

Abstract

Quantitative analysis of anions and cations is an important aspect of drug development and quality control. This includes confirmation of stoichiometry, counter-ion identity and completeness of pharmaceutical salt formation as well as detection of ionic impurities. Ion exchange chromatography is often used for these analyses but a variety of specialized columns, ion suppressors and modes of operation are required to address the range of analytes of interest. In this study, hydrophilic interaction chromatography (HILIC) was used in combination with charged aerosol detection (CAD). A polymeric zwitterionic stationary phase operated in HILIC mode allowed separation of anions and cations (API and counter-ion) in a single chromatographic run. Mobile phase conditions such as pH, buffer strength and organic modifier concentration could be manipulated to effect a given separation including changing the elution order of API and counter-ion. A single chromatographic method allowed quantitative analysis of positive and negative counter-ions in a variety of pharmaceutical salts with close agreement between theoretical and experimental values. HILIC in conjunction with CAD allowed quantitation over at least 3 orders of magnitude with precision (RSD) <3%. The ability to simultaneously analyze counter-ions and their parent pharmaceutical compounds, in addition to ionic impurities from drug formulations is also reported.

Introduction

Ion chromatography with conductivity detection (ICCD), is the most common approach for the measurement of ions. ICCD typically uses a weak ionic resin for its stationary phase and a high ionic strength mobile phase. An additional neutralizing suppressor column is often required to remove background eluent ions. ICCD cannot measure both anions and cations simultaneously, so either the operator must wait for a considerable period when switching chemistries or multiple instruments must be used. Due to the amount of time to recondition the column and detector, gradient methods are typically not implemented with ICCD.

HILIC, a variation of normal phase HPLC, uses a polar stationary phase (e.g., zwitterionic) and a mobile phase that is highly organic but contains a small amount of aqueous/polar solvent. Ions are separated both by partitioning and electrostatic interaction.¹ Once separated, anions and cations can then be measured simultaneously using the universal Corona[®] Charged Aerosol Detector (CAD[®]).

The use of a binary gradient system in this study allows for the analysis of a range of anions and cations along with the parent API. The retention time of both the ions and the API can be altered by adjusting the initial percentage of the high-aqueous mobile phase (mobile phase B). The majority of the work in this study was done with an initial mobile phase containing 45% B composition unless otherwise indicated to be starting at 20%. This use of the binary gradient, the Sequant ZIC-pHILIC HPLC column, and the Corona CAD detector allowed for detection limits in the low nanogram on column level for both anions and cations.

Method Conditions

Column	Sequant ZIC-pHILIC; 4.6 x 150mm, 5µm
Column Temperature	30°C
Mobile Phase A:	15% 100mM Ammonium Acetate pH=4.68, 5% Methanol, 20% IPA, 60% Acetonitrile
Mobile Phase B:	50% 30mM Ammonium Acetate pH=4.68, 5% Methanol, 20% IPA, 25% Acetonitrile
Flow Rate	0.5mL/min
Injection Volume	10µL
Gradient Method 1	T=0 min 45%B, T=15 min 65% B, T=20 min 65%, T=23 min 40%, T=24 min 45%B, T=30 min 45%
Gradient Method 2	T=0 min 20%B, T=3 min 20% B, T=24 min 70%, T=26 min 70%, T=32 min 15%B, T=34min 20%
Corona	100pA range, no filter
Sample Vial	Polypropylene or certified borosilicate

Range and Linearity

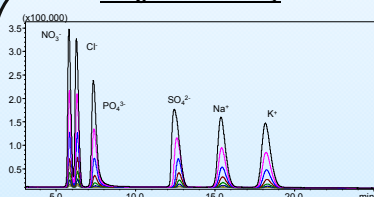


Figure 1: Overlay of eight different concentrations, (~ 1mg to ~8ng o.c.) for the ammonium or acetate salts of the ions.

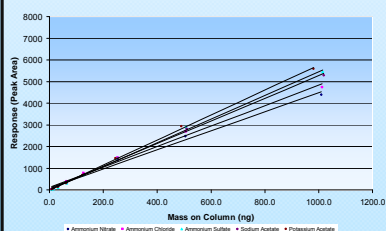


Figure 2: Calibration curves (average of three injections) for 8 Levels (~ 1µg to ~8ng o.c.) for the ammonium or acetate salts of the ions, analyzed using linear regression.

Table I. Correlation Results for Full Range and Target Curves

Analyte	8 point curve		3 point curve
	polynomial	linear	linear
Nitrate	0.9998	0.9937	0.9995
Chloride	0.9998	0.9931	0.9989
Phosphate	0.9991	0.9984	0.9986
Sulfate	0.9999	0.9983	0.9972
Sodium	1.0000	0.9985	0.9994
Potassium	0.9999	0.9991	0.9980

Table 1: Correlation coefficients for the data presented in Figure 2 using a 2nd order polynomial fit and linear fit along with linear results for three point target curves

Reproducibility

Table II. Inter and Intra-Day Reproducibility of Retention Times and Peak Areas

Analyte	Day 1 (n=5)		Day 2 (n=5)		Day 4 (n=6)		Day 7 (n=6)		All Points	
	Area	R.T.	Area	R.T.	Area	R.T.	Area	R.T.	Area	R.T.
Nitrate	1.2	0.04	1.4	0.16	1.0	0.02	0.9	0.02	2.7	0.83
Chloride	0.5	0.05	1.3	0.16	1.2	0.02	0.6	0.02	2.6	1.3
Phosphate	4.2	0.05	2.4	0.22	3.1	0.05	3.4	0.03	5.3	2.2
Sulfate	1.7	0.06	0.8	0.25	2.2	0.07	1.3	0.08	4.7	2.7
Sodium	1.9	0.02	1.3	0.05	1.7	0.03	1.5	0.09	3.3	1.0
Potassium	1.2	0.02	1.9	0.13	1.2	0.05	1.4	0.15	3.5	0.99

Table 2. Displays the %RSD for raw peak areas and retention times for each of the ions during the study. The (n) value indicates the number of injections made on that day. The all day data represents all 22 points.

Accuracy

Table III. Accuracy Results

Sample	Injection Concentration µg/mL	Calculated Ion	Percent Recovery
Sodium Phosphate monohydrate	46	Na ⁺	96.5
Quinine sulfate dihydrate	227	PO ₄ ³⁻	96.3
Diclofenac Sodium Salt	120	SO ₄ ²⁻	101.4
Acesulfame K	75	Na ⁺	99.8
Lysine dihydrochloride	46	K ⁺	99.0
Ammonium Nitrate	15	Cl ⁻	103.6

Table 3. The percent recovery was calculated using the linear standardization curves. The values are based on the theoretical ion content for each of the compounds.

Sensitivity

Table IV. Limits of Detection and Quantification

Analyte	LOQ	LOD	Solution Concentration
	(ng O.C.)	(ng O.C.)	
Nitrate	4	1.3	* 100 ppb
Chloride	4	1.3	* 90 ppb
Phosphate	12	7	** 150 ppb
Sulfate	7	2.5	** 85 ppb
Sodium	4	1.3	** 40 ppb
Potassium	5	3	** 60 ppb

* maximum injection volume used 20µL
** maximum injection volume used 50µL

Table 4. Limits of Detection and Quantification observed for ions as both mass on column and minimum solution concentration

Improving Selectivity with Gradient Adjustments

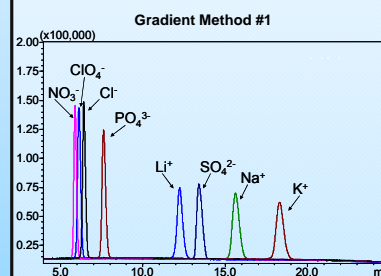


Figure 3: Overlays of anions and cations (10µL, each ~300ng o.c.) analyzed using the gradient method described in the methods section.

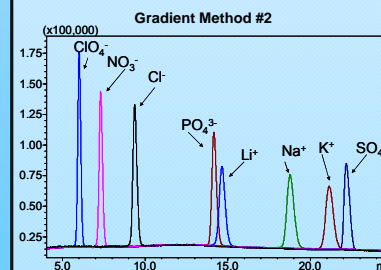


Figure 4: Improved resolution of anions and cations (10µL, each ~300ng o.c.) using a gradient method # 2

Discussion

The initial gradient method enabled the simultaneous analysis of inorganic cations and anions. A validation of this method evaluated linearity, reproducibility, accuracy, robustness, and intermediate precision. The results are listed in Tables 1-3. The standardization curve from 1µg to 8ng could be analyzed by either a linear regression or by a 2nd order polynomial fit. The correlation coefficient by both these approaches are listed in Table 1. Over the range tested the Corona CAD demonstrates R² for the linear correlations ≥0.993 for the ions tested. The linear regression was used to calculate the experimental percentage of the inorganic counter-ions in an amino acid, API, sugar, and salt sample within 5% of the expected value for each. The LOD and LOQ for each of the ions are listed in Table 4.

Minor adjustments to the gradient allowed marked improvement in ion resolution (compare Figures 3 and Figure 4) without the need for changing mobile phase composition, illustrating the chromatographic power of this approach. Modified gradients enable the measurement of several analytes including: anions, cations, amino acids, organic acids, and numerous APIs using the same instrument platform. This allows for a faster and cheaper method development which can be fully optimized to include all the components of interest with only minor mobile phase composition adjustments.

Regulatory authorities like ICH and USFDA are placing emphasis on the purity requirements and identification. The methods presented in this poster are also capable of measuring low level counter-ion impurities to the 0.1% w/w level which simplifies impurity analysis.

Conclusions

A flexible gradient method for the simultaneous determination of anions and cations in a single analysis, using common HPLC equipment and the Corona Charged Aerosol Detector was developed. The method uses a relatively simple sample preparation, requiring sufficient acetonitrile to maintain peak shape, and it can resolve many ions individually and away from other organic compounds, such as APIs.

The method, without sample extractions, can determine ion concentrations in the high ppb range, with sufficient accuracy and precision to meet pharmaceutical testing requirements. Ion analyses were shown to be quantifiable over a large range of concentrations, covering 800ppb to 100 ppm. The amount of variance at the 12.5ppm points was found to be less than 5% RSD over a 7 day period, indicating that the method is also precise.

Although the data presented here will be of most interest to the pharmaceutical industry, this approach is also applicable to other industries where ion monitoring is important e.g., analysis of environmental samples. However, like with ICCD, sample pre-concentration may be required to measure the ultra-low levels of the ions of concern in these samples.

References

- D.S. Risley, B.W. Pack, LCGC North America, August 1, 2006