

Simultaneous Measurement of Anions And Cations: Method Validation

The analysis of ions is typically done by ion exchange chromatography using a conductivity detector (ICCD). This technique tends to be specific to analytes, and anions and cations must be measured individually using platform specific systems. A newer approach using the Corona[®] Charged Aerosol Detector (CAD[®]) with a polymeric zwitterionic HILIC column offers reproducible detection of both anions and cations in a single run with detection in the low nanogram on column levels. This approach allows for complete method optimization by using gradients to enable the measurement of several analytes including: anions, cations, amino acids, organic acids, and numerous APIs on the same instrument platform. This provides a faster and less expensive analytical method which can be simply optimized to include all the components of interest with only minor mobile phase composition adjustments. The sensitivity level of the CAD allows for determination of 0.1% ion impurities in most products which is a regulated requirement during pharmaceutical product development.

The details for the generic gradient method and validation results are presented in this application note.

Time (mins)	% Mobile Phase B
0	45
15	65
20	65
25	40
26	45
30	45

Table 1. Gradient Parameters.

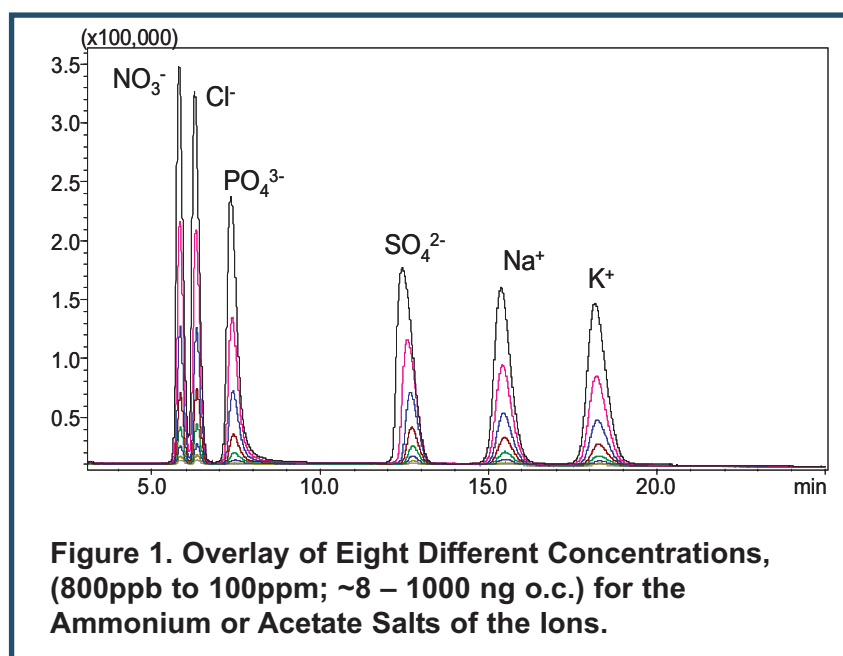


Figure 1. Overlay of Eight Different Concentrations, (800ppb to 100ppm; ~8 – 1000 ng o.c.) for the Ammonium or Acetate Salts of the Ions.

Method Parameters

Column: Sequant ZIC[®]-pHILIC; 4.6 x 150mm, 5µm
 Column Temp: 30 °C
 Injection Volume: 10 µL
 Flow Rate: 0.5 mL/min
 Mobile Phase A: 100 mM Ammonium Acetate (pH 4.6), Methanol, Isopropyl Alcohol, Acetonitrile (15:5:20:60, v/v/v/v)
 Mobile Phase B: 30 mM Ammonium Acetate (pH 4.6), Methanol, Isopropyl Alcohol, Acetonitrile (50:5:20:25, v/v/v/v)
 Gradient: Table 1
 Corona: 100pA range, no filter
 Sample Vial: Polypropylene or certified borosilicate

Sample Preparation and Analysis

The following six salts were prepared at a concentration of 10 mg/mL in deionized water: ammonium nitrate, ammonium chloride, ammonium sulfate, ammonium phosphate, sodium acetate, and potassium acetate. Aliquots of these samples were

combined and then diluted in 80/20 acetonitrile/water to approximately the following concentrations of each ion salt; 100, 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8 µg/mL. Additional samples were prepared to test the accuracy of the method for each of the ions of interest. The compounds and injection concentrations are listed in Table 2.

The eight point standard curve and accuracy samples were transferred to Agilent polypropylene vials. Standard 4 (~12.5mg/mL) was injected 22 times over the course of the study to check reproducibility and intermediate precision of the analysis. Mobile phase was prepared three different times in the course of the study as a check of assay robustness.

Results and Discussion

In Figure 1, overlays of eight different concentration points are illustrated on the same scale. The data was fit using a 2nd order polynomial fit and a linear fit over the range of 8 to 1000ng on column as shown in Figures 2B and 2A, respectively. The Accuracy of the analysis was examined by comparing either inorganic salts or organic compounds which contain an inorganic counter ion. Table 2 lists the compounds tested and the % recovery for each of the ions examined. The experimental values for all of the ions were consistently within 5% of their theoretical values, based on the linear standardization fit shown in Figure 2A. The limit of detection for the phosphate ion was greater than

	Injection Concentration (µg/mL)	Theoretical Counter-ion %	Experimental Counter-ion %	% Recovery
Sodium Salicylate	88	14.4	15.1	105.0
Quinine Sulfate Dihydrate	227	10.2	10.4	101.4
Potassium Iodide	23	23.6	22.8	97.0
Lysine Dihydrochloride	46	19.3	20.0	103.6

Table 2. Method Accuracy. Experimental Recovery Results for Counter-ion Determination.

	Limit of Quantification (ng)	Limit of Detection (ng)
Nitrate	6.1	~1
Chloride	5.2	~1
Phosphate	25.0	~10
Sulfate	9.6	~2
Sodium	4.5	~1
Potassium	6.1	~2

Table 3. Method Sensitivity (mass on column, not salt injected).

	% RSD Day 1 (n=5)	% RSD Day 2 (n=5)	% RSD Day 4 (n=6)	% RSD Day 7 (n=6)	% RSD All Days (n=22)
Nitrate	0.04	0.16	0.02	0.02	0.83
Chloride	0.05	0.16	0.02	0.02	1.27
Phosphate	0.05	0.22	0.05	0.03	2.16
Sulfate	0.06	0.25	0.07	0.08	2.70
Sodium	0.02	0.05	0.03	0.09	1.05
Potassium	0.02	0.13	0.05	0.15	0.99

Table 4. Retention Time Precision for Ions Tested at Four Concentrations over Seven Days.

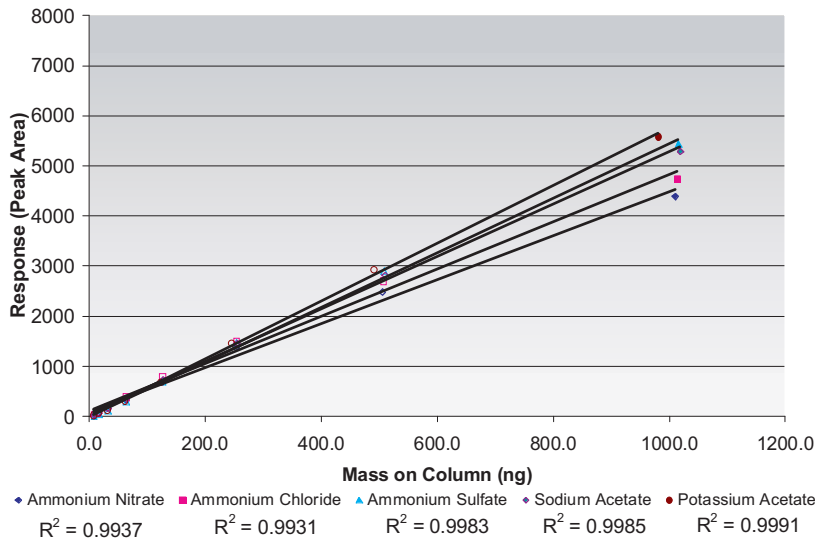


Figure 2A. Calibration Curves (Average of Three Injections Each for 8 Levels ~ 8 to 1000 ng o.c.) for the Ammonium or Acetate Salts of the Ions, Analyzed Using a Linear Fit.

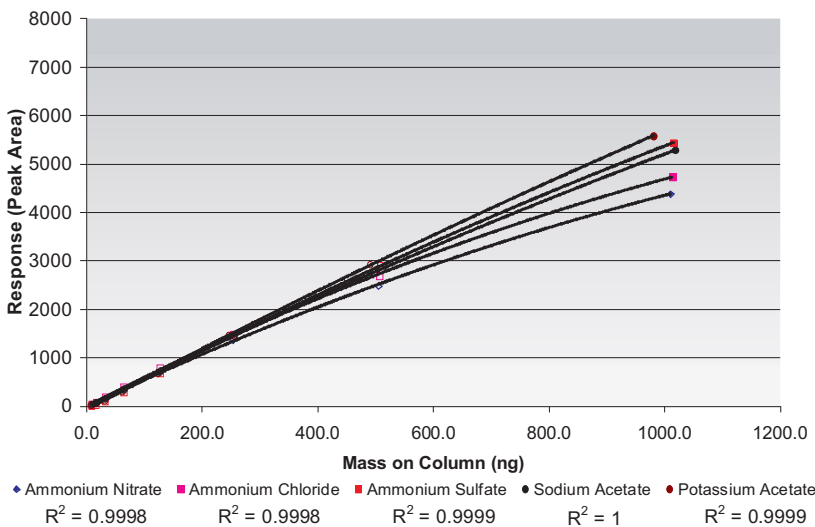


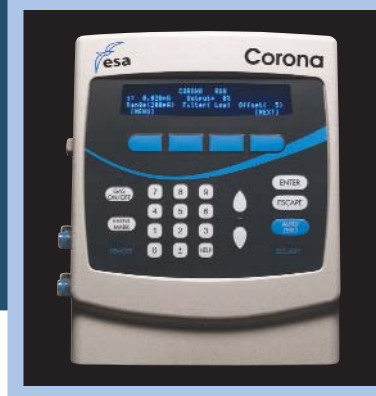
Figure 2B. Calibration Curves (Average of Three Injections Each for 8 Levels ~8 to 1000 ng o.c.) for the Ammonium or Acetate Salts of the Ions, Analyzed Using a 2nd Order

the 8 ng point. Therefore, for phosphate only the highest six points (3.2 – 100 µg/mL) for the concentration curve were used. All eight concentration points on the curve were used with the other ions. The correlation coefficients for each individual calibration curve were greater than 0.9998, using 2nd order polynomial fit, and greater than 0.993, using linear regression analysis for the six ions.

The limits of quantification and detection for each of the ions tested were in the low nanogram on-column levels and are listed in Table 3.

The reproducibility and intermediate precision of the analysis was examined by looking at peak areas and retention times of Standard 4 (~12.5 µg/mL of each ion salt) over the length of the study. Five injections of Standard 4 were made on Days 1 and 2. A fresh vial was then prepared on Day 4 and on Day 7 of the analysis, and this standard was re-injected six times each day. The peak area reproducibility as well as the retention time reproducibility was examined over this 22-point data set for the six ions. The Day 7 data also used a new mobile phase which resulted in a slight shift in the retention time data. The inter- and intra-day reproducibility results are listed in Tables 3 and 4, respectively.

The Corona[®] Charged Aerosol Detector



	% RSD Day 1 (n=5)	% RSD Day 2 (n=5)	% RSD Day 4 (n=6)	% RSD Day 7 (n=6)	% RSD All Days (n=22)
Nitrate	1.25	1.45	1.00	0.88	2.67
Chloride	0.45	1.33	1.23	0.56	2.55
Phosphate	4.18	2.38	3.08	3.41	5.33
Sulfate	1.71	0.77	2.21	1.31	4.68
Sodium	1.94	1.35	1.69	1.54	3.30
Potassium	1.16	1.93	1.19	1.39	3.47

Table 5. Peak Area Precision (~125ng o.c.) for Ions Tested Over Seven Days.

Conclusions

The data collected during this study demonstrate that the analysis of ions using the Corona detector can be done accurately and reproducibly down to low nanogram levels. All major validation components were tested for chloride, sulfate, sodium, and potassium. The analysis was not a complete validation with all robustness levels needed according the FDA Q7A. However, it does demonstrate the accuracy, linearity, LOD, and intermediate precision of the detector and method. The other application notes in this series will discuss these ions, additional ions, and other components. The ability to validate this type of analysis along with the versatility of the method makes this a new and viable alternative to many traditional techniques. The Corona CAD is a strong alternative to ICCD for the simultaneous analysis of both cations and anions.

Ordering Information

Corona Charged Aerosol Detector	70-6350
or Corona Plus	70-7041
Thermal Organizer Module	70-5499TA
Optional Equipment:	
Nitrogen generator	70-6003
Pump, model 584, RS232	70-7058
Gradient Upgrade	70-5057
Degasser	70-7666
Mixer	70-4000
Autosampler, model 542	70-4152
EZChrom Elite(TM) for ESA software including PC	70-5073



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