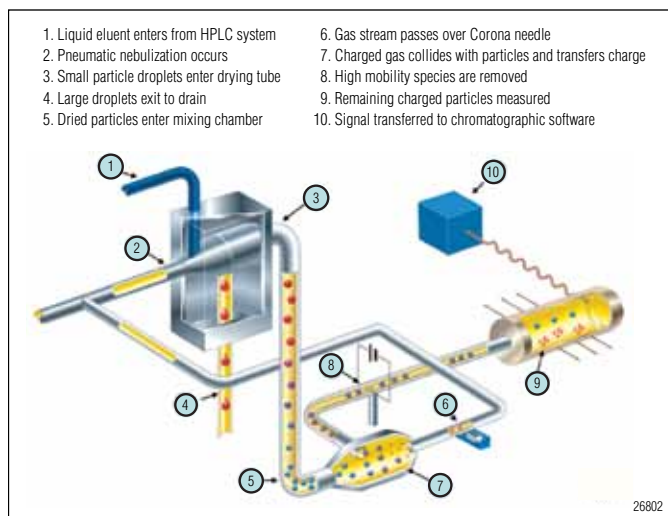


Figure 1. Corona CAD schematic.

## METHOD

### Chemicals and Standards

All chemicals for standards were purchased from Sigma. HPLC-grade solvents were purchased from EMD.

### HPLC Instrumentation

A Dionex UltiMate® 3000 system consisting of a dual-gradient pump (DGP-3600RS), an autosampler (WPS-3000TRS), a thermostatic column compartment (TCC-3000RS), a diode array detector (DAD-3000RS) with a 2.5 µL flow cell, and Chromeleon® Chromatography Data System (CDS) was used in this study. All tubing connections were made with fingertight Viper™ fittings. To introduce an inverse gradient, the column outlet was connected to a secondary pump connected together using a Valco® stainless steel T-union. The connector was positioned after the UV and connected to the CAD with a short length of PEEK™ 0.005" tubing.

### HPLC Method Conditions

#### ELSD vs CAD experiments

HPLC Column: Waters ACQUITY® BEH C18, 2.1 × 50 mm (1.7 µm)  
Mobile Phase: 50 mM Ammonium formate, pH = 3, 10% acetonitrile  
Flow Rate: 1.2 mL/min, isocratic  
Column Temp.: 40 °C  
Sample Temp.: 10 °C  
Injection Volume: 1 µL

#### Inverse Gradient Experiments

Column: Dionex Acclaim® 300 C18, 300 Å,  
4.6 × 150 mm, 3 µm  
Mobile Phase: A) 100 mM Ammonium acetate, pH 4.6  
B) Acetonitrile  
Pump Right Flow Rate: 0.8 mL/min  
Pump Left Flow Rate: 0.8 mL/min  
Gradient: Time observed for void volume was 2.4 min

Pump Right		Pump Left	
Time (min)	%B	Time (min)	%B
0	2	0	98
0.1	2	2.4	98
15.1	98	17.4	2
15.6	98	17.9	2
16.6	2	18.9	98
20.0	2	20.0	98

Detector: Corona *ultra*™  
CAD Filter Setting: Medium

## RESULTS AND DISCUSSION

CAD uses a high-voltage Corona needle to charge nitrogen gas molecules that collide with analyte particles resulting in the formation of charged particles in the mixing chamber. The charge from the analyte particles is collected and then measured by a sensitive electrometer (Figure 1).

The CAD response was more consistent (uniform) among analytes than ELSD. In Figure 2, ELSD response for four phenolic compounds varied greatly, with 4-hydroxyphenylacetic acid (4-HPAC) giving minimal signal. CAD was also more sensitive than ELSD. For example, the limit of detection (S/N = 3) for gallic acid was 31 ng by ELSD and 4 ng (S/N = 3) by the Corona *ultra* detector.

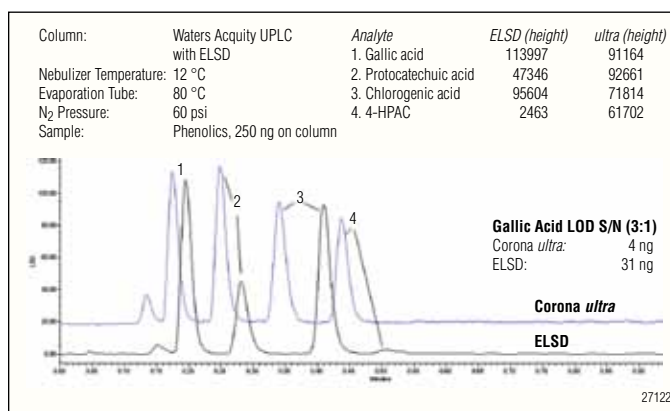


Figure 2. CAD vs ELSD response for phenolics.

Regulatory standards specify the use of mass balance to assess the appropriateness of analytical methods as a stability-indicating methods to determine whether all degradants have been accounted for.<sup>1-4</sup> The Corona *ultra* detector is ideally suited for the routine analysis of APIs, impurities, and for mass balance studies. The CAD provides more uniform response factors for different analytes, and when combined with an inverse gradient, this variance is further reduced. Consistent analyte response is critical for making compositional estimates which contain unknown analytes.

Discrepancies in mass balance studies can result when HPLC-UV is used, as different analytes can have drastically different extinction coefficients. In some cases, an impurity may have no absorbance, and this mass would be missed entirely. Here, seven test compounds (citric acid, phenylalanine, theophylline, propanolol, naproxen, diclofenac, and progesterone) were analyzed using gradient chromatography with both dual-wavelength UV (Figure 3) and CAD detection (Figure 4). For the analysis of these test compounds, the CAD was first run with the same gradient conditions used for UV analysis. When the organic solvent proportion increases, a significant response change of the CAD occurs

due to enhanced nebulizer efficiency (Figure 4A). The system was then adapted to include a postcolumn T-union and a makeup flow of solvents using an inverse gradient. Compound response was more consistent when inverse gradient conditions were employed (Figure 4B) due to a consistent solvent composition during solvent nebulization. No net loss in sensitivity occurs since the CAD is a mass-sensitive detector unlike the UV detector which is sensitive to analyte concentration. Any makeup solvent evaporates within the CAD. Uniform nebulizer efficiency results in an increased response of early-eluting analytes (due to a higher percentage organic solvent) and relative response changes across the gradient chromatogram are minimized. Figures 5A and 5B illustrate the calibration curves for the test compounds without and with inverse gradient, respectively. The inverse gradient minimizes variation in response as demonstrated by more uniform calibration curves.

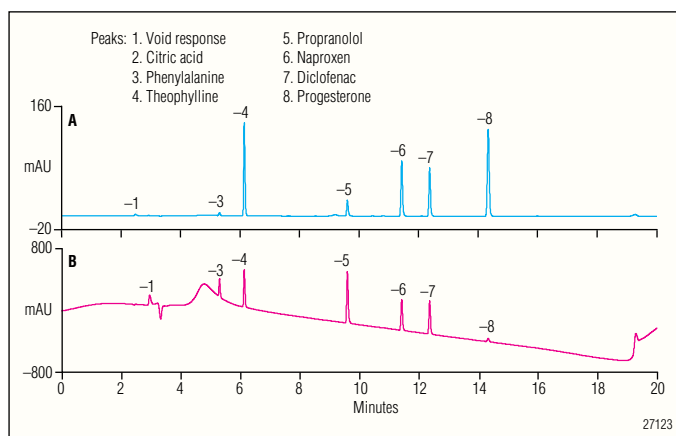


Figure 3. UV response for test compounds at (3A) 254 and (3B) 210 nm. Citric acid did not respond at either wavelength.

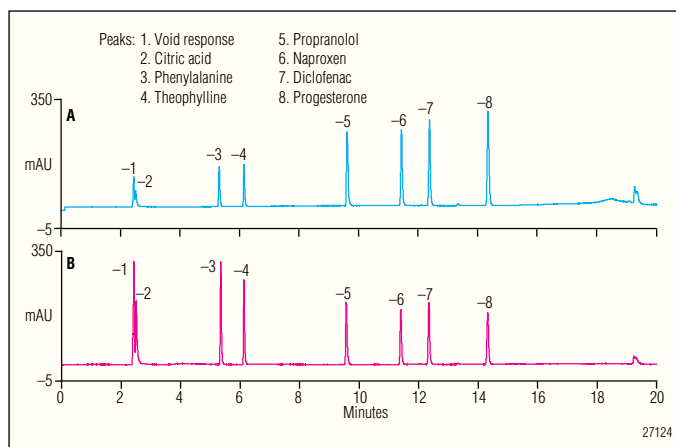


Figure 4. CAD response without (4A) and with inverse gradient (4B) conditions.

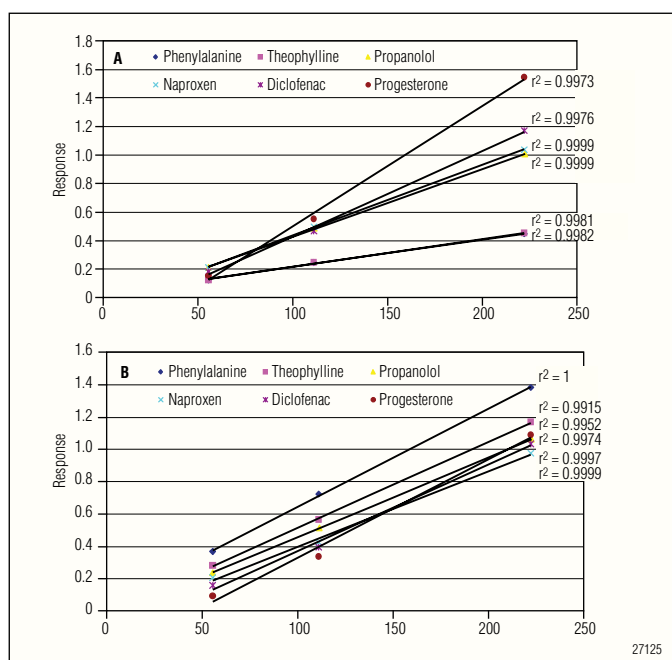


Figure 5. CAD calibration curves without (5A) and with inverse gradient (5B) conditions.

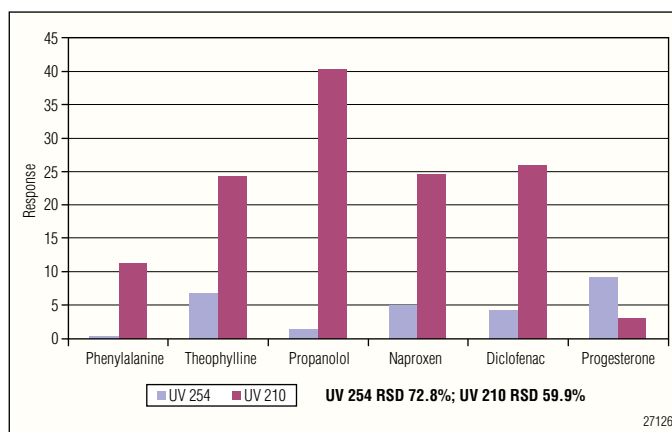


Figure 6. UV response at 210 and 254 nm using gradient conditions.

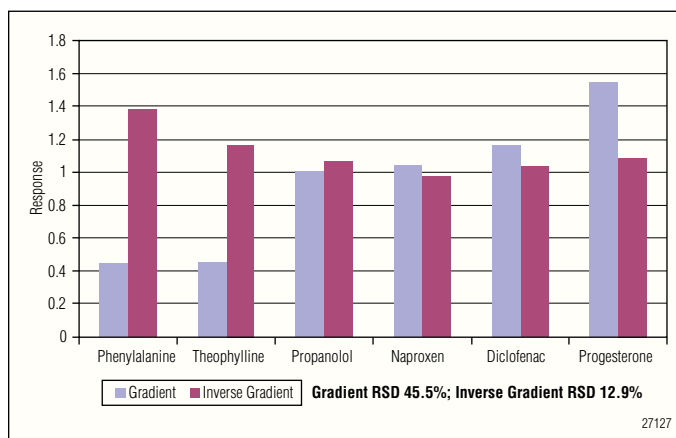


Figure 7. CAD response factor variance without and with inverse gradient conditions.

The variation for compound response when using UV detection is clearly seen in Figure 6 where the response for the test compounds shows nearly a 60% RSD at 210 nm and 73% RSD at 254 nm. This improves when the CAD is used since the variation is reduced to 45% when gradient conditions are used and down to 12.9% when an inverse gradient is employed (Figure 7). This compares favorably to the 10.7% RSD in CAD response observed when the influence of column effects is minimized by using flow injection analysis (FIA, data not shown). The low RSD for compound response using charged aerosol detection is due to the fundamental technology where charge on the analyte particle is being measured and particle composition is unimportant. Others have reported similar improvements in response factors using CAD with inverse gradients.<sup>5</sup> Previous studies using condensation nucleation light scattering detection (CNLS) showed > 40% RSD for 26 compounds when using FIA (data not shown).<sup>6</sup> This is hypothesized to be due to the complexity of the interaction (e.g., wettability and solubility) between the analyte particle and the water condensation used to increase the particle size prior to detection.

## CONCLUSIONS

- Charged aerosol detection offers inherently more consistent response than that obtained from ELSD or UV detection for various compounds. This makes the Corona *ultra* detector ideally suited for the routine analysis of actives, impurities, and for mass balance studies. The use of inverse gradient enables consistent response for analytes detected during gradient elution HPLC (12.9% RSD for different compounds). CAD provides a broad dynamic range, excellent sensitivity, and uniformity of response independent of chemical structure while also being the easiest to operate.
- The Dionex DGP dual HPLC pumping system under the Chromeleon system control offers an inexpensive platform to facilitate method development and operate the system with inverse gradients. This provides a suitable method to prevent dramatic changes in nebulizer efficiency due to changes in solvent composition. The uniformity of CAD response was improved by mitigating gradient effects on nebulizer efficiency by using an inverse gradient.

## REFERENCES

- International Conference on Harmonization (ICH) and US Food and Drug Administration, *Q1B: Photostability Testing of New Drug Substances and Products* (Geneva, Switzerland, and Rockville, MD, Nov. 1996).
- ICH and FDA, *Q1A(R2): Stability Testing of New Drug Substances and Products* (Geneva, Switzerland, and Rockville, MD, Feb. 2003).
- ICH and FDA, *Q2A: Validation of Analytical Procedures, Text for Validation of Analytical Procedure* (Geneva, Switzerland, and Rockville, MD, Oct. 1994).
- ICH and FDA, *Q2B: Validation of Analytical Procedures, Methodology* (Geneva, Switzerland, and Rockville, MD, Nov. 1996).
- Gorecki, T.; Lynen, F.; Szucs, R.; Sandra, P. Universal Response in Liquid Chromatography Using Charged Aerosol Detection. *Anal. Chem.* **2006**, *78* (9), 3186–3192.
- ESA Application Note 70-8913.

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