

# Universal Approach to Analysis of Pharmaceutical Salts Including Inorganic and Organic Counterions

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## ABSTRACT

In general, multiple analytical techniques are required to address the diverse nature of analytes (inorganic/organic, anionic/cationic, as well as differences in chromophores and hydrophobicity) used in pharmaceutical preparations. The objective of this study was to develop and investigate the use of a more generic approach for the analysis of this broad range of analytes using HPLC with Charged Aerosol Detection<sup>®</sup> (CAD<sup>®</sup>). A polymeric ZIC-pHILIC HPLC column with a polar (zwitterionic) stationary phase was employed with a mobile phase of acetonitrile/methanol/IPA/water buffered with ammonium acetate. Our studies demonstrate the ability to rapidly analyze 13 commonly used inorganic, cationic, and anionic counterions, as well as seven organic acids and five organic bases simultaneously along with several active pharmaceutical ingredients (APIs) in < 25 min. Limits of detection were in the low ng (ppb) range with a linear response over three orders of magnitude and intra-assay precision of less than 3% RSD. The recoveries of counterions calculated for six different, commercially available standards were shown to be within 5% of the theoretical results. The sensitivity of this approach could readily detect impurities at < 0.1% (w/w), while simultaneously measuring the API and counterion. As the approach uses LC-MS compatible phases, the flow was split between the CAD and a single quad MS to generate mass/charge data for positive identification of APIs, organic counterions, and impurities. The method allows for the simultaneous measurement of cations and anions, as well as an API and its counterion(s), and ionic impurities on a platform-independent HPLC system without restrictions on organic content of the sample solvent.

## INTRODUCTION

Inorganic cation and anion analyses are required by pharmaceutical formulations, product characterization, and environmental analyses. API salts influence their solubility, stability, and hygroscopicity so appropriate counterion choice is an important part of drug development process. Ten of the most common pharmaceutical counterions are: chloride, bromide, nitrate, ammonium, sulfate, tosylate, phosphate, tartrate, ethylenediamine, and maleate.

Typically, ion chromatography with conductivity detection (ICCD) is employed, but individual IC techniques require dedicated, platform-dependent instruments for each suite of analytes. HILIC 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. Inorganic cations and anions, organic acids and bases, as well as APIs can be separated using a polymeric zwitterionic column (ZIC) from Sequant with a binary gradient. Anions and cations in the same mixture can be measured simultaneously using the universal Corona<sup>®</sup> CAD detector. The LC system can also be configured with a postcolumn flow splitter to a MS detector for further characterization of the API.

Sensitivity and reproducibility are a requirement for the pharmaceutical industry. The wide dynamic range and the high sensitivity of CAD enables ionic impurities to be detected down to the 0.1% level. For each compound class, examples are shown for the calculations of experimental counterion concentration versus theoretical values.

## METHOD CONDITIONS

Column	Sequant ZIC-pHILIC; 4.6 × 150 mm, 5 μm
Column Temperature:	30 °C
Mobile Phase A:	15% 100 mM ammonium acetate pH=4.68, 5% methanol, 20% IPA, 60% acetonitrile
Mobile Phase B:	50% 30 mM ammonium acetate pH=4.68, 5% methanol, 20% IPA, 25% acetonitrile
Flow Rate	0.5 mL/min
Injection Volume	10 μL
Gradient	t=0 min 20% B, t=3 min 20% B, t=24 min 70% B, t=26 min 70% B, t=32 min 15% B, t=34 min 20% B, t=40 min %B
Corona	100 pA range, no filter
Sample Vial	Polypropylene

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## INORGANIC IONS

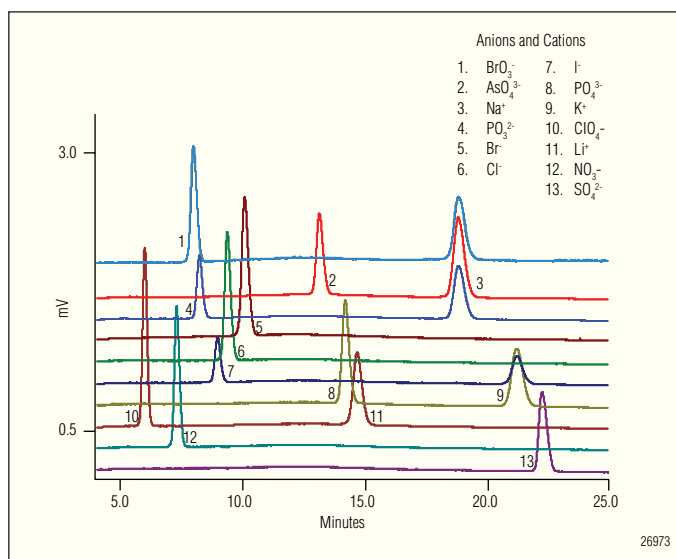


Figure 1. Overlays of 10 anions and three cations (10  $\mu$ L injections of ~25 ppm salt solutions) analyzed using gradient method.

## ORGANIC ACIDS

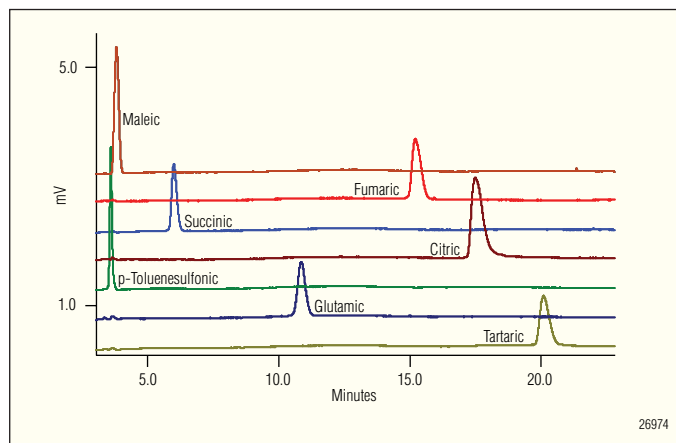


Figure 2. Overlays of seven organic acids (10  $\mu$ L injections of ~60 ppm solutions) analyzed using gradient method.

## ORGANIC BASES

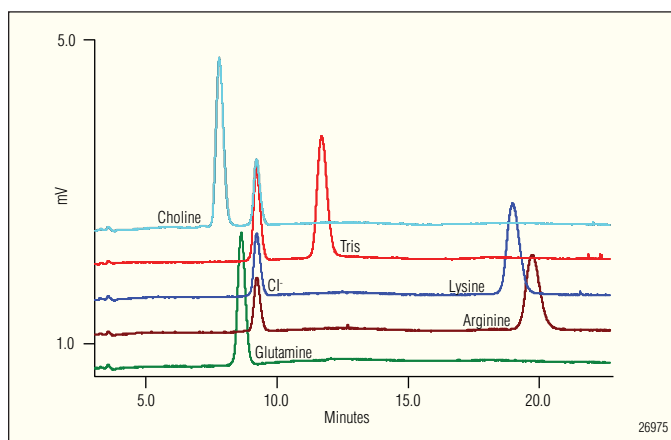


Figure 3. Overlays of five organic bases (10  $\mu$ L injections of ~60 ppm solutions) analyzed using gradient method.

## CAD-MS METHOD

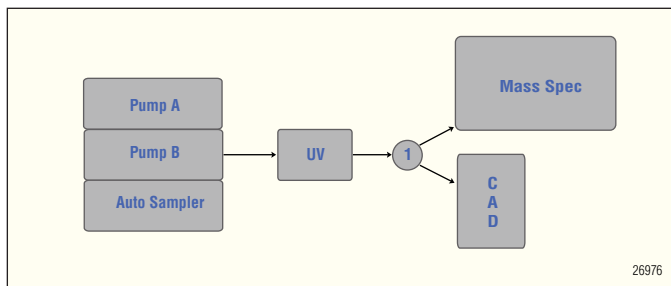


Figure 4. Schematic of the instrumentation setup used for the analysis. The 1 represents an adjustable flow splitter set to deliver 80 mL/min to the Shimadzu mass spec and 0.42 mL/min to the Corona CAD when running the method at 0.5 mL/min total flow.

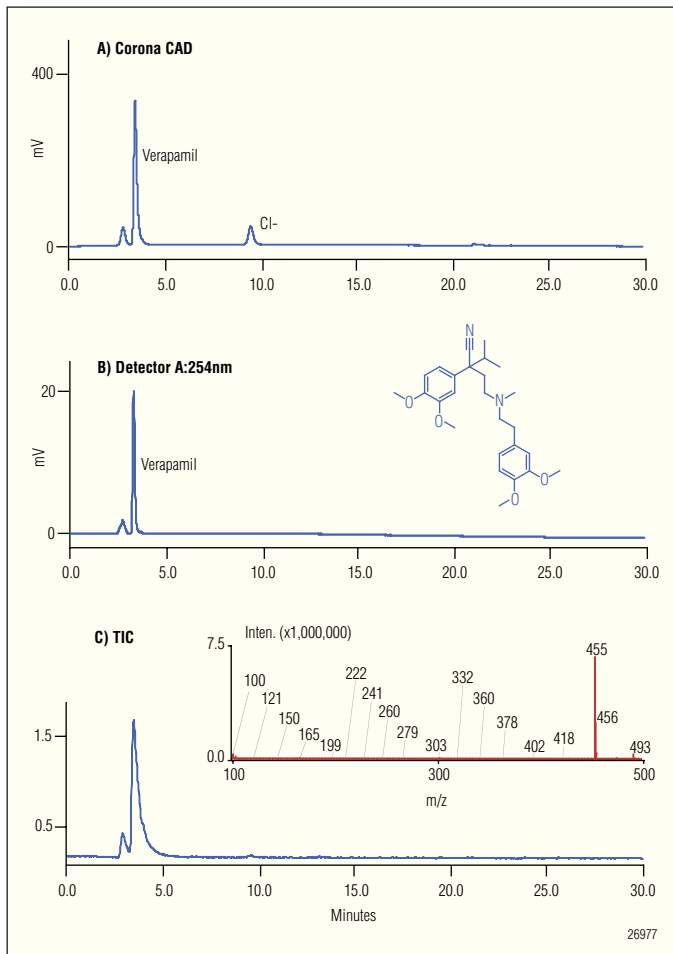


Figure 5. Verapamil hydrochloride at ~1.2 µg on column (a) Corona CAD detection 100 pA full scale. (b) UV/Vis detection at 254 nm with structure shown. (c) TIC mass spectrum scanning from 100-500 in positive ion mode. (Inlay) Mass spectrum for verapamil (MW=455.6) retention time 3.6 min.

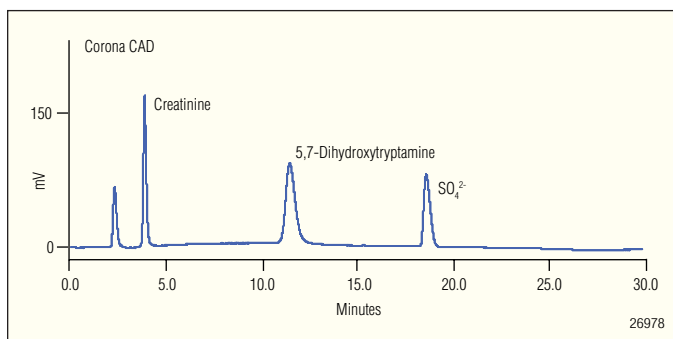


Figure 6. 5,7-Dihydroxytryptamine creatinine sulfate salt at ~2 µg on column with Corona CAD detection 100 pA full scale.

## ANALYSIS OF APIS AND COUNTERIONS

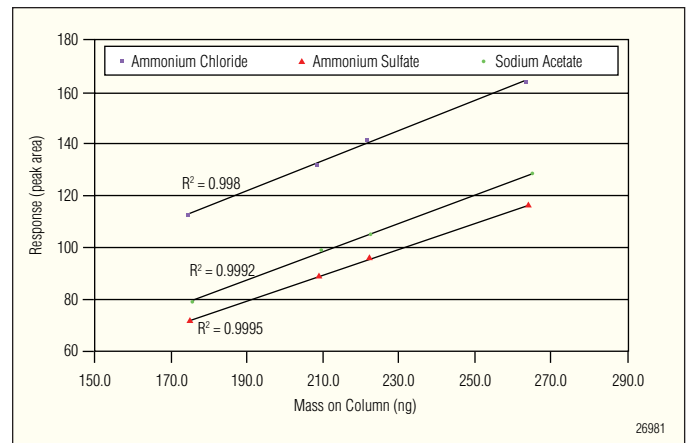


Figure 7. Target concentration curves for counterion analysis used for chloride, sulfate, and sodium.

Table 1. Counterion Comparison to Theoretical Values for APIs

Active Pharmaceutical Ingredient	Counter-ion	k' (relative retention)		Counterion %	
		API	Counterion	Theoretical	Experimental
Verapamil Hydrochloride	Cl <sup>-</sup>	0.27	2.4	7.2	7.0
Procainamide Hydrochloride	Cl <sup>-</sup>	1.3	2.4	13	12.7
Dextromethorphan Hydrobromide	Br <sup>-</sup>	N/D	2.8	21.3	22.4
Quinine Sulfate Dihydrate	SO <sub>4</sub> <sup>2-</sup>	0.86	7.0	12.3	11.9
Diclofenac Sodium Salt	Na <sup>+</sup>	0.11	6.0	7.2	7.2
Enalapril Maleate Salt	C <sub>4</sub> H <sub>3</sub> O <sub>4</sub> <sup>2-</sup>	0.06	0.5	23.6	24.3

## 0.1% ION IMPURITIES

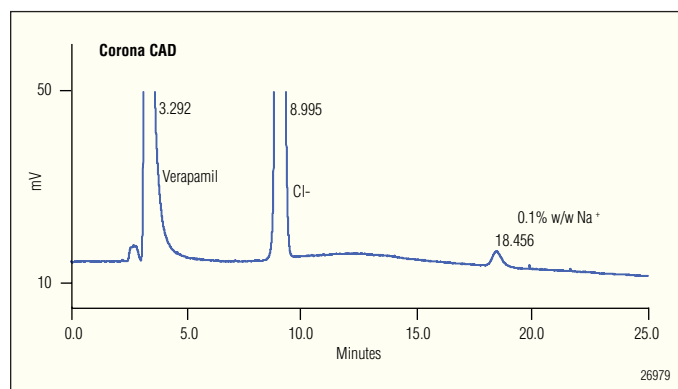


Figure 8. Chromatogram of a 10  $\mu$ L injection verapamil hydrochloride (0.7 mg/mL) in 80/20 acetonitrile/water with 0.1% by weight of sodium added.

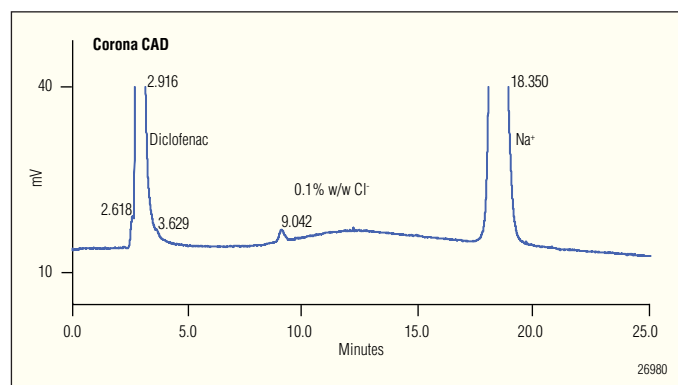


Figure 9. Chromatogram of a 10  $\mu$ L injection diclofenac sodium salt (0.3 mg/mL) in 80/20 acetonitrile/water with 0.1% by weight of chloride added.

## DISCUSSION

As shown in Figure 1, this system, using a ZIC-pHILIC column and the CAD, enabled the detection and partial resolution of 10 anions and three cations. The same single gradient system was able to resolve seven organic acids and five organic bases (presented in Figures 2 and 3, respectively). These data demonstrate the ability to measure eight of the top ten most common counterions in a single method on a single HPLC system.

The HPLC-Corona CAD system can be used to measure a large range of organic compounds and ionic salts. However, it can also be used in conjunction with other detectors to provide an orthogonal technique to more fully qualify the composition of a sample. For example, the multi-detector platform, presented in Figure 4, was used to characterize the composition of a verapamil hydrochloride sample (Figure 5) and a dihydroxytryptamine creatinine sulfate sample (Figure 6). In these examples, the Corona CAD detected all components in the sample, whereas the UV and the MS detected only the API. The MS data was used to better qualify the API. Such information may be used to assist the method development process.

The experimental percentages of the counterion for five APIs were calculated using calibration curves bracketed around the target concentration of the counterion. This allows a simple, linear regression ( $R^2 \geq 0.998$  for all ions) to be used for the analysis of API counterions (Figure 7). The calculated experimental values for counterions correlated to within 5% of the theoretical values for all the components tested (see Table 1).

Regulatory authorities, including ICH and USFDA, are placing greater emphasis on purity requirements and identification of all analytes and impurities contained in a formulation. This gradient HILIC-CAD approach is well suited for the measurement of low-level impurities. For example, Figures 8 and 9 show the measurement of low-level counterion impurities at the 0.1% w/w level.

## CONCLUSION

- A single chromatographic method enabled quantitative analysis of inorganic and organic counterions, API, and impurities at the 0.1% w/w level.
- The Corona CAD approach is able to measure both anions and cations simultaneously on a single platform. It offers both time and cost savings when compared to conventional ICCD approaches.
- The Corona CAD method uses conditions compatible with LC-MS. An HPLC-CAD-UV-MS platform can be used to more fully characterize a sample.

## REFERENCES

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2. Risley, D.S.; Pack, B.W. Simultaneous Determination of Positive and Negative Counterions Using a Hydrophilic Interaction Chromatography Method. *LC-GC* **2006**, *24*, 776-785.

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