

Measurement of Ionic Impurities at <0.1% w/w Level by HILIC-CAD

Early studies of the salt and polymorphic form of a research compound are an important part of the information required for candidate selection of the optimum drug substance. Once the appropriate candidate is selected, various impurities may arise during manufacture and/or subsequent storage. These impurities can arise from reagents, ligands, catalysts, and equipment. The source of water and type of reactors and other processing materials (celite, activated carbon) are also important. The evaluation of potential impurities in a drug candidate is often related to organic compounds, residual solvents, and inorganic impurities such as counter-ion impurities.

Various authorities like ICH, USFDA, US and European Pharmacopoeia, Canadian Drug and Health Agency are placing emphasis on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's). The ICH Guideline on Specifications (Q6A) lists the measurement of impurities that should always be conducted on both drug substance and product. The concepts and thresholds adopted by the FDA and European Pharmacopoeia are found in ICH Q3A (R2) for reporting, identification and quantitation of these impurities. Even minute amounts of impurities may influence efficacy, safety and side effects of the drug. Impurity profiling is increasingly more important and regulatory bodies are including limits to allowable levels of impurities present in the APIs [Roy, (2002) AAPS PharmSciTech., **3**, 1-8]. Detection, quantitation and profiling of these impurities and any others is thus critical. This application note describes the use of a zwitterionic stationary phase operated in hydrophilic interaction chromatography (HILIC) mode [see Risley and Pack, (2006) LCGC, **24**, 776-785] with a Corona[®] Charged Aerosol

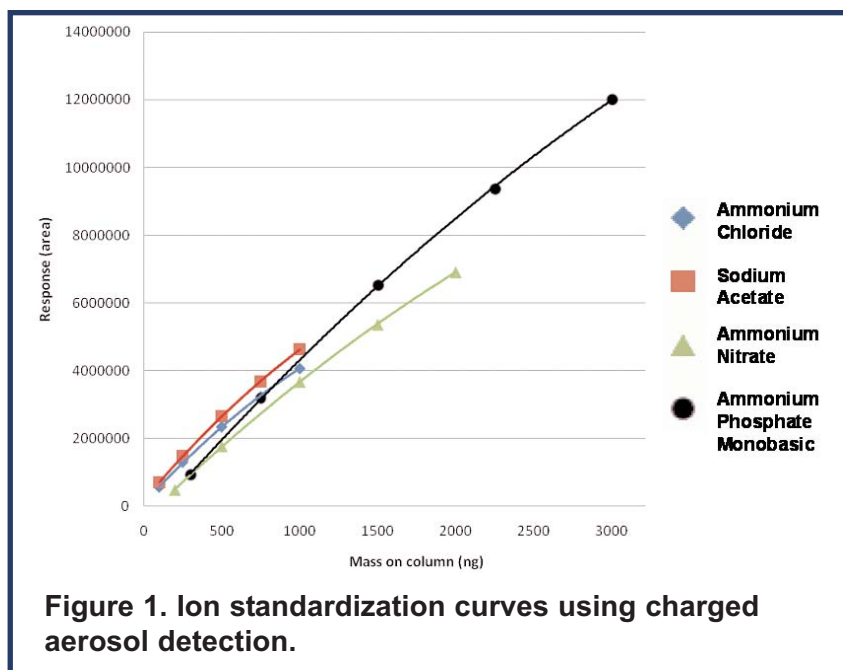


Figure 1. Ion standardization curves using charged aerosol detection.

Detector (CAD[®]), for the measurement of inorganic, ionic impurities in solutions of APIs. In the first example, chloride acting as a representative impurity, was spiked into a commercial preparation of Diclofenac sodium salt to examine the lowest level of ionic impurity that could be measured using this approach. Secondly, the impurity sodium was determined in different commercially available API-hydrochloride salts.

Method Parameters

Column:	ZIC [®] -pHILIC; 4.6 x 150mm, 5µm
Column Temp:	30°C
Mobile Phase:	A) 75:25 acetonitrile/100mM ammonium acetate (pH 7.00) or B) 70:30 acetonitrile/100mM ammonium acetate (pH 7.00)
Flow Rate:	1.0mL/min
Injection Volume:	10µL
Run Time:	10mins
Corona:	100pA range, no filter
Sample Vial:	Polypropylene or certified borosilicate

Sample Preparation

Chloride (ammonium chloride) and sodium (sodium acetate) ions were prepared in mobile phase. All subsequent dilutions were in mobile phase. Diclofenac sodium salt (D6899) was obtained from Sigma-Aldrich (St. Louis, MO) and was prepared in mobile phase at 1.0mg/mL. Chloride (as ammonium chloride) was spiked into the diclofenac sodium salt solution at a level of 0.1% (w/w). Adenine hydrochloride (A8751) and verapamil hydrochloride (V4629), both from Sigma, were prepared as individual stock solutions (1mg/mL) in mobile phase.

Results and Discussion

The measurement of the API and sodium counter-ion (Figure 2) used the same conditions described in Application Note #70-8290, "Simultaneous Measurement of An Anion and Cation". Analytes were separated in less than eight minutes, showed good correlations (Figure 1), and both inter- and intra-day precision (Table 1). The limit of detection was <20ng (on column). A chromatogram of the Diclofenac sodium salt solution spiked with chloride impurity at 0.1% v/v is presented in Figure 2. Mobile phase A was modified slightly, by increasing the amount of water, in order to improve the peak shape of both sodium and chloride. As shown in Table 2, the actual spike level was

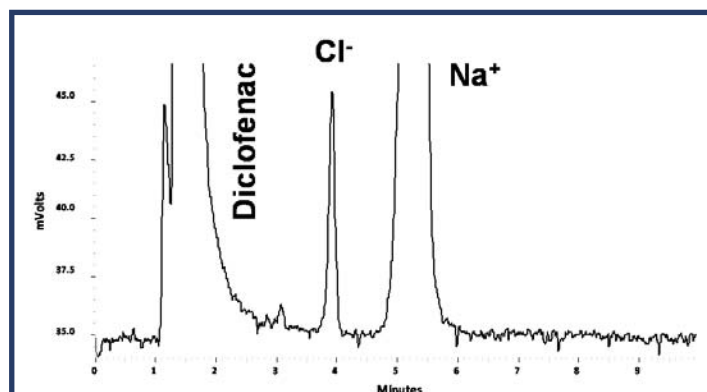


Figure 2. Chromatogram of diclofenac and sodium counter-ion spiked with chloride (as ammonium salt) at 0.1% w/w.

found to be 0.11% v/v. With a signal/noise ratio of 20:1, it is theoretically possible to measure impurities at the 0.05% level (s/n = 10)*, well below the current mandate. The HILIC-CAD approach was also used to measure sodium impurity in two commercially available hydrochloride salts: Adenine and Verapamil. The actual % impurity of sodium in Adenine HCl and Verapamil HCl, was found to be 0.11% and 0.28%, respectively (Table 3).

*NB: LOQ, S/N = 10, LOD, S/N = 3 (detectable, but not for quantitation).

Nitrate				Chloride			
Correlation	Injection	Interday %RSD	Intraday %RSD	Correlation	Injection	Interday %RSD	Intraday %RSD
0.999	1µg	0.52	0.95	0.999	1µg	0.23	1.13
	100ng	1.59	0.44		100ng	0.89	1.03
Phosphate				Sodium			
Correlation	Injection	Interday %RSD	Intraday %RSD	Correlation	Injection	Interday %RSD	Intraday %RSD
0.999	3µg	0.47	0.43	0.999	2µg	0.36	0.02
	300ng	0.88	0.07		200ng	2.09	1.78

Table 1. Correlation and precision data for ions.

The Corona[®] Charged Aerosol Detector

% Na ⁺ by mass of salt	7.2
Reported % Na ⁺	5-9
Determined % Na ⁺	6.6
Signal/noise ratio sodium	84
Theoretical % Cl ⁻	0.1
Determined % Cl ⁻	0.11
Signal/noise ratio chloride	20

Table 2. Theoretical (added) and determined (found) levels of chloride impurity spiked into diclofenac sodium salt standard.

Conclusions

These data demonstrate that HILIC HPLC with CAD is highly versatile and can be used to measure the API, its counter ion, and ionic impurities at <0.1% levels. Levels of salt impurities were identified in manufactured products using this technique, as demonstrated in Table 3.

Ordering Information

Corona *ultra*[™] Charged Aerosol Detector 70-8773
Nitrogen generator 70-6003

Example	% Chloride by Mass of salt	Determined % Chloride	Determined % Sodium Ion Impurity %
Adenine HCl	20.7	19.1	0.11
Verapamil HCl	7.20	8.60	0.28

Table 3. Measurement of sodium ion impurity in adenine and verapamil standards.



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