New Approaches for Simultaneous API and Counterion Analysis Using Charged Aerosol Detection

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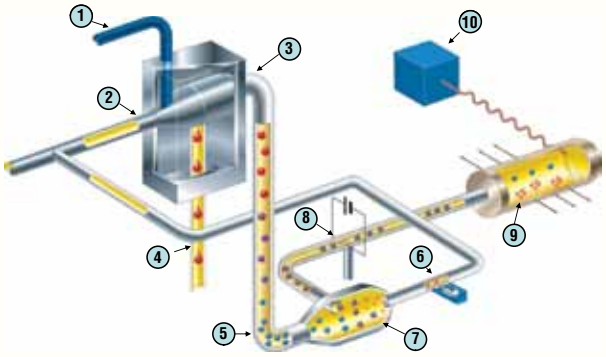
ABSTRACT

Approximately 50% of all drug molecules used in medicinal therapy are administered as salts. Salt forms are used to improve the biological and physiochemical properties of active pharmaceutical ingredients (APIs) including: aqueous solubility, hygroscopicity, solution pH, melting point, dissolution rate, chemical stability, crystal form, and mechanical properties. Traditional approaches for salt analysis use several analytical methods to measure the API and various counterions. Recent innovations in mixed-mode column technology and universal detection techniques enable the rapid, sensitive, and simultaneous analysis of an API together with its inorganic or organic counterion. The work in this study uses HPLC and the Corona® *ultra*™ detector for the analysis of several common pharmaceutical salts. A Dionex Acclaim® Trinity™ P1 column, which combines anion-exchange, cation-exchange, and reversed-phase functionalities, allows separation of positive and negative counterions, as well as lipophilic APIs in a single run. Different methods are presented, including an isocratic approach capable of simultaneously measuring the API and its counterion in less than 3 min. Figures of merit are reviewed, including accuracy, precision, linear range, LOD, and LOQ. Comparison of experimental and theoretical values are within 1%. The ability to test for ionic impurities of < 0.1% is demonstrated. The combination of the Trinity column and the Corona *ultra* detector offers a new, versatile, and powerful tool for counterion analysis with applications for salt selection, drug formulation, and drug impurity analysis.

INTRODUCTION

Counterions influence an API's solubility, stability, and hygroscopicity, so appropriate counterion selection is an important part of drug development process. The most common pharmaceutical counterions include: chloride, sodium, sulfate, acetate, phosphate, potassium, maleate, calcium, citrate, bromide, nitrate, ammonium, tosylate, phosphate, tartrate, and ethylenediamine.[1,2] Methods for the analysis of 13 of the 16 most common pharmaceutical salts are demonstrated on the zwitterionic or the nanopolymer silica hybrid column technologies. The SeQuant polymeric zwitterionic column (ZIC®-*p*HILIC) uses zwitterionic stationary phase on porous polymer particles. The separation is achieved by a hydrophilic interaction mechanism superimposed on weak electrostatic interactions.[3] The Acclaim Trinity P1 column uses silica particles where the inner-pore area is modified with an organic layer that provides both reversed-phase and anion-exchange properties. The outer surface is modified with cation-exchange functionality. The Charged Aerosol Detector (CAD®) uses a patented detection technique described in Figure 1 which is able to achieve near uniform response over a broad spectrum of analytes. The analysis of inorganic ions involves the use of volatile buffers, such as ammonium acetate or formate, to create salt particles that can then be charged and detected.



1. Liquid eluent enters from HPLC system
2. Pneumatic nebulization occurs
3. Small particle droplets enter drying tube
4. Large droplets exit to drain
5. Dried particles enter mixing chamber
6. Gas stream passes over corona needle
7. Charged gas collides with particles and transfer charge
8. High mobility species are removed
9. Remaining charged particles measured
10. Signal transferred to chromatographic software

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Figure 1. Corona ultra *detector workflow.*

SIMULTANEOUS SEPARATION OF CATIONS AND ANIONS

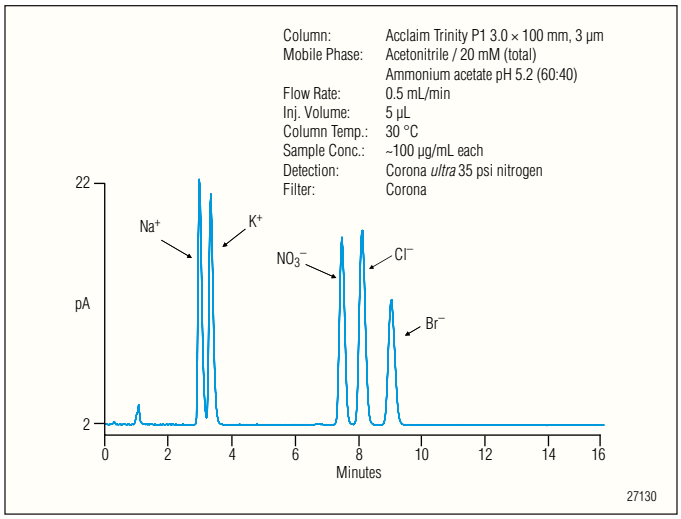

Figure 2. Isocratic analysis on the Acclaim Trinity P1, 3.0 × 100 mm, 3 µm column.

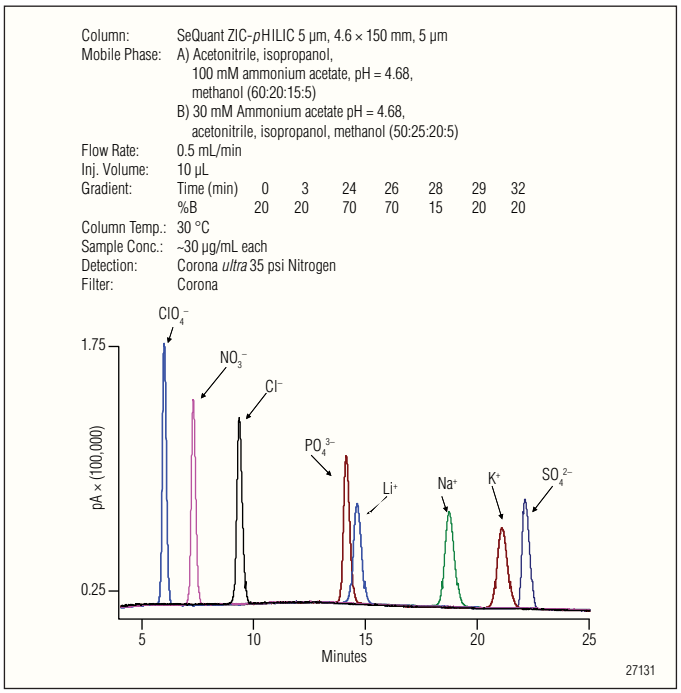

Figure 3. Gradient analysis on the SeQuant ZIC-pHILIC, 4.6 × 150 mm, 5 µm column.

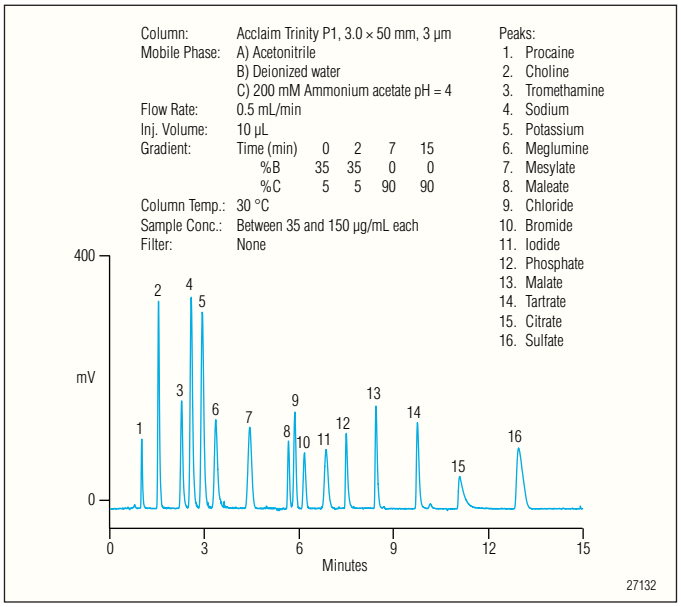

Figure 4. Gradient analysis on the Acclaim Trinity P1, 3.0 × 50 mm, 3 µm column.

REPRODUCIBILITY AND RANGE

Table 1. Intra- and Between-Day Reproducibility of Five Analytes

	% 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
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

Percentage RSD of area averages of anions and cations using the method described in Figure 3.

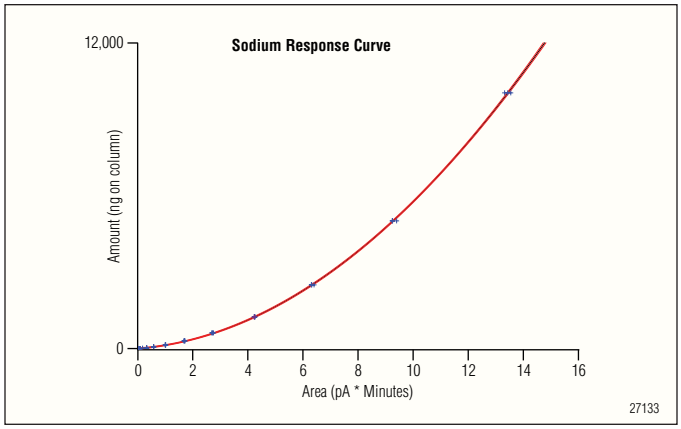

Figure 5. Sodium acetate from 5–10,000 ng on column. Three injections, at each of the 12 concentrations, fit with a polynomial inversed x and y axis (r^2 = 0.9997) overlaid with the 95% confidence limits.

API AND COUNTERIONS

The simultaneous measurement of an API and its counterion was illustrated using diclofenac sodium salt. Figure 6 shows the analysis on the Acclaim Trinity P1 column while Figure 7 shows the analysis on the ZIC-*p*HILIC column. In this example, the API and counterion elution order was reversed for the two columns with diclofenac retaining better on the Trinity column with a shorter run time.

This approach can be used to measure other APIs with counterions shown in Figures 2–4 on both the Trinity and ZIC columns.

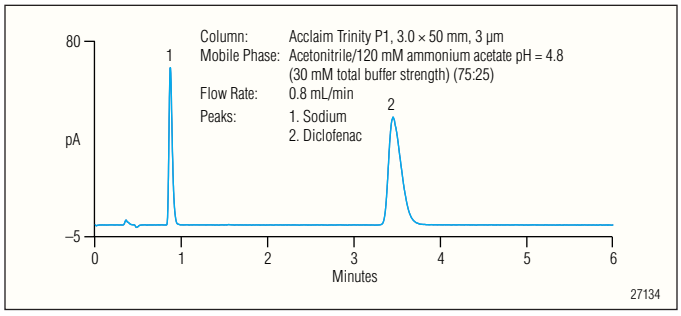

Figure 6. Diclofenac sodium salt, 1.3 µg on column.

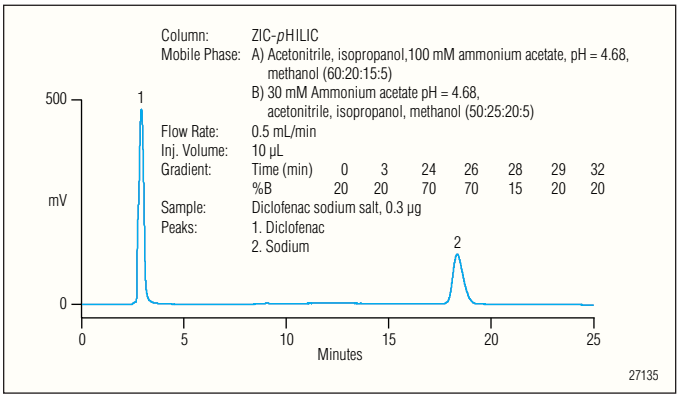

Figure 7. Chromatogram of a 10 µL injection of diclofenac sodium salt with the method described in Figure 3.

FAST COUNTERION ANALYSIS

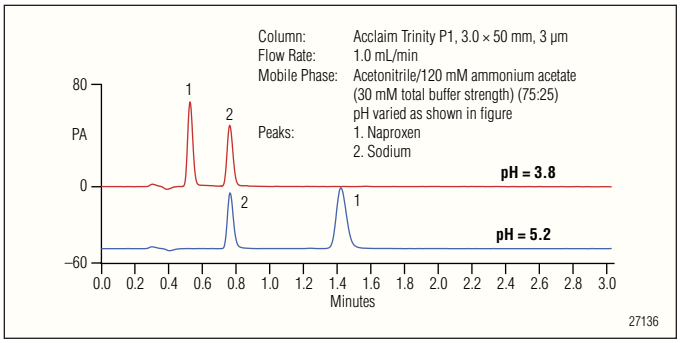

Figure 8. Naproxen sodium salt, ~500 ng on column.

LIMITS OF DETECTION AND 0.1% IMPURITIES

Table 2. LODs for Sodium and Chloride Using the Corona *ultra* Detector

	ZIC-*p*HILIC	Trinity P1
Sodium	1.3 ng	0.9 ng
Chloride	1.3 ng	0.2 ng

Limits of detection using the methods described in Figures 2 and 3. Limit defined as signal-to-noise > 3.

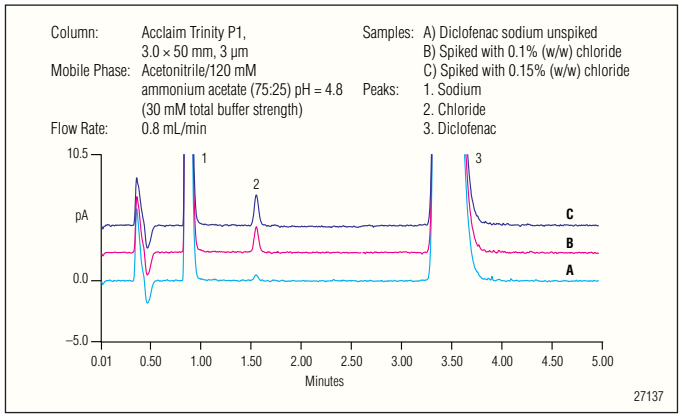

Figure 9. Three injections of diclofenac sodium salt, 5.7 µg on column.

Table 3. Area Values and Deviation for Chloride in Diclofenac

Sample	Raw Area (pA *min)	RSD (n = 5)	Calculated Chloride (w/w)
Diclofenac Standard	0.0231	13.01%	0.03%
Diclofenac + 0.1% Cl^-	0.1069	2.39	0.14%
Diclofenac + 0.15% Cl^-	0.1318	1.49%	0.17%

Average of the chloride raw area values and the corresponding deviation along with the calculated weight percent of chloride in dicolfenac sodium samples run as described in Figure 9.

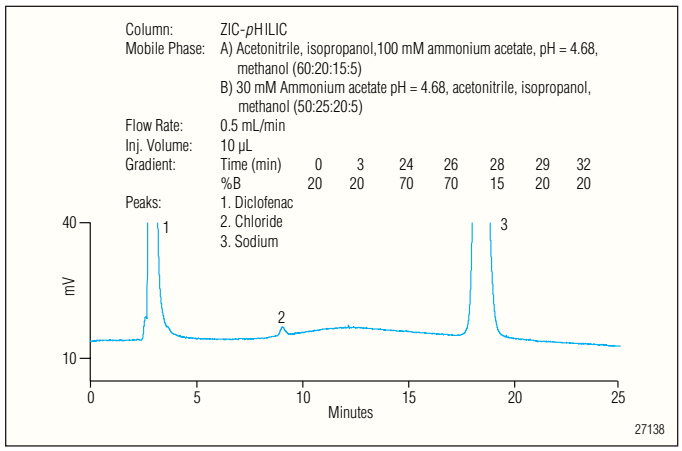

Figure 10. Chromatogram of a 10 µL injection of diclofenac sodium salt 0.3 µg in acetonitrile/water (80:20) with 0.1% w/w of chloride using ZIC-pHILIC column and the method described in Figure 3.

DISCUSSION

The two columns evaluated (ZIC-pHILIC and Trinity P1) both provide the ability to simultaneously resolve anions and cations as shown in Figures 2–4. These columns use very different packing materials for their separations, which lead to different advantages depending on application requirements.

The ZIC-pHILIC column was found to operate best at a flow rate of 0.5 mL/min with a gradient method. It is possible to run the column isocratically at flow rates up 1 mL/min. However, long-term stability was compromised with a loss in peak shape and resolution. This was possibly due to build-up of trace ionic materials which do not fully elute under weak isocratic conditions.

Both columns were able to provide good reproducibility data (e.g., Table 1, ZIC data; Trinity data not shown) with large dynamic ranges (e.g., Figure 5, Trinity data; ZIC data not shown).

The focus of the work presented here was the analysis of an API and counterion on a single platform. This can be achieved using either columns as demonstrated in Figures 6–7. In these examples, compound specificity is reversed for the different columns. The API (diclofenac) is more retained on the Trinity column and the sodium peak elutes earlier. However, on the ZIC-pHILIC column, the diclofenac elutes very quickly, close to the void, and the sodium is more retained. Because the ZIC-pHILIC column uses a HILIC approach, the initial mobile phase composition needs to contain a high level of organic content, generally > 70%. For APIs that do not contain strong polar groups and are retained primarily by reversed-phase properties, the high organic mobile phase can lead to peaks eluting in or close to the void volume.This makes the analysis of hydrophobic APIs on the ZIC column difficult.

Figure 8 illustrates the seperation of another common API, naproxen, and its counterion sodium using the Trinity column. Baseline resolution can be obtained in less then 1 min. A simple change in the pH from 3.8 to 5.2 determines the elution order of the two major components. One of the major advantages found with the Trinity column was its ability to run fast isocratic methods. This resulted in better peak shape and sharper peaks which leads to lower limits of detection on the Corona *ultra*, as shown in Table 2.

Using the improved chromatography discussed here, an experiment was conducted to determine whether a significant difference between a diclofenac sodium sample spiked with 0.1% versus a sample spiked with 0.15% (w/w) chloride could be identified. Figure 9 is an overlay of chromatograms at each corresponding level. As shown in Table 3, the calculation of the chloride content with a four-point linear regression curve from 3 to 25 ng chloride on column yielded recoveries of 100 ± 10% of the expected values. The method was sensitive enough to measure a trace amount of chloride contamination in the injection of the unspiked standard.

CONCLUSION

- Analysis using CAD and either the Acclaim Trinity P1 or the SeQuant ZIC-*p*HILIC column provided sensitive and reproducible results for the determination of API counterions.
- The HILIC chemistry and column packing material for the ZIC-*p*HILIC column made it necessary to use longer gradient runs to achieve optimal performance. The need to run under HILIC conditions make it unsuitable for analyzing hydrophobic APIs.
- The mixed-mode, Nanopolymer Silica Hybrid (NSD) technology employed in the Trinity P1 column allowed for fast isocratic API counterion analysis required when screening counterions and in QC functions.

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3. Merck website on ZIC-HILIC column http://www.sequant.com.

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