Generic Approach to Analysis of Pharmaceutical Salts Including Inorganic and Organic Counter-ions

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Abstract

The characterization of pharmaceutical salts is critical to the drug development process, for example, in salt selection, confirmation of stoichiometry and detection of impurities. In general, multiple analytical techniques are required to address the diverse nature of analytes (inorganic, organic, anionic, cationic, absorbtivity, hydrophobicity, etc.). The objective of this study was to investigate the use of HPLC with Charged Aerosol Detection (CAD) as a generic approach to this broad range of analytes. A zwitterionic stationary phase was operated in HILIC mode using a acetonitrile-water mobile phase pH buffered with ammonium acetate. CAD response was obtained for all analytes, with or without a chromophore. Our initial studies demonstrated the ability to analyze commonly used inorganic positive and negative counter-ions simultaneously along with several active pharmaceutical ingredients (APIs). Limits of Detection were in the low ng range with linear response over at least 3 orders of magnitude and intra-assay precision <3% RSD. This has recently been extended to include measurement of organic counter-ions including acids (maleic, succinic, fumaric, tartaric) and bases (choline, Tris, lysine). These data demonstrate the capability to quantify a wide range of analytes that are relevant to pharmaceutical formulation development and characterization.

Introduction

The need to analyze inorganic cations and anions stretches across many fields, ranging from pharmaceutical formulations and product characterization, to environmental analysis. The use of ion chromatography with a conductivity detector (ICCD) for the analysis of inorganic anions is the most common technique employed today. Ion chromatography techniques by their very design do not permit the simultaneous analysis of anions and cations in a single run. Due to time requirements to change an IC system from one ion to another, many laboratories accept the expense of having dedicated, platform-dependent instruments for each suite of analytes

One of the fields requiring sensitive and reproducible methods for the analysis of ions, is the pharmaceutical industry. Their use of inorganic counter-ions is now an important part of the drug development process. The salt formation is used to selectively alter physicochemical characteristics of the drug, such as solubility, stability, and hygroscopicity. According to the data from the Cambridge Structural Database presented in Haynes *et. al.* the top ten most common counter-ions in ranked order are: chloride, bromide, nitrate, ammonium, sulfate, tosylate, phosphate, tartrate, ethylenediamine, and maleate.[1]

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.[2] In this study a binary gradient system with a Sequant polymeric Zwitterionic column (ZIC-pHILIC) separates inorganic cations and anions, organic acids and bases as well as APIs and there respective counter-ions. Once separated, anions and cations can then be measured simultaneously using the universal Corona® charged aerosol detector (CAD®). Examples are illustrated for each compound class, as well as calculations of counter-ion concentration versus their theoretical values. Additionally, the Corona CAD has the dynamic range and sensitivity to detect ionic impurities to the 0.1% level. All the data presented here were obtained without changing mobile phase composition or column.

Inorganic Ions

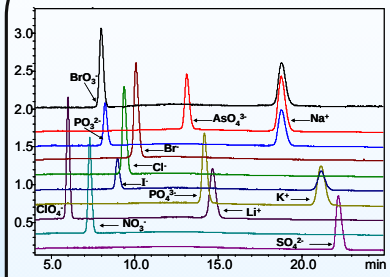

Figure 1: Overlays of 10 anions and 3 cations (10µL injections of ~25ppm salt solutions) analyzed using gradient method.

Organic Acids

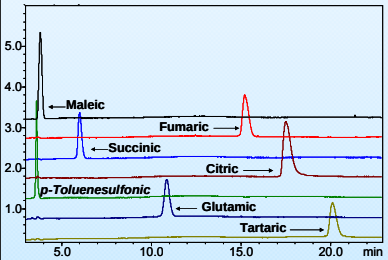

Figure 2: Overlays of 7 organic acids (10µL injections of ~60ppm solutions) analyzed using gradient method.

Organic Bases

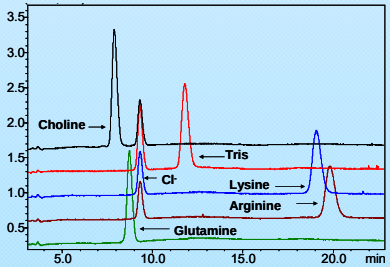

Figure 3: Overlays of 5 organic bases (10µL injections of ~60ppm solutions) analyzed using gradient method.

Method Conditions

Column	Sequant ZIC-pHILIC; 4.6 x 150mm, 5µm
Column Temperature	30ºC
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	T=0min 20%B, T=3min 20%B, T=24min 70% B, T=26min 70%B, T=32min 15%B, T=34min 20%B, T=40min %B
Corona	100pA range, no filter
Sample Vial	Polypropylene

CAD-MS Method

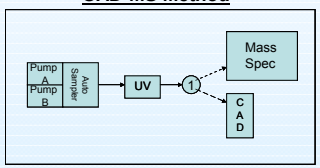

Figure 4: Schematic of the instrumentation setup used for the analysis. The 1 represents an adjustable flow splitter set to deliver 80µL/min to the Shimadzu Mass Spec and 0.42mL/min to the Corona CAD when running the method at 0.5mL/min total flow.

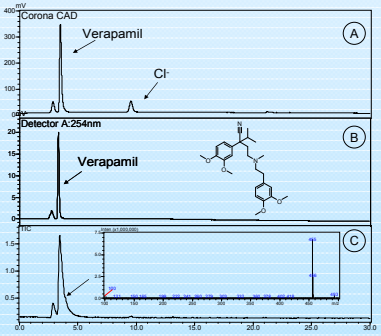

Figure 5: Verapamil Hydrochloride at ~1.2µg on column **(A)** Corona CAD detection 100pA full scale. **(B)** UV/Vis detection @254nm with structure shown. **(C)** TIC mass spectrum scanning from 100-500 in positive ion mode. (Inlay) Mass spectrum for Verapamil (MW=455.6), retention time 3.6 min.

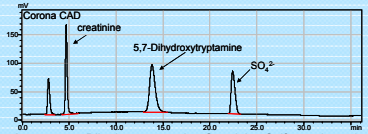

Figure 6: 5,7-Dihydroxytryptamine Creatinine Sulfate salt at ~2µg on column with Corona CAD detection 100pA full scale

Analysis of APIs and Counter-Ions

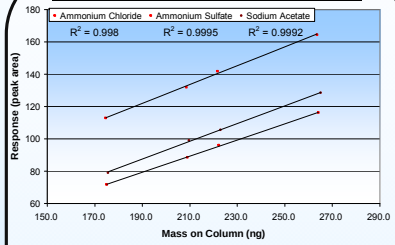

Figure 7: Target concentration curves for counter-ion analysis used for chloride, sulfate and sodium.

Table I: Counter-Ion Comparison to Theoretical Values for APIs

Active Pharamceutical Ingredient	Counter Ion	k' (relative retention) API	k' (relative retention) Counter Ion	Counter Ion % Theoretical	Counter Ion % Experimental
Verapamil Hydrochloride	Cl^-	0.27	2.4	7.2	7.0
Procainamide Hydrochloride	Cl^-	1.3	2.4	13	12.7
Dextromethorphan Hydrobromide	Br^-	N/D	2.8	21.3	22.4
Quinine sulfate Dihydrate	SO_4^{2-}	0.86	7.0	12.3	11.9
Diclofenac Sodium Salt	Na^+	0.11	6.0	7.2	7.2
Enalapril Maleate Salt	$C_4H_3O_4^{2-}$	0.06	0.5	23.6	24.3

0.1% Ion Impurities

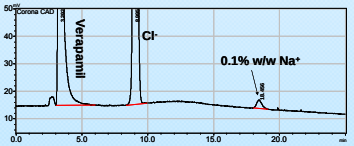

Figure 8: Chromatogram of a 10µL injection Verapamil Hydrochloride (0.7mg/mL) in 80/20 Acetonitrile/Water with 0.1% by weight of Sodium added.

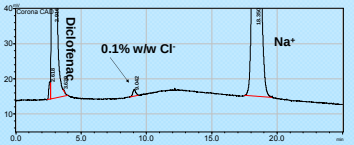

Figure 9: Chromatogram of a 10µL injection Diclofenac Sodium Salt (0.3mg/mL)in 80/20 Acetonitrile/Water with 0.1% by weight of Chloride added.

Discussion

As shown in Figure 1, this method using a ZIC-pHILIC column and the CAD provides detection and partial resolution of 10 anions and 3 cations. The same single gradient system is also able to resolve 7 organic acids and 5 organic bases (presented in Figures 2 and 3, respectively). These data demonstrate the ability to measure 8 of the top 10 most common counter-ions 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 that can used 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 chloride sample (Figures 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 counter ion for five APIs were calculated using standardization curves bracketed around the target concentration of the counter-ion. This allows a simple linear fit technique (R^2 ≥0.998 for all ions) to be used for the quantitation of API counter ions (Figure 7). The calculated experimental values for counter-ions 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 contained in a formulation. The gradient HILIC-Corona CAD approach is well suited for the quantitation of both counter-ions and low level ionic impurities. For example, Figures 8 and 9 show the measurement of low level counter-ion impurities at the 0.1% w/w level.

Conclusion

- A single chromatographic method provides quantitative analyses of inorganic and organic counter-ions, API salts, 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. The HPLC-CAD-UV-MS platform can be used to more fully characterize a sample.

References

1) D.A. Haynes, W. Jones, and W.D.S Motherwell. Occurrence of pharmaceutically acceptable anions and cations in the Cambridge structural database. J. Pharm Sci. **94:** 2111-2120 (2005).
2) D.S. Risley and B.W. Pack. Simultaneous determination of positive and negative counter-ions using a hydrophilic interaction chromatography method. LC-GC **24:** 776-785 (2006).