Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 809–814

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of inorganic pharmaceutical counterions using hydrophilic interaction chromatography coupled with a Corona® CAD detector

Z. Huang*, M.A. Richards, Y. Zha, R. Francis, R. Lozano, J. Ruan

Bristol-Myers Squibb, Research and Development, 1 Squibb Drive, New Brunswick, NJ 08901, USA

ARTICLE INFO

Article history:
Received 27 April 2009
Received in revised form 18 June 2009
Accepted 19 June 2009
Available online 27 June 2009

Keywords:
Counterion analysis
HILIC
Charged aerosol detection
Pharmaceutical
Chromatography

ABSTRACT

A simple generic approach was investigated for the determination of inorganic pharmaceutical counterions in drug substances using conventional high performance liquid chromatographic (HPLC) instruments. An intuitive approach combined Corona® charged aerosol detection (CAD) with a polymer-based zwitterionic stationary phase in the hydrophilic interaction chromatography (HILIC) mode. Two generic methods based on this HILIC/CAD technique were developed to quantitate counterions such as Cl^-, Br^-, SO_4^{2-}, K^+, Ca^{2+} and Mg^{2+} in different pharmaceutical compounds. The development and capability of this HILIC/CAD technique analysis were examined.

HILIC/CAD was compared to ion chromatography (IC), the most commonly used methodology for pharmaceutical counterion analysis. HILIC/CAD was found to have significant advantages in terms of: (1) being able to quantitate both anions and cations simultaneously without a need to change column/eluent or detection mode; (2) imposing much less restriction on the allowable organic percentage of the eluents than IC, and therefore being more appropriate for analysis of counterions of poorly water-soluble drugs; (3) requiring minimal training of the operating analysts. The precision and accuracy of counterion analysis using HILIC/CAD was not compromised. A typical precision of <2.0% was observed for all tested inorganic counterions; the determinations were within 2.0% relative to the theoretical counterion amount in the drug substance. Additionally, better accuracy was shown for Cl^- in several drug substances as compared to IC. The main drawback of HILIC/CAD is its unsuitability for many of the current silica-based HILIC columns, because slight dissolution of silica leads to high baseline noise in the CAD detector.

As a result of the universal detection characteristics of Corona® CAD and the unique separation capabilities of a zwitterionic stationary phase, an intuitive and robust HPLC method was developed for the generic determination of various counterions in different drug substances. HILIC/CAD technique is a useful alternative methodology, particularly for determination of counterions in low-solubility drugs.

1. Introduction

Many drug substances are developed in salt form for improvement of solubility, physicochemical stability, processing properties and biopharmaceutical characteristics without altering their basic chemical structures [1–4]. The content of the salt counter ion usually needs to be determined as part of release testing of the drug substance for use in clinical supplies. Therefore, analytical methodologies are required to measure the counterion content in the drug substance during pharmaceutical development.

Several techniques for inorganic counterion analysis exist, including potentiometric titration [5,6], liquid chromatography with indirect UV and evaporative light scattering detection (ELSD) [5–11], capillary electrophoresis with indirect UV or conductivity detection [12–15], ion chromatography (IC) with conductivity detection [6,16–20], and spectroscopic methods such as inductively coupled plasma-atomic emission spectrometry (ICP-AES) [21]. IC is the most commonly used methodology for quantitating pharmaceutical counterions, but it may require extensive method development for each new application. In order to analyze a wide variety of counterions that commonly occur in pharmaceutical salts, a typical IC procedure requires separate conditions: different columns, mobile phases, and detection modes. IC instruments have more restrictions on mobile phase compositions and sample diluents as compared to conventional chromatography, and the conditioning and changeover time for IC is usually lengthy. The ideal counterion method would be able to determine a variety of ions, be adaptable to any drug substance analyte regardless of its solubility, use non-specialized and cost-effective instrumentation, and employ generic experimental conditions for maximum flexibility. Based on our previous successful experiences with Corona® charged aerosol detector (CAD) and hydrophilic interaction chromatography (HILIC), we investigated these two techniques for their applicability to counterion analysis and found that using them

* Corresponding author. Tel.: +1 732 227 5419.
E-mail address: zongyun.huang@bms.com (Z. Huang).

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doi:10.1016/j.jpba.2009.06.039

in combination resulted in satisfying all the criteria described above.

Corona® CAD, an aerosol-based detector, has become increasingly useful in the analytical chemistry field [23–33]. In Corona® CAD, non-volatile analytes are nebulized into aerosol particles that are charged subsequently with an ionized gas (typically nitrogen). After passing through a negative ion trap to remove excessive cations (mainly the charged gas molecules), the charged particles are transferred into a sensitive electrometer in which the number of electrical impacts is measured. Thus, CAD response is proportional to the number of similar-sized analyte particles that reflect the analyte mass in the original sample. It has been reported that Corona® CAD can provide greater sensitivity, wider linear response range and better precision than other aerosol-based detectors such as ELSD [22,23,30]. Moreover, Corona® CAD is quite user-friendly since it does not require any optimization of operating parameters; therefore it can be operated by any chromatographer without significant additional training [30].

A generic chromatographic method for counterions should be able to accommodate any ions regardless of their charge type. A zwitterionic stationary phase, containing both negative and positive functional groups, has been used to retain both cations and anions. The retention mechanism of this type of stationary phase, known as electrostatic ion or zwitterionic chromatography, is somewhat complicated and has not been completely understood. The mechanism proposed by Hu and Cook [34–36] has been gaining acceptance and is summarized as follows. The retention of ionic analytes generally follows the principles of both cation and anion exchanges. The retention of anions depends on cation concentration in the mobile phase. This is because the association of cations in the mobile phase with negatively charged functionality on the stationary phase improves the access of anionic analytes to interior cationic centers. The retention mechanism is further complicated when the separation is operated in HILIC mode, which employs polar stationary phases with mixed aqueous–organic mobile phases to establish a stagnant enriched water layer around the polar stationary phase, allowing analytes to partition between the two phases based on polarity. The partitioning function between the two phases permits easier access for anionic analytes to the positively charged group, hence enhancing retention of anions.

Risley and Pack [11] reported a comprehensive investigation on how the organic composition, pH, and buffer concentration of the mobile phase impacted retention of ionic analytes using zwitterionic stationary phase in HILIC mode. The effect of the mobile phase organic strength in HILIC mode is generally opposite to that of reversed phase chromatography. As the percentage of organic in the HILIC mobile phase is increased, the retention times of both anions and cations are increased. In addition, the resolution between the ions typically increases with increasing organic composition in isocratic mode. They also found that pH value of the mobile phase containing ammonium acetate or formate plays an important role in the zwitterionic retention mechanism. When pH decreases, cations elute more rapidly following cationic exchange principles. In the case of anions, in addition to standard anion-exchange mechanism, the increasing protons in the mobile phase also reduce the repulsion effect by the negatively charged groups of the stationary phase and provide easier access for anions to interact with the positively charged groups. Hence, anionic analytes are retained longer at lower pH. Buffer composition (e.g., ammonium acetate versus formate) and concentration in the aqueous fraction also have a significant impact on retention and peak shape of ions. When buffer concentration was increased from 0.01 M to 0.2 M, anions eluted more slowly and cations were not retained as long. The symmetry of peak shapes worsened with very low (0.01 M) or very high (0.2 M) buffer concentration. Risley's study suggested that a buffer concentration between 0.05 M and 0.1 M was optimal for these analyses.

Directed by these principles, we combined the detection technique of Corona® CAD and zwitterionic chromatography in HILIC separation mode (HILIC/CAD technique) to develop a generic and user-friendly chromatographic method to analyze different counterions in a variety of drug substances without changing stationary phase, eluent, or detection mode. Our study on HILIC/CAD technique was first presented at CAD Symposium 2007 and HPLC Symposium 2008. The application of this technique was subsequently examined by Santiago et al. [37]. This paper describes the development and capability of the HILIC/CAD technique for pharmaceutical counterion analysis and compares its results to those obtained using IC in detail.

2. Experimental

2.1. Materials

ACS reagent grade chemicals were used unless otherwise indicated. High purity grade (>99.5%) sodium chloride, potassium chloride, sodium bisulfate, calcium chloride, sodium hydrogen sulfate, potassium phosphate monobasic, and ammonium nitrate (Sigma–Aldrich, St. Louis, MO) were used to prepare standard solutions of inorganic ions. The drug substances were provided by Bristol-Myers Squibb Process R&D (New Brunswick, NJ). Water purified through a Barnstead purification unit (Barnstead International, Dubuque, IA) was used to prepare HPLC mobile phases and diluents. Ammonium acetate, acetic acid (J.T. Baker, Phillipsburg, NJ), ammonium formate, formic acid (Fluka, St. Gallen, Switzerland), and HPLC grade acetonitrile (J.T. Baker) were used for the preparation of mobile phases and diluents.

2.2. Chromatographic conditions

The HPLC system used was a Waters Alliance (Waters Corporation, Milford, MA) in-line with an ESA Corona® charged aerosol detector (Corona® CAD, ESA Inc., Chelmsford, MA). Chromatographic data were recorded and processed using Waters Empower software. ZIC-*p*HILIC columns (150 or 50 mm × 4.6 mm, 5 μm) from SeQuant (Umea, Sweden) were used for the separation. The mobile phase flow rate was set at 1.0 mL/min and injection volumes of 10 μL were used. Although gradient elution was evaluated, a typical analysis was performed under isocratic conditions with a mobile phase of buffer–acetonitrile (25:75). The buffer was composed of 0.1 M ammonium acetate or formate; pH was adjusted with acetic or formic acid. Depending upon the specific application, the organic composition of mobile phase and buffer pH were varied as indicated in the text. Columns used for the evaluation of this application were: SeQuant ZIC HILIC column (150 mm × 4.6 mm, 3.5 μm), SeQuant ZIC-*p*HILIC (150 mm × 4.6 mm, 5 μm). Waters Atlantis HILIC (150 mm × 4.6 mm, 3 μm), and Phenomenex Luna HILIC column (150 mm × 4.6 mm, 5 μm).

2.3. Standard and sample preparation

Unless otherwise indicated, all standard and sample solutions were prepared in diluents that consisted of 0.1 M buffer (either ammonium acetate or formate):acetonitrile (50:50). The pH values of the buffers were consistent with the buffers that were used to make the mobile phases.

The standard solutions were prepared using the inorganic salts that contained the anions or cations to be analyzed. In a typical preparation, three working standard solutions at levels of 80%, 100%, and 120% of nominal standard concentration were prepared from a standard stock solution. The standard calibration

curve was calculated by least-squares regression analysis of peak area versus concentration. The drug substance samples were accurately weighed and diluted with diluent. The sample weights were determined by matching the standard range to the theoretical concentrations of the counterions. The counterion was quantitated by comparing the peak area versus the standard curve.

3. Results and discussion

3.1. Method development

The Corona® CAD detector requires volatile mobile phases to analyze non-volatile or semi-volatile analytes. The presence of ammonium acetate/formate ($CH_3COO^-NH_4^+$/$HCOO^-NH_4^+$) buffer in the mobile phase is not only critical for the retention behaviors of ions but also allows the inorganic ions to form non-volatile salt particles that enhances their detectability. In the case of NaCl, the non-volatile particles of CH_3COONa and NH_4Cl are formed during the desolvation in the drying tube and subsequently detected.

As described in the introduction, various counterions can be retained and separated using HILIC stationary phases. The separation mechanism and retention order in HILIC mode are opposite to that of reversed phase chromatography. Very polar compounds including inorganic ionic species can be retained by an eluent containing a high amount of organic solvent, typically from 70% to 95%, which in fact provides a significant solubility advantage over the reversed phase mode for the analysis of counterions in poorly soluble drug substances.

To evaluate the compatibility of HILIC columns with CAD, we studied two column types categorized by their substrates: silica-based (including SeQuant ZIC-HILIC, Waters Atlantis HILIC, and Phenomenex Luna HILIC) and polymer-based (SeQuant ZIC-*p*HILIC). Fig. 1 shows a plot of Corona® CAD baseline response versus the aqueous percentage of the mobile phase using different HILIC stationary phases. The magnitude of the baseline noise varied significantly among the columns. When operating on bare silica-based columns (ZIC-HILIC and Atlantis HILIC) with eluent containing 0.1 M ammonium acetate buffer:acetonitrile (25:75), significant baseline noise of the Corona® CAD was observed, higher than 50 pAmp, which is about 10% of the maximum scale of the Corona® CAD (500 pAmp). This noise is believed to be a result of column bleeding. Unlike reversed stationary phases, where the silica substrates are well protected by a bonded organic layer (e.g. ODS), slight silica dissolution or column bleed may occur on silica-based HILIC phases [38–40]. Since Corona® CAD is a mass-dependent "universal" detector with substantial sensitivity, the dissolved silica or column bleed is easily detected, causing an increase in detector baseline response. Meanwhile, baseline levels lower than 5 pAmp were observed using a silica-based diol column (Phenomenex Luna HILIC) with the same mobile phase. Protection of the silica particles, afforded by this column's unique cross-linked diol bonded phase, likely minimized silica dissolution and column bleed.

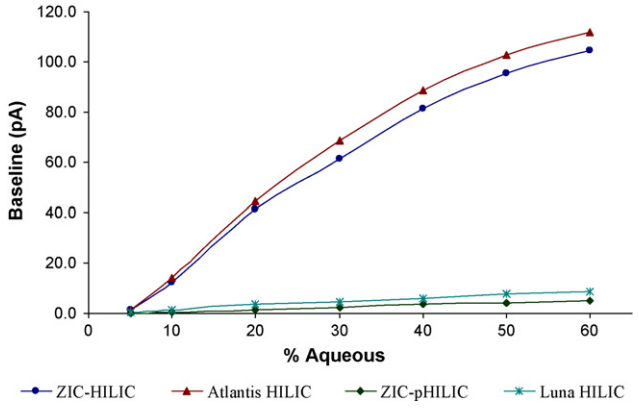

Fig. 1. Plots of Corona® CAD baseline response as a function of aqueous fraction of mobile phase that flowed through Atlantis HILIC, Luna HILIC, ZIC-HILIC and ZIC-*p*HILIC columns. Column temperature: ambient; aqueous portion of the mobile phase: 0.1 M ammonium acetate buffer at pH 7.0 or water; organic portion of the mobile phase: acetonitrile; flow rate 1 mL min^{-1}.

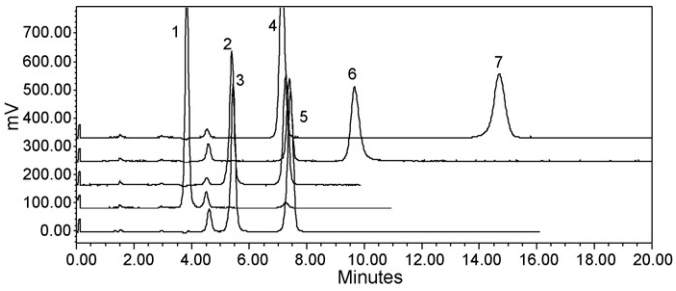

Fig. 2. Chromatographic overlay of a number of inorganic ions using SeQuant ZIC *p*HILIC (5 μm particle) 150 mm × 4.6 mm column. Column temperature 30 °C; mobile phase: acetonitrile–0.1 M ammonium acetate buffer, pH 7.0 (75:25, v/v); flow rate: 1 mL min^{-1}; injection volume: 10 μL; diluents: acetonitrile–0.1 M ammonium acetate buffer, pH 7.0 (50:50, v/v); detection: Corona® CAD. List of sample solutions (top to bottom): 1, potassium hydrogen sulfate; 2, sodium phosphate; 3, sodium bromide; 4, ammonium nitrate; 5, sodium chloride. Peak identities: 1 = nitrate, 2 = bromide, 3 = chloride, 4 = potassium, 5 = sodium, 6 = phosphate, and 7 = hydrogen sulfate.

When operating on ZIC-*p*HILIC columns, the baseline noise was negligible (<3 pA) and not affected by increasing the aqueous percentage of the eluent. This is because the substrates for ZIC-*p*HILIC stationary phase are polymeric particles and very stable in high aqueous eluent. For counterion analyses using Corona® CAD, the detector output of the ionic analyte peaks typically ranged between 100 pA and 300 pA. With the typical silica-based HILIC phases such as ZIC-HILIC and Atlantis HILIC, precision and accuracy can be significantly impacted since the detector baseline noise was above 50 pA due to the column bleed. Therefore, these two silica-based columns were less amenable for counterion analysis in combination with Corona® CAD and were not selected for further method development. Both ZIC-*p*HILIC and Luna HILIC stationary phases, for which column bleeding and baseline noise were negligible, appeared to be good candidates for this application and were thus selected for further method optimization.

The selection of mobile phase was based on the retention behaviors of inorganic ions on ZIC-HILIC column that were described in the introduction (e.g., increased retention and resolution of both anions and cations with higher organic percentage). A mobile phase of 0.1 M ammonium acetate buffer, pH 7.0:acetonitrile (25:75, v/v) was capable of retaining typical inorganic ions (NO_3^-, Cl^-, Br^-, Na^+, K^+, PO_4^{3-}, and SO_4^{2-}) in less than 15 min on a ZIC-*p*HILIC column (Fig. 2). Separation of most of these ions was achieved, except for coelution of the Br^- and Cl^- peaks and the K^+ and Na^+ peaks, respectively. For unusual cases where complete separation of these ions might be desired (e.g., both Na^+ and K^+ ions being present in a single sample, such as a fixed-dose combination product whose drug substances have different counterions), the method conditions only need to be fine-tuned by adjusting the mobile phase to ammonium formate buffer, pH 3.5:acetonitrile (20:80) while the column and other conditions remain the same (Fig. 3). For the particular analysis of multivalent ions (Ca^{2+} Mg^{2+}, SO_4^{2-} and PO_4^{3-}), which were retained considerably longer than the monovalent ions, a shorter 50-mm column with a mobile phase of pH 3.5 ammonium formate:acetonitrile (30:70) was employed for less retention (Fig. 4).

A chromatographic condition using Phenomenex Luna HILIC column with a mobile phase of pH 4.0 ammonium formate:acetonitrile

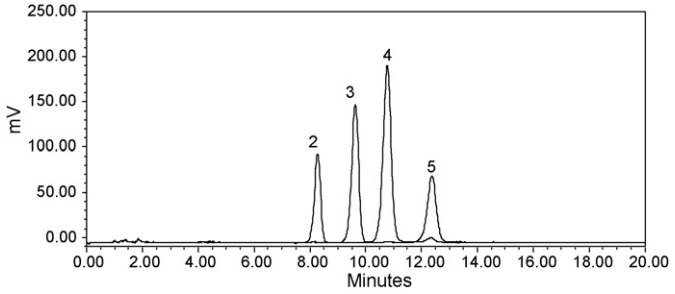

Fig. 3. Chromatographic overlay demonstrating separation of bromide (peak #2) from chloride (peak #3) anions and potassium (peak #4) from sodium (peak #5) cations. Conditions were the same as in Fig. 1 except for the mobile phase: acetonitrile–0.1 M ammonium formate buffer, pH 3.5 (80:20, v/v).

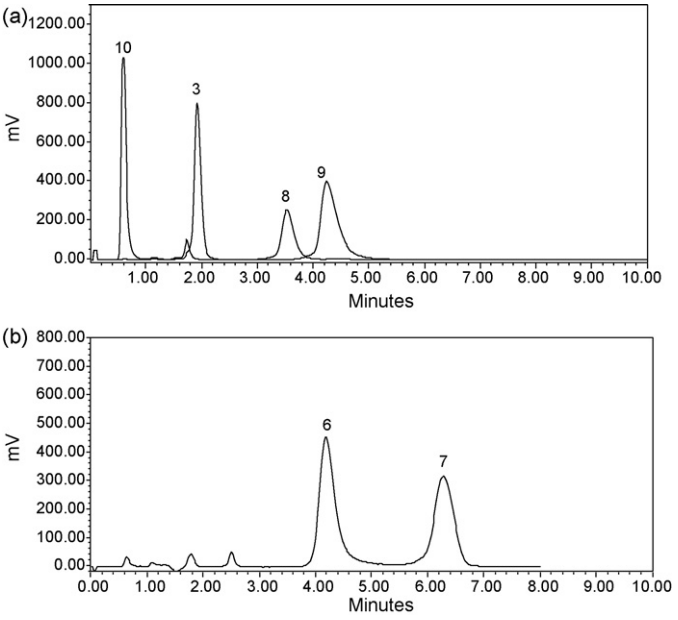

Fig. 4. Chromatograms of (a) calcium chloride and magnesium stearate, and (b) phosphate and hydrogen sulfate ions (b) using SeQuant ZIC *p*HILIC (5 μm particle) 50 mm × 4.6 mm column. Conditions were the same as in Fig. 1 except for the mobile phase: acetonitrile–0.1 M ammonium formate buffer, pH 3.5 (70:30, v/v). Peak identities: 3 = chloride, 6 = phosphate, 7 = hydrogen sulfate, 8 = magnesium, 9 = calcium, and 10 = stearate.

(30:70) was examined for its ability to retain ionic species. As shown in Fig. 5, all the cations and multivalent anions (SO_4^{2-} and PO_4^{3-}) were retained substantially while monovalent anions were only slightly retained but still eluted after the column dead time. The retention mechanism of ionic species on a silica-based HILIC phase without negative and positive functional groups may be best described as a combination of HILIC effects and ion-exchange interactions with the active silanol sites [10]. However, the peak shapes of all the ionic species on this column (Fig. 5) were inferior to those observed on the ZIC-*p*HILIC stationary phase (Figs. 2–4). As a result, the ZIC-*p*HILIC column was selected for our final method and used in all the subsequent studies.

3.2. Linearity studies

Linearity was evaluated for all of the ions that were used for counterion analysis. Although the relationship of Corona® CAD response as a function of ion concentration was quadratic at ranges of approximately two orders of magnitude, linear regressions with $r^2 > 0.995$ were achieved at a smaller range (75–125%) of the nominal concentration (typically 50 mg L^{-1}) for the inorganic ions. Fig. 6 shows a typical linearity plot that was used for counterion analysis of Cl^- at different ranges. However, the percent bias between the theoretical peak areas determined from a single point line that is forced through zero and the raw peak areas cannot be neglected. Therefore, the quantitation of counterions was performed using a three-point standard calibration curve by the equation shown in Fig. 6(b).

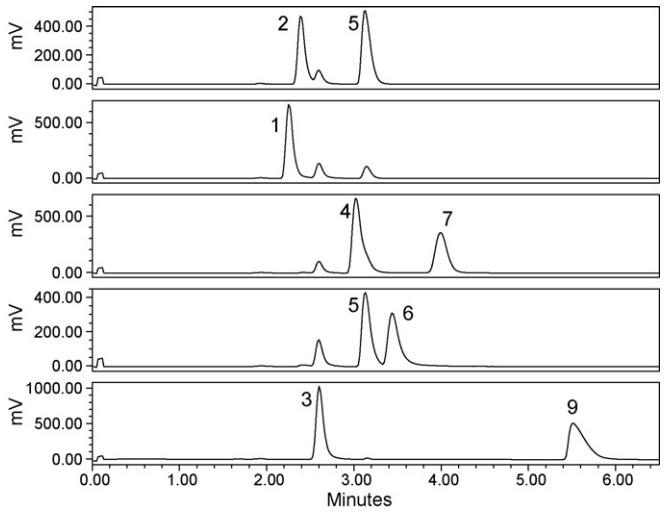

Fig. 5. Chromatogram overlays of a number of inorganic ions using Phenomenex Luna HILIC (3 μm particle) 150 mm × 4.6 mm column. Column temperature 30 °C; mobile phase: acetonitrile–0.1 M ammonium formate buffer, pH 4.0 (70:30, v/v); flow rate: 1 mL min^{-1}; injection volume: 10 μL; diluents: acetonitrile–0.1 M ammonium acetate buffer, pH 7.0 (50:50, v/v); detection: Corona® CAD. Peak identities: 1 = nitrate, 2 = bromide, 3 = chloride, 4 = potassium, 5 = sodium, 6 = phosphate, 7 = hydrogen sulfate, and 9 = calcium.

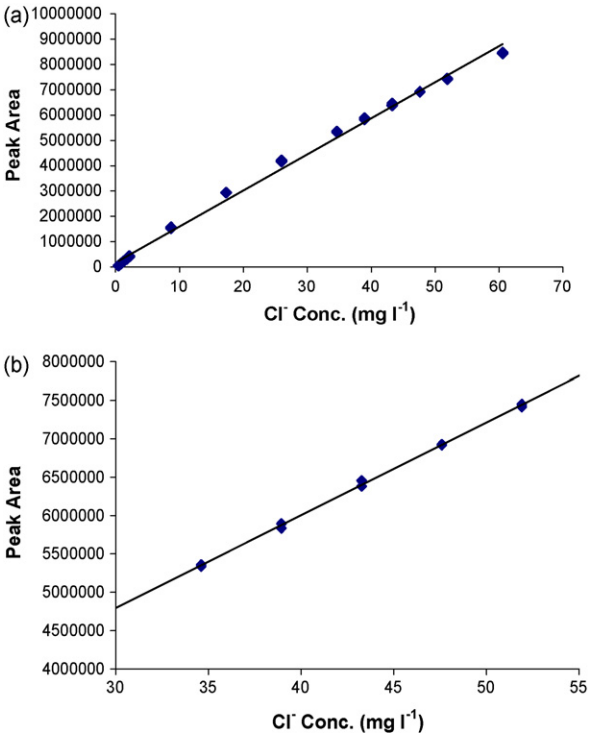

Fig. 6. Linearity plot of Corona® CAD peak response versus standard concentration of Cl^- (nominal concentration = 44 mg L^{-1}): (a) plot at Cl^- concentration range from 0.1 mg L^{-1} to 60 mg L^{-1}, $y = 142{,}376x + 18{,}673$, $r^2 = 0.996$; (b) the same plot at a smaller range from 32 mg L^{-1} to 55 mg L^{-1}, $y = 120{,}969x + 1{,}164{,}917$, $r^2 = 0.999$.

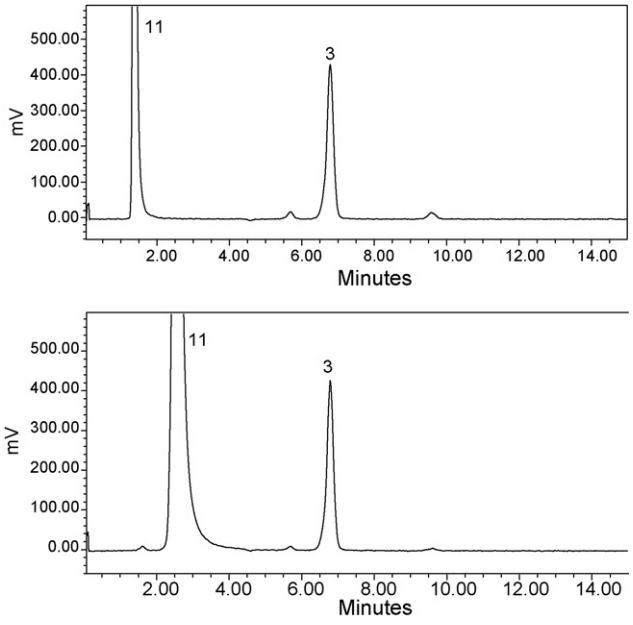

Fig. 7. Chromatograms for the analysis of chloride counterions in drug substances. Conditions are the same as in Fig. 1. (a) Compound A with concentration 500 mg L^{-1}; (b) compound B with concentration 815 mg L^{-1}. Peak identities: 3 = chloride and 11 = drug substance peak.

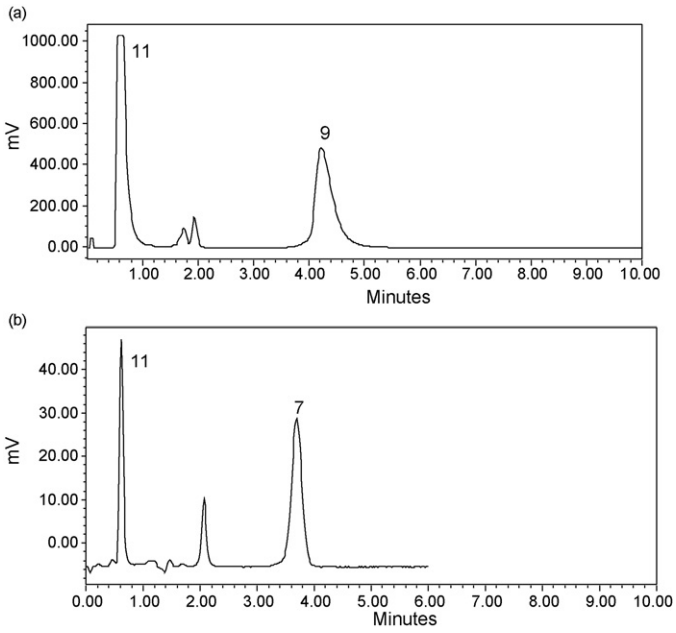

Fig. 8. Chromatograms for the analysis of calcium and sulfate counterions in drug substances. Conditions are the same as in Fig. 3. (a) Compound F with concentration 1200 mg L^{-1}; (b) an undisclosed drug substance. Peak identities: 9 = calcium, 7 = sulfate, and 11 = drug substance peak.

3.3. Counterion analysis

We conducted analyses of four different ionic species (Cl^-, K^+, Ca^{2+} and SO_4^{2-}) as counterions in six drug substances (Compounds A through F and an undisclosed drug substance) using the HILIC/CAD technique (separation by ZIC-*p*HILIC stationary phase combined with detection by Corona® CAD) and compared the results with those obtained by IC. The chromatographic conditions were selected based on the valence of the individual counterions: monovalent Cl^- and K^+ were analyzed using the chromatographic conditions described in Fig. 2, while divalent Ca^{2+} and SO_4^{2-} were analyzed using the more strongly eluting conditions in Fig. 4. Typically, the theoretical concentrations of the counterions in these drug substances were within the target standard concentration range of 30–75 mg L^{-1} for individual ions. Excellent system precision was obtained for all tested ionic species with an RSD of <2.0% for each set of six standard injections. As shown in Fig. 7, the Cl^- counterion peaks in drug compounds A and B were well separated from the drug substance peaks that were eluted early by the mobile phase of high organic strength. Similarly, the divalent Ca^{2+} and SO_4^{2-} ions were well separated from the drug substance peaks and eluted within a reasonable 6-min run time (Fig. 8).

Table 1 summarizes the accuracy results determined by HILIC/CAD in comparison with results obtained by IC for the same samples. The % recovery of counterions for all six drug substances by HILIC/CAD fell between 98% and 102% versus the theoretical concentrations, which was well within the typical acceptance criterion of 95–105% for counterion determination. The accuracy for SO_4^{2-} by HILIC/CAD was also found to be equivalent to that obtained by IC and within the acceptance criterion (data is not listed in Table 1).

HILIC/CAD generally provided better accuracy than IC, especially for the Cl^- level in Compound B where the IC result (94.8% of theory) was significantly lower than the HILIC/CAD result (99.3%). This result can be explained by the restrictions on eluents for IC instruments coupled with the poor aqueous solubility of Compound B. A typical IC method using conductivity detection with ion suppression requires the organic percentage of the eluent to be kept at the lowest level (typically less than 20%) to avoid damage to the ion suppressor membranes and minimize eluent interference on conductivity detection. When Compound B at the same concentration was introduced to the high aqueous IC eluent, it is likely that the drug substance would partially precipitate in the eluent along with a small portion of Cl^-, since the counterions sometimes tend to association with their parent compounds. Thus, low recovery was observed for the Cl^- in Compound B due to the mass

Table 1
Accuracy data for counterion analysis in various drug substances.

Compound	Counterions				
	Name	Theoretical conc. (%)	Results by ZIC-CAD		Results by IC[a] (% of theory)
			% of theory	%RSD (n = 3)	
A	HCl	9.0	98.0	0.52	97.8
B	HCl	5.4	99.3	0.32	94.8
C	HCl	7.3	98.9	0.54	97.3
D	HCl	7.7	100.0	0.68	Not determined
E	K^+	5.3	101.6	0.68	98.5
F	Ca^{2+}	3.8	98.7	0.89	98.1

[a] Data by IC was obtained from internal studies.

balance deficit. Alternatively, HILIC/CAD requires high organic percentage in the eluent (typically greater than 70%) to retain the ionic species; poorly water-soluble drug substances such as Compound B will not precipitate in such an eluent and will elute much earlier than their counterions, which is an indicator of the complete dissociation between counterions and their parent drug substances. For this reason, it is believed that the use of high organic eluents improved the accuracy of counterion analysis for Compound B. Furthermore, under IC conditions, different detection and separation modes including columns, eluents and ion suppressor had to be used to quantitate Cl^- in Compounds A, B and C, and K^+ in Compound E, which required additional preparation and lengthy system changeover time. Therefore, in addition to providing better accuracy, the obvious advantage using HILIC/CAD technique over IC is that many different counterion species over a broad range can be quantitated using a single chromatographic condition. It is also feasible to use the same method to quantitate the counterion and the parent drug substance simultaneously. As a result, this technique greatly enhances laboratory efficiency for inorganic counterion analysis.

Based on the results of this study, a generic methodology was developed for inorganic counterion analyses using a HILIC/CAD technique. Most of the commonly used inorganic counterions can be quantitated accurately by two similar chromatographic conditions coupled with Corona® CAD detection: (1) a 15-cm SeQuant ZIC-*p*HILIC (5 μm particle size) column with eluent of pH 7.0 ammonium acetate buffer:acetonitrile (25:75, v/v) for monovalent ions such as NO_3^-, Cl^-, Br^-, Na^+ and K^+; (2) a 5-cm same-type column with a mobile phase of pH 3.5 ammonium formate:acetonitrile (30:70, v/v) for multivalent ions such as Ca^{2+} Mg^{2+}, SO_4^{2-} and PO_4^{3-}. The sample preparation procedure for different drug substances can also be a generic one with the target concentration of individual ions ranging between 30 mg L^{-1} and 75 mg L^{-1}. Since HILIC/CAD technique permits the use of sample diluents that can accommodate the diverse solubilities of different compounds, sample solutions can be prepared easily at the target concentration, regardless of the drug substances that are analyzed, particularly those that are poorly water-soluble. Therefore, the task of quantitating inorganic counterions can be accomplished by a simple approach utilizing mostly conventional HPLC components and eluents in combination with the relatively novel CAD detector.

4. Conclusions

The HILIC/CAD technique was shown to be a convenient and widely applicable technique for analyzing inorganic counterions in drug substances. Compared to IC with conductivity detection, HILIC/CAD technique was easier and less expensive to implement, required much less time for method development, and provided adequate precision and overall better accuracy. Simple and generic chromatographic conditions were developed to determine a variety of inorganic counterions in different drug substances using a conventional HPLC system with a unique separation and detection mode. Operation of such a system by a typical chromatographer required no specialized training. More importantly, the high percentage of organic solvent tolerable in the HILIC/CAD mobile phase made this method more appropriate than IC for low-solubility drugs. This approach could greatly increase laboratory productivity for counterion analysis. The drawback of this technique is that Corona® CAD is a "universal" detector with substantial sensitivity that is able to detect slight silica dissolution or column bleed, which leads to high detector baseline noise. However, two HILIC stationary phases were identified that had negligible column bleeding and worked well with the CAD detection.

As a result of the universal detection characteristics of Corona® CAD and the unique separation capabilities of a zwitterionic stationary phase, intuitive and robust HPLC methods can be developed to allow generic determination of various counterions in different drug substances, as an alternative to the more commonly used methodologies. In particular, the HILIC/CAD technique is a more effective method for determination of counterions in poorly soluble drugs.

Acknowledgements

The authors gratefully acknowledge the valuable insights provided by Anne-Francoise Aubry, Pankaj Shah and Steven Klohr of Bristol Myers Squibb Co. in their review of this article.

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