Application of Ion Chromatography in Pharmaceutical and Drug Analysis

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Abstract

Ion chromatography (IC) has developed and matured into an important analytical methodology in a number of diverse applications and industries, including pharmaceuticals. This manuscript provides a review of IC applications for the determinations of active and inactive ingredients, excipients, degradation products, and impurities relevant to pharmaceutical analyses and thus serves as a resource for investigators looking for insights into the use of the IC methodology in this field of application.

Introduction

Since its introduction in 1975 (1), ion chromatography (IC) has developed and matured into an important analytical methodology that is applied in a number of diverse applications and industries. The technique, a type of high-performance liquid chromatography (HPLC), has gained popularity in laboratories for determining inorganic anions and cations, organic acids, carbohydrates, sugar alcohols, and aminoglycosides in environmental, agricultural, clinical, pharmaceutical, metal plating, power generation, semiconductor fabrication, and industrial samples. IC is a complimentary technique to the more commonly employed reversed-phase and normal-phase HPLC and atomic spectroscopic [such as atomic absorption spectrometry (AAS) and inductively coupled plasma atomic spectroscopy (ICP-AS)] techniques in pharmaceutical analysis. Broadly defined, the term ion chromatography applies to any method that combines the processes of analyte separation, accomplished via differences in ion migration, and analyte detection, accomplished via any number of applicable means. More traditionally, IC involves separations performed with a column containing an ion exchange stationary phase and detection performed by a number of means including electrochemical (for example, conductivity, amperometry, coulometry) and spectroscopic (for example, direct and indirect UV absorption and mass spectrometry) methods. Several books and chapters on IC furnish a detailed review of IC principles and instruments (2,3,4). Presently, IC-based procedures are cited in several USP monographs (see Table I: all tables appear in the appendix.), and many more IC methods have been successfully developed and validated for pharmaceutical applications (Tables II–VI). Because the technique has matured to the point that it is readily recognized and accepted as a standard method and that it can be readily and reproducibly implemented by appropriately trained technical staff members, one anticipates that as the technique gains greater acceptance and wider application the utilization of IC in USP procedures will increase. For example, IC methods coupling strong anion exchange separations with UV detection have been recently developed and adopted for the characterization of heparin for impurities whose presence in commercial products has been linked to adverse events.

This manuscript provides a review of IC applications for the determinations of active and inactive ingredients, excipients, degradation products, and impurities relevant to pharmaceutical analyses and thus serves as a resource for investigators looking for applications of the IC methodology in this field of application. This review is presented in a tabular format that highlights the major operational parameters of the IC methods utilized and summarizes the major findings summarized in the individual cited references.

Applications of IC in Pharmaceutical and Drug Analysis

As dictated by the nature of the analyte, IC has been applied to all aspects of the manufacturing and disposition of pharmaceutical products, including the characterization of drug substances and active ingredients, excipients and other "inert" product components, degradation products and/or impurities and process streams components. The following sample types are analyzed: starting raw materials, intermediates (including media and culture broths), pharmaceutical raw materials, diluents, formulated products, production equipment cleaning solutions, and waste streams. The method is especially valuable in the pharmaceutical industry for ionic analytes (in products containing non-ionic components) that have little or no native UV absorbance. However, the ability to couple the ion exchange separation with numerous detection strategies expands IC applications to instances were analyte-specific detection strategies can provide the required degree of sensitivity and/or specificity. Utilization of such strategies allows for IC applications to be implemented on appropriately configured HPLC systems. Additionally, ion exclusion separations expand the range of application of IC to non-ionic analytes of significant pharmaceutical interest including alcohols and carbohydrates. The wide dynamic range of the methodology makes it applicable for the quantitation of trace contaminants as well as major product components.

Examples of the application of IC to pharmaceutical analysis have been considered previously (5) and are provided in Tables

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II–V. While these Tables do not exhaustively capture the entire database of pharmaceutical applications of IC, they do provide relevant examples of the types of separation/detection strategies that are used in this field.

Method Validation

As IC is a liquid chromatographic method, guidelines for the validation of IC applications in the pharmaceutical industry are readily available (6–8) and most of the applications cited in this manuscript contain some degree of assay validation information. Comprehensive validation information, specifically with respect to the common validation parameters (e.g., accuracy, linearity, precision, specificity, sensitivity, and ruggedness), is summarized in Table VI for the various pharmaceutical applications of IC. Consistent with the nature of the application (trace analysis versus content/potency), the operating characteristics are similar to those that are routinely obtainable in more classical applications of liquid chromatography (e.g., HPLC).

IC is extensively employed in the environmental and food industries. As the requirements for quality in these disciplines are similar to those in the pharmaceutical industry, applications in these fields have been extensively validated, including the utilization of inter-laboratory collaborative assessments (9–16, 99–102).

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Appendix: Tables I–VI

Table I. IC in USP monographs*

Title	Test	Separation	Detection†
Amikacin	Assay	Column: Anion Exchanger, 8 µm, L47 packing, available as Dionex's CarboPac MA1; Eluent: 0.115 N NaOH	PAD
Bethanicol chloride injection	Assay and limit of 2-hydroxypropyl trimethyl ammonium chloride	Column: Weak cation Exchanger, 3 to 15 µm, L53 packing, available as Dionex's CS14; Eluent: 20 mM methanesulfonic acid	NS, CD
Erythromycin ointment	Erythromycin assay	Column: Cation Exchange, 5 to 10 µm, L47 packing, available as TSK IC SW cation from TosoHaas; Eluent: mixture of ACN, NaOH, and water	PAD
Fludeoxyglucose F18 injection	Limit of 2-chloro-2-deoxy-D-Glucose	Column: Anion Exchange, 10 µm, L46 packing; Eluent: 0.8% NaOH	PAD
Fenoldopam mesylate	Limit of iodide	Column: Anion Exchange, packing not specified; Eluent: 2.8 mM $NaHCO_3$ + 2.2 mM Na_2CO_3 + 0.8 mM 4-cyanophenol + 2% acetonitrile	S, CD
Kanamycin sulfate	Assay	Column: Anion Exchange, 8 µm, L47 packing, available as Dionex's CarboPac MA1; Eluent: 0.115 N NaOH eluent	PAD
Mg-carbonate, citric acid, and K-citrate for oral solution (proposed)	Assay for citrate	Ion Exchange separation. No further details since the assay is yet to be published.	CD
Oil and water soluble vitamins with minerals oral solution	Assay for fluoride	Column: Ion Exclusion, 7 to 11 µm, L17 packing; Eluent: 10% (v/v) Ethanol + 2 mN H_2SO_4	CD
Oil and water soluble vitamins with minerals tablets	Assay for fluoride	Column: Ion Exclusion, 7 to 11 µm, L17 packing; Eluent: 10% (v/v) Ethanol + 2 mN H_2SO_4	CD
PEG3350 and electrolyte oral solution	Assay of sodium and potassium	Column: Strong Cation Exchanger, about 10 µm, L22 packing; Eluent: 1.9 mM HNO_3 eluent	NS, CD
	Assay of chloride and sulfate	Column: Weak Anion Exchanger, about 10 µm, L23 packing; Eluent: Mixture of borate, gluconate, glycerine, and acetonitrile	
Potassium Perchlorate	Assay of perchlorate	Column: Weak Anion Exchanger, about 10 µm, L23 packing; Eluent: 10 mM phthalic acid + 10% methanol, pH 4.5	CD
Sodium fluoride F18 injection	Radiochemical purity (Purity of N18F)	Column: Ion Exclusion, 7 to 11 µm, L17 packing; Eluent: 3 mN H_2SO_4	Gamma ray detector in series with CD
Streptomycin sulfate (proposed)	Assay	Ion Exchange separation. No further details since the assay is yet to be published.	PAD
Heparin Sodium‡	Chromatographic Identity and Organic Impurities	Column: strong anion-exchange resin, L61 packing (Dionex Ion Pac As-11). Eluent = Phosphate buffer gradient	UV at 202 nm

* Information revised from reference 17.
† Detection: PAD, pulsed amperometric; NS, CD, non-suppressed conductometric; CD, direct conductometric; S, CD, suppressed conductometric
‡ See reference 103.

Table II A. Pharmaceutical Applications of Ion Chromatography, Active Ingredients

Analyte	Column	Eluent	Detection	Other	Ref.
Disodium clodronate tetrahydrate	Dionex IonPac AS7, 250 × 4 mm, 10 µm particles	40 mM HNO_3 at 0.5 mL/min	UV at 300 nm after post-column derivatization with acidic iron (III)	Stability-indicating versus degradation products with some validation data provided	18
1-(butylamino)-1-deoxy-D-glucitol*	Dionex IonPac CS3, 250 × 4 mm, 10 µm particles	10 mM HCl with 0.01 mM DL-2,3-diaminopropionic acid, 2 mL/min	Suppressed conductivity, 0.1M TBAOH† regenerant	Stability indicating versus common impurities with some validation data provided.	19
Alendronate ‡	Waters IC-PAK HR (75 × 4.6 mm, 6 µm particles) or a Dionex OmniPac PAX-100 (250 × 4 mm, 8 µm particles)	1.6 mM HNO_3 or 1.76 mM HNO_3 with 20% acetonitrile at 0.5 mL/min	Direct conductivity	Analysis in intravenous solution, validation data provided	20
Aredia §	Alltech Universal Anion	5 mM potassium nitrate, pH 3.5, 1.2 mL/min	Refractive index	Quantitation in dosage forms, stability indicating versus formulation components, impurities and degradation products	21
Alendronate sodium†, etidronate disodium#, clodronate disodium**	Waters IC-PAK HR anion (75 × 4.6 µm, 6 µm particles) and others ††	Nitric acid, or nitric acid, potassium nitrate mixtures, 0.5–1.0 mL/min	Indirect UV at 235–245 nm	Validation of method for drug analysis in tablets of i.v. formulations	22
Alprenolol ‡‡, atenolol §§, acebutolol ##, meto-propolol ***, oxprenolol †††, propranolol‡‡‡	Waters IC-PAK CM/D (5 µm particles)	50 mM HNO_3 in 4% acetonitrile, 1 mL/min	UV at 270 nm	Validation of method for drug analysis in tablets and i.v. formulations	23
Alendronate, Clodronate, Etidronate	Hamilton PRP-X100, 250 × 4.1 mm, 10 µm particles	1 mM trimesic acid, pH 5.5 at 1 mL/min	Indirect UV at 254 nm	Stability-indicating versus thermal degradation products, influence of mobile phase composition explored	24
Sodium salicylate, Ampicillin sodium, Potassium guaiacolsulfonate, Benzylpencillin potassium	Bio Tech Research Carbon B1-01, 100 × 4.6 mm	0.1M Pyrocatechol Violet–2 mM HNO_3 at 0.8 mL/min	Suppressed conductivity with TBAOH as regenerant	Indirect drug quantitation via the counterions (Na, K). Some validation data provided.	25
Caffeine, theobromine, theophylline§§§	Dionex HPIC-CS3 (cation, 2 columns in series) Dionex OmniPac PAX-100 (anion)	100 mM HCl at 1 mL/min (cation) 15 mM KOH in 1% acetonitrile at 1 mL/min (anion)	Direct UV at 274 nm	Quantitation in injections and tablets, some method optimization and validation data provided.	26
Paracetamol###	Waters IC-PAK A HR (10 cm, 6 µm particles)	5 mM LiOH in 5% acetonitrile at 1 mL/min	Direct UV at 300 nm	Quantitation in solid dosage forms.	27
Oxytetracycline, Tetracycline, Chlortetracycline, Doxycycline	Dionex OmniPac PCX-100 (250 × 4 mm)	0.2 M HCl in ~ 28% acetonitrile at 1 mL/min	Direct UV at 300 nm	Method developed primarily for residuals testing	28
Benzethonium chloride, Cetylpyridium chloride, Chlorhexidine digluconate, Cetrimonium bromide, Domiphen bromide	Shim-Pack IC-A1 (100 × 4.6 mm, 10 µm)	0.94 mM sodium carbonate + 0.31 mM sodium bicarbonate or 0.25 mM phthalic acid + 2.4 mM tris(hydroxymethyl)aminoethane, pH 4.31, both at 1.5 mL/min	Direct conductivity	Sample combusted and analyzed via the liberated anion (Cl or Br). Analytical recoveries reported.	29

* A polyhydroxy aliphatic amine synthetic reaction intermediate.
‡ Monosodium monohydrate salt of 4-amino-1-hydroxybutane-1,1-bis-phosphonic acid.
# 1-hydroxyethane-1,1-bisphosphonic acid disodium salt.
†† Other columns include Dionex AS7 and AS4A, MetaChem HEMA 1000Q.
§§ l,4-(2′-hydroxy-3′-isopropylamino-propoxy)phenylacetamide.
*** 1-isopropylamiono-3-(p-(B-methyloxyethyl)phenoxy)-2-propanol.
‡‡‡ 1-isopropylamino-3-(1-napththyoxy)-2-propanol.
acetominophen, *N*-acetyl-p-aminophenol.
† TBAOH = tetrabutylammonium hydroxide.
§ Disodium-3-amino-1-hydroxy-propylidene-1,1-biphosphonate pentahydrate.
** 1,1-dichloromethane-1,1-bisphosphonic acid, disodium tetrahydrate salt.
‡‡ 1-(o-allylphenoxy)-3-isopropylamino-2-propanol.
N-3-acetyl-4-(2-hydroxy-3-(isopropylamino)-propoxy)-phenylbutanamide.
††† 1-(2-(allyloxy)-phenoxy)-3-isopropylamino-2-propanol.
§§§ Separations as anions and cations reported.

Table II B. Pharmaceutical Applications of Ion Chromatography, Active Ingredients

Analyte	Column	Eluent	Detection	Other	Ref.
Copper, manganese, and zinc	Dionex HPICE-CS5	0.05 M oxalic acid (pH 5.24) at 1 mL/min	UV–vis at 520 nm after post-column reaction with PAR	Used to characterize multivitamin supplements.	67
Chloride, bromide	Dionex IonPac AS4A	0.75 mmol/dm^3 sodium bicarbonate, 2.2 mmol/dm3 sodium carbonate at 1 mL/min	Suppressed conductivity with 25 mmol/dm^3 sulfuric acid as regenerant	Used to assess batch to batch variation in an ion exchange bile acid sequestrant	66
Anion scan*	Dionex AS11HC with AG11 guard	KOH gradient: 3 mM, 0–5 min; 5 mM at 12 min; 35 mM at 36 min; 3 mM, 36.2–50 min.	Suppressed conductivity, electrolytic generation	Used to measure counter-ions for API salts	69
Carbocisteine	Dionex AS-14 with AG-14 guard	0.25 mM trifluroacetic acid at 1.2 mL/min	Suppressed conductivity, electrolytic generation	Used to measure active in cough syrups and oral granules	70
Flucloxacillin and Amoxicillin	Zorbax 300-SCX (Agilent), 250 × 4.6 mm, 5 µm particles	0.025 M ammonium dihydrogen phosphate (pH 2.6)–acetonitrile (95/5) at 1.5 mL/min	UV at 225 nm	Used in QC test in pharmaceutical injection products	71
Ephedrine, Pseudoephedrine, Norephedrine	Metrohm Metrosep cation 1–2 (125 × 4.0 mm)	2.0 mM HNO_3 with 2% acetonitrile, 1.2 mL/min	Direct conductivity	Used to assay pharmaceutical preparations and raw materials	72
Methenamine, Methenamine mandelate, Methenamine hippurate	Zorbax SCX-300 (Agilent), 150 × 4.6 mm, 5 µm	Acetonitrile–sodium perchlorate monohydrate (0.1M, pH 5.8), 1 mL/min	UV at 212 nm	Used to assay pharmaceutical tablets	73
Salbutamol, Fenoterol, Clorprenaline, Clenbuterol	Metrohm Metrosep cation 1–2 (125 × 4.0 mm)	1.8 mM HNO_3 with 2% acetonitrile, 1.0 mL/min	Direct conductivity	Used to test tablets and biological (clinical) samples	74
Anion suite†	Dionex IonPac AS18 (250 × 2 mm) with AG18 guard	KOH gradient; 1.0–3.5 mM from 0 to 5 min; 4.0 mM from 5 to 12 min; 4.0 to 12 mM from 12 to 20 min; 12 to 28 mM from 20 to 28 min; 80 mM from 28 to 40 min; 0.25 mL/min	Suppressed conductivity	Used to test toothpaste-like formulations and to follow the decomposition of monoflurophosphate and glycerphosphate	75
Etidronate, Clodronate,	Phenomenex Phenosphere SAX (150 × 2.0 mm, 5 µm)	20 mM Sodium citrate, pH 3.6, 0.3 mL/min	Indirect UV at 226 nm	Used to assay tablets	76
Pamidronate, Alendronate	Phenomenex Sphereclone SAX (250 × 2.0 mm, 5 µm)	20 mM Sodium citrate, pH 4.6, 0.25 mL/min	Indirect UV at 222 nm	Used to assay tablets	76
Chromium (III) and Chromium (VI)	Shodex RSpak NN-614, 150 × 6 mm	90 mM am monium sulfate + 10 mM ammonium nitrate, pH 3.0–3.5, 0.3 mL/min	ICP-MS	Used to establish Cr speciation in homeopathic drugs	77
Iodine	Chromsep (Varian) LC-Varian	0.1 M HNO_3 with 20% acetonitrile and 0.5 mM EDTA, 0.6 mL/min	Amperometric at a Ag electrode	Potentially applicable to assay of iodine-containing drugs	78
Ascorbic acid	Dionex IonPac AS11-HC	1.25 mM NaOH, 1.5 mL/min	Amperometric at a Pt electrode	Used to test vitamins	79

* Separation of anions including acetate, chloride and phosphate; elution properties of other common anions reported.
† Including fluoride, chloride, nitrite, nitrate, glycerophosphate, monoflurophosphate, sulfate, oxalic acid, and phosphate.

Table III A. Pharmaceutical Applications of Ion Chromatography, Excipients, and Inactive Formulation Components

Analyte	Column	Eluent	Detection	Other	Ref.
Acetate, lactate, chloride, phosphate citrate, sulfate	Hamilton PRPX-100, 250 × 4.1 mm; Waters IC PAK A, 50 × 4.6 mm, Vydac 300 IC, 50 × 4.1 mm	Various potassium hydrogen phthalate solutions (some with acetonitrile at 1.3–2.0 mL/min)	Indirect UV at 254 nm	Used to measure these anions in i.v. solutions	30
Oxalate	Dionex AS-1	1 mM potassium hydrogen phthalate, 2 mM sodium borate, pH 9.1 at 2–4 mL/min	Indirect UV at 250 nm pharmaceutical LVP solution	Recovery data provided from a generalized	30
Lactic acid and lactic acid lactate	Dionex HPICE-AS1 (ion exclusion)	1 mM sodium octanesulfonate	Suppressed conductivity	Used to characertize Amrinone Lactate Injection	31
Sodium, potassium, cesium, magnesium, calcium	Zorbax SCX-300, 250 × 4.6 mm	2.5 mM copper sulfate at 1.2 mL/min	Indirect UV at 230 nm	Used for the analysis of a cardioplegic solution, some validation data	32
Sodium lauryl sulfate	Dionex Omnipac PAX-500, 250 × 4 mm	Gradient used* at 1 mL/min	Suppressed conductivity, 12.5 mM sulfuric acid as regenerant	Characterization of tablet dosage form	33
Chloride, sulfate, phosphate, citrate	Dionex Omnipac PAX-500, 250 × 4 mm	40 mM NaOH in 5% methanol at 1 mL/min	Suppressed conductivity, 12.5 mM sulfuric acid as regenerant	Characterization of a liquid veterinary drug	34
Citrate	Hamilton PRP-X100, 150 × 4.1 mm, 10 µm particles	0.875 mM trimesic acid, pH 10.0 at 1.5 mL/min	Indirect UV at 280 nm	Used to characterize a number of commercial pharmaceutical products, validation data provided	35
Methane-through octane sulfonic acids	Dionex PAX-500	Gradient used† at 1 mL/min	Suppressed conductivity with 12.5 mM sulfuric acid regenerant	Validation data provided	36
Methanesulfonic acid, fumaric, maleic, succinic, tartaric acids	Dupont Zorbax NH2, 250 × 4.6 mm, 5 µM particles	20 mM sodium dihydrogen phosphate, 0.13 mM phosphoric acid (pH 4.2) in 5% acetonitrile	Direct conductivity	Method development data provided	37
Chloride, bromide, sulfate	Bio Tech Research Carbon BI-01, 100 × 4.6 mm	2 mM sodium carbonate, 1 mM tetrabutylammoniumhydroxide, 5% acetonitrile at 0.8 mL/min	Suppressed conductivity, 12.5 mM sulfuric acid regenrant	In D&C color additives. Sample analysis by direct injection or after oxygen flask combustion	38
Oxalate, citrate	Dionex AS-1	10 mM TRIS, 30 mM sulfate, pH 6.5 at 1 mL/min	Direct UV at 210–220 nm	Accuracy documented in a number of i.v. products	39
Acetic, malic, lactic acids	Bio-Rad Aminex HPX-87H, 300 × 7.8 mm	0.15% sulfuric acid or 0.005% phosphoric acid at 0.8 mL/min	Direct UV at 210 nm	Ion Exclusion method. Assay validation data provided for use in TPN solutions	40
Malic, citric and tartaric acids	Shimpack IC-A1, 100 × 4.6 mm, 12.5 µm particles	1.5 mM phthalic acid at pH 4.0 at 1.2 mL/min	Series bulk acoustic wave detector	Reported to be 5X more sensitive than direct conductivity. Used to characterize a homeopathic drug	41
Phosphate, Phosphite, hydrophosphite	Waters IC-PAK A HR, 6 µm particles	Gluconate/borate buffer containing acetonitrile and *n*-butanol at 1 mL/min	Direct conductivity	Used to characterize several tablet and syrup products	42

* This separation utilized a 60 minute gradient formed with four eluents including water, acetonitrile–water (90:10), 200 mM NaOH, and methanol–water (45:55).
† A = 5 mM sodium borate, 5% acetonitrile, B = 20 mM sodium borate, 40% acetonitrile. Time = 0 min, 100% A. Time = 10 min, 100% B. Time = 15 min, 100% B.

Table III B. Pharmaceutical Applications of Ion Chromatography, Excipients, and Inactive Formulation Components

Analyte	Column	Eluent	Detection	Other	Ref.
Sulfite	Wescan Anion Exclusion H/S, 250 × 4.6 mm	20 mM sulfuric acid at 0.75 mL/min	Direct conductivity	Used to characterize several herbal medicines.	43
Additives in foods and pharmaceutical preparations *	Shimadzu Shim-pack IC-A3, 150 × 4.6 mm, 5 µm particles	5 mM sodium phosphate monobasic (pH 8.2) with 4% acetonitrile at 1 mL/min	Direct UV, switched between 205 and 227 nm	Some assay validation and performance data provided (tablet dosage form).	44
Acetic and lactic acids	Alltech Anion Exclusion, 300 × 7.8 mm	2 mM sulfuric acid at 0.7 mL/min	Direct conductivity	Validation of an ion exclusion method for use in characterizing LVP products.	45
Silicic acid, calcium, magnesium, aluminum	Tosoh TSK gel IC-Anion-PW (50 × 4.6 mm, 10 µm particles)	1 mM NaOH with 10% methanol at 1.2 mL/min	Conductivity	Cations separated as their EDTA complexes. Validation data provided.	46
EDTA	Dionex AS-14 (250 × 4 mm) and AG-14 guard	10 mM carbonate buffer (pH 10.5 or 11.0) at 1 mL/min.	Suppressed conductivity	Method applied to analysis of contact lens care solutions and injections solutions. Validation data provided.	80
Anions (Cl, Br) in quaternary ammonium salts	Shim-Pack IC-A1, 100 × 4.6 mm, 10 µm particles	# 1: 0.94 M sodium carbonate (pH 9.85). # 2: 2.5 mM phthalic acid + 2.4 mM tris(hydroxymethyl)–aminoethane (pH 4.31). Flow rate: 1.5 mL/min	Direct conductivity	Method applied to the analysis of dental hygiene solutions (mouth rinses and disinfectants).	81
Sulfate	Dionex IonPac AS14, 250 × 4 mm, with AG14 guard	7.0 mM sodium carbonate + 2 mM sodium hydrogen, carbonate 1.2 mL/min.	Suppressed conductivity	Method, with appropriate sample preparation, used to assess total sulfate and sulfate inside and outside the liposome.	82
Fl, Cl, Br, sulfate in seawater	Dionex IonPac AS14, 250 × 4 mm, with AG14 guard	$NaHCO_3$ (3.5 mM) + Na_2CO_3 (1 mM) at 1.2 mL/min	Suppressed conductivity	Method applied to the analysis of seawater as a nasal spray diluent. Separation optimization is discussed. Validation data provided.	83
Citrate, acetate	Bio-Rad HPX-87H, 300 × 7.8 mm.	25 mM sulfuric acid at 0.8 mL/min	UV at 210 nm	Method uses SPE sample treatment. Method is used in production of medical fluids. Validation data provided.	84
Citrate, phosphate	Dionex IonPac AS-11, 250 × 4.6 mm, with AG-11 guard.	20 mM sodium hydroxide, 2 mL/min.	Suppressed conductivity.	Assessment of USP method for these analytes. Method optimization discussed. Validation data provided.	85
Citrate, phosphate	Dionex IonPac AS-11, 250 × 4.6 mm, with AG-11 guard.	20 mM sodium hydroxide, 2 mL/min.	Suppressed conductivity.	Assessment of USP method for these analytes. Method optimization discussed. Validation data provided.	86
Counter ion of ionized pharmaceutical salts	Various	Various	Various	This is a general review of IC, CE and realated methods.	87

‡ Analytes included saccharin, aspartame, acesulfame-K, benzoic acid, sorbic acid, caffeine, theobromine, and theophylline.

Table IV A. Pharmaceutical Applications of Ion Chromatography, Degradation Products and Impurities

Analyte	Column	Eluent	Detection	Other	Ref.
5-Hydroxy-methylfurfural (5-HMF)	Wescan Anion Exclusion HS, 100 × 4.6 mm	10 mM sulfuric acid at 0.8 mL/min	UV detection at 285 nm	Performance contrasted favorably with reversed phase HPLC method.	47
Aluminum	Waters Protein-Pak SP-5PW, 75 × 7.5 mm	0.01M potassium sulfate, pH 3.0 at 1 mL/min	Fluorescence (395 nm excitation, 500 nm emission) with post column reaction	Used for the quantitation of trace levels of Al in pharmaceutical diluents.	48
Cyanamide*	Dionex Ion-Pac AS-10	50 mM NaOH in 1% acetonitrile	PAD	Validation information provided.	49
Common anions†	Dionex IonPac AS4A	1.75 mM sodium bicarbonate, 2.5 mM sodium carbonate at 1 mL/min	Suppressed conductivity	Characterization of high purity water for sub-ppb trace ionic contaminants with pre-concentration.	50
Common cations‡	Dionex IonPac CS12	20 mM methanesulfonic acid at 1 mL./min	Suppressed conductivity	Characterization of high purity water for sub-ppb trace ionic contaminants with pre-concentration.	51
Sulfamate, sulphate	Hamilton PRP-X100 (150 × 4.6 mm, 10 µm particles)	5.8 mM *p*-hydroxybenzoic acid, 2.5% methanol at pH 9.4, 1.5 mL/min	Indirect UV at 310 nm	Degradation products of Topiramate.	51
Methansulfonic acid	Hamilton PRP-X100 (150 × 4.1 mm, 10 µm particles)	Acetonitrile/60 mM NaOH (20/80) at 2 mL/min	Suppressed conductivity with 50 mN sulfuric acid as regenerant.	Measured this drug synthesis intermediate in drug raw materials, some validation data provided.	52
Citric, malic, ascorbic acid	Shim-pack IC-A1 (100 × 4.6 mm)	20 mM potassium hydrogen phthalate at 1 mL/min	Direct conductivity and Quartz Crystal detector	Used to determine these components in a Chinese herbal medicine.	53
Amylamine, tert-butylamine	Dionex CS-14 (250 × 4 mm, 5 µm particles)	Acetonitrile/50 mM methanesulfonic acid (5/95) at 1 mL/min	Suppressed conductivity with 100 mM tetrabutyl ammonium hydroxide as regenerant.	Amylamine is a synthetic residual and t-butylamine is the active's counter-ion (measured for purity assessment).	54
Nitrite, nitrate	Exsil SAX (125 × 4.6 mm)	22 mmol/dL potassium dihydrogen phosphate, 3 mmole/dL phosphoric acid with 20% acetonitrile at 1.6 mL/min	Direct UV at 214 nm, or electrochemical	Used to study the oxidative denitrification of hydroxyguanidines.	55
Carbonate	Dionex IonPac ACE-AS1 (ion exclusion)	Water at 1 mL/min	Conductivity	Used to study the oxidative decarboxylation of aromatic carboxylic acids	55
Sulfate	Dionex AS4-SC	1.8 mmol/dL sodium carbonate, 1.7 mmol/dL sodium bicarbonate at 1.5 mL/min	Direct UV at 214 nm or suppressed conductivity	Used to study the generation of sulfate from perthiol drugs.	55
Methanesulfonic acid	Dionex IonPac AS4A-SC	0.015% sodium hydrogen carbonate at 2 mL/min	Suppressed conductivity with 0.075% sulfuric acid as regenerant	Hydrolysis of busulfan tablets, validation data provided.	56

* Synthetic residue
† Including chloride, nitrite, bromide, nitrate, orthophosphate, and sulfate.
‡ Including lithium, sodium, ammonium, potassium, magnesium and calcium.

Table IV B. Pharmaceutical Applications of Ion Chromatography, Degradation Products and Impurities

Analyte	Column	Eluent	Detection	Other	Ref.
Oxalic acid, oxamic acid, and oxamide	Dionex IonPac ICE-AS1 (250 × 9 mm, 7.5 µm particles)	5:95 acetonitrile–0.1% sulfuric acid at 0.8 mL/min	UV at 205 nm	Impurity products in synthetic processes, some validation data provided.	57
Sulfate and sulfamate	Dionex IonPac AS5A-5m (150 × 4 mm)	Gradient with mobile phase A being water and mobile phase B being 50 mM NaOH at 1 mL/min	Suppressed conductivity	Assay used to measure these analytes as degradation products of Topiramate. Extensive validation information provided.	65
Trifluoroacetate	Dionex IonPac AS18, 250 × 4 mm, with AG18 guard	KOH gradient at 1 mL/min: 0–6 min, 22 mM; 6–12 min, 28 mM, 12–15 min, 50 mM, 15–20, 22 mM	Suppressed conductivity	Assay is used to measure residual TFA in injection solutions and peptides. Method optimization and evaluation data provided.	88
Azide	Dionex IonPac AS11, 250 × 4 mm, with AG11 guard	NaOH gradient at 1 mL/min: 0–14 min, 1. mM; 15–23 min, 15 mM; 23–24 min, 1.2 mM	Suppressed conductivity	Assay is used to measure residual azide in protein samples. Method validation data provided.	89
Monoethylsulfate	Metrohm Metrosep A Supp5, 250 × 4 mm, 5 µm particles	Sodium carbonate–sodium hydrogen carbonate mixture (339 mg and 84 mg per 1000 mL) at 0.7 mL/min	Suppressed conductivity.	Assay is used to measure residual synthesis byproduct. Method validation data provided; method compared to CE.	90
Monomethylamine	Metrohm Metrosep C3-250	1 mM nitric acid at 1 mL/min	Suppressed conductivity	Method is coupled with in-line column matrix elimination for the determination of 0.05 mg/L of amines in cationic drugs.	91
N-methylpyrrolidine	Metrohm Metrosep C2-150	6 mM nitric acid with 10% acetonitrile	Direct conductivity	Method proposed as an alternate for the USP method for determining this analyte in cefepime hydrochloride. Method optimization and validation details provided.	92
Azide	Metrohm Metrosep A Supp10-250	5 mM each of sodium carbonate and sodium bicarbonate, 1 mL/min	Suppressed conductivity	Method proposed as an alternate for the USP method for determining this analyte in Irbesartan drug. Method optimization and validation details provided. Method employs in-line sample preparation for matrix elimination.	93
Tetrabutylammonium bromide	Metrohm Metrosep Cation C2-150	7.5 mM nitric acid with 35% acetonitrile, 1 mL/min.	Direct conductivity	Method separates tetramethyl-through tetrapropyl-ammonium bromides. Method employs on-line preconcentration. Method optimization and validation data provided	94
Formic acid		90% 0.5mM sulfuric acid, 10% acetone		Method is used to measure analyte in ceftazidime drug substance. Method validation data provided.	95
Sulfated glycosaminoglycans (GAGs)	Dionex IonPac AS-11 or AS-11HC	Various gradients. The USP method utilizes a gradient based on a phosphate buffer containing sodium perchlorate (pH 3) while the literature references utilizes a gradient based on a mobile phase containing 2.5 M NaCl and 20 mM Tris . (pH 3, phosphate buffer)	UV detection at low wavelength (e.g., 202, 215 nm)	Method is utilized to characterize Heparin Sodium and Heparin Sodium Injection for impurities such as oversulfated chondrotin sulfate and additional GAGs. The reported LODs are on the order of 0.1% by weight for the individual impurities. Some method validation is provided in the individual references.	103–107

Table V. Pharmaceutical Applications of Ion Chromatography, Process Streams

Analyte	Column	Eluent	Detection	Other	Ref.
Carbohydrates (galactose, glucose, ribose, fructose)	Dionex CarboPac PA1 (250 × 4 mm)	150 mM NaOH at 1 mL/min*	PAD (Au electrode)	Methods used to quantitate substrates and metabolites in fermentation broth.	58
Inorganic cations (calcium, magnesium, ammonium, potassium, sodium)	Dionex IonPac CS10 (250 × 4 mm) acid at 1 mL/min	20 mM HCl, 4 mM d,l-2,3-diaminopropionic	Suppressed conductivity with 0.1 M tetrabutylammonium hydroxide as regenerant		59
Sugar alcohols (glycerol, inositol, mannitol, sorbitol)	Dionex HPICE-AS1 (250 × 4 mm), ion exclusion	100 mM perhloric acid at 0.8 mL/min	PAD (Pt electrode)		59
Sodium, ammonium, potassium , magnesium, calcium	Dionex IonPac CS12 (250 × 4 mm)	4 mM methanesulfonic acid at 1 mL/min	Suppressed conductivity	Used to optimize culture media composition.	59
Chloride, nitrate, sulfate	Ion exclusion: Biorad Organic Acids column HPAH (100 × 7.8 mm, 9 µm particles) Ion exchange: Dionex AS4A	Ion Exclusion: 1 mM octanesulfonic acid with 2% isopropyl alcohol Ion Exchange: 2.8 mM sodium carbonate, 1.7 mM sodium bicarbonate, both at 2 mL/min	Suppressed conductivity with suppression for both the ICE and IC separations	Coupled ion exclusion (on-line sample preparation) with ion exchange (analytical separation) to analyze these analytes in fermentation broth containing high levels of organic acids.	60
Dimethyl-benzenesulfonate	Dionex OmniPack PAX-100	5 mM sodium chloride, 0.2 mM sodium hydroxide, 32% acetonitrile at 1 mL/min	Direct UV at 220 nm	Used to perform residuals testing during cleaning validation.	61
Magnesium	Shim-pack IC-C1 (150 × 5 mm, 10 µm particles)	4 mM tartaric acid, 2 mM ethylenediamine at 1.5 mL/min	Double cell bulk acoustic wave detector	Used to follow decrease in magnesium levels in cell culture media.	62
Hydroxylamine	Dionex CS14 (250 × 4 mm)	11 mM sulfuric acid at 1 mL/min	PAD, Au working electrode	Analyte is a mutagenic active ingredient raw material whose levels were monitored in final waste streams before disposal.	63
Chloride, nitrate, sulfate	Dionex AS4A-SC	1.8 mM sodium carbonate, 1.7 mM sodium bicarbonate at 2 mL/min	Suppressed conductivity	Validated as the EP method for characterizing Purified Water.	64
Ammonium, magnesium, calcium	Dionex CS 12	20 mM methanesulfonic acid at 1 mL/min	Suppressed conductivity	Validated as the EP method for characterizing Purified Water.	64
Hydroxyamine	Dionex IonPac CS14, 250 × 4 mm, with CG14 guard	11 mM sulfuric acid at 1 mL/min	Pulsed amperometric detection at an Au electrode	Analyte is measured in waste streams as it may interfere with sewage plant operation. Method optimization and validation discussed.	96
CIP-100 detergent (EDTA as target analyte)	Metrohm Metrosep A Supp5-150, 150 × 4 mm, 5 µm particles	3,2 mM sodium carbonate + 1.0 mM sodium hydrogen carbonate at 0.7 mL/min	Direct conductivity	Method proposed for cleaning validation applications. Method validation data reported.	97

* A gradient separation for the carbohydrates is also reported.

Table VI A1. Examples of Validation Data For Quantitative Ion Chromatography Methods

Application	Performance Parameters: Accuracy	Precision	Linearity	Specificity	LOQ/LOD	Ruggedness	Ref.
A. Active Ingredient Analysis.							
Alendronate in i.v. and tablet formulation by suppressed conductivity detection	Spiked drug into placebo, at 80, 90, 100, 110, and 120% of formulation level. Mean recovery, 100.2%. Demonstrated equivalence versus an HPLC–fluorescence method.	Injection, *n* = 10 at 0.05 mg/mL, 1% RSD by peak height. Total method, *n* = 10 at 2.5 mg/mL 1.1% RSD or less.	Coefficients of determinations of 0.999 or greater for range of 40–160% of assay level. Non-zero intercepts observed.	Tested against formulation placebo and known thermal decomposition products	Not evaluated.	Evaluated by performing testing on four analytical systems	20
Biphosphonate drugs with indirect UV detection	Spiked drug into placebo, at 50, 75, 100, 125, and 150% of formulation level. Mean recovery for three drugs, 100 ± 1% at sample level of 0.05 mg/mL.	Injection to injection, *n* = 10 at 0.05 mg/mL (25 mL injection) or 0.4 mg/mL (50 mL injection). %RSD less than 1%. Total method, 1–2% RSD for *n* = 10.	Coefficients of determinations of 0.999 or greater for range of 20–200% of formulation level. Areas better than heights, non-zero intercepts observed	Tested against formulation placebo and known thermal decomposition products	LOD of 0.001 mg/mL at S/N of 4 for all analytes	Evaluated via two analysts/two system testing, testing using five columns, and examining performance on one column after at least 500 injections	22
Disodium clodronate in bulk materials pharmaceuticals by UV with post-column derivatization	Spiked drug into placebo capsules and tablets at 80, 100 and 120% of nominal formulation level (300–1000 mg). Mean recovery = 99.5–100.7%.	Six samples of bulk material and pharmaceuticals assayed. %RSD from 0.8% to 1.3% for 60–820 mg of drug.	50-175% of nominal analyte conc. (0.02–0.07 mg/mL), r^2 = 0.9999 (*n* = 6 at each level).	Tested against formulations degraded by acid, base, peroxide, heat, UV light) and against mixture of known impurities.	Not evaluated .	Stability of sample solutions = 60 h at RT. Tested two columns with system suitability tests	18
SPE removal of non-polar compounds, ion exclusion separation and UV detection of citrate and acetate in medical fluids	Recovery, compared with non-SPE treated, ranged from 99.9 to 100.3% for 80 to 120% concentration	%RSD ranging from 0.0 to 0.3%.	$r^2 \geq 0.999$, with % y-intercept ≤ 0.6	The SPE treatment did not introduce citrate or acetate.	Not evaluated.	Two analysts on separate systems. Pooled data, 99 to 101% recovery, and % RSD ≤ 0.5	68
Quantitation of carbocistine (acidic amino acid) in syrup formulations	Standard additions into syrup formulations (95–105% of label); recovery ranged from 96–105%.	%RSD of triplicate injections = 1.5%; %RSD of triplicate preparations of sample = 1.8–3.6%.	*r* = 0.99994 over range of 50–400 µg/mL.	Not evaluated.	LOD = 0.14 µg (5.6 µg/mL)	Not reported	70
Quantitation of anionic constituents of APIs in early product development	Recovery determined in spiked portions of dissolved APIs at 80–120% of anticipated level; results compared to data obtained by other methods. Recovery ranged from ~93–104%.	Triplicate preparations (dissolutions); %RSD ranging from 0.2% to 2.0% RSD.	Linear ranges as follows (mg/L): F: 0.2–10; Cl: 0.3–30; NO_2: 0.4–20; Br: 4–100; NO_3: 4–100; SO4: 6–150; PO_4: 1.5–150	No blank responses noted for the APIs tested.	Not reported.	Chloride and phosphate standards and samples stable for 9 days at RT; acetate standards and samples stable for 2 days at RT, 9 days refrigerated	69
Simultaneous quantitation of flucloxacillin and amoxicillin in injection products; QC applications	Recovery in synthetic mixtures prepared at 80–120% of label; ranged from 100 ± 1%.	Six individual weighings; % RSD < 1%.	r^2 = 1.0000 over range of 50–150 µg/mL (50–150% of nominal)	Chromatograms of samples aged for 7 days at RT exhibited no interfering peaks.	LOQ = 0.2 µg/mL	Sample and standard solutions stable for at least 4 h at RT	71

Table VI A2. Examples of Validation Data For Quantitative Ion Chromatography Methods

	Performance Parameters						
Application	**Accuracy**	**Precision**	**Linearity**	**Specificity**	**LOQ/LOD**	**Ruggedness**	**Ref.**
A. Active Ingredient Analysis.							
Quantitation of ephedrine and related compounds in injections solutions, powders, and herbs	Analysis of spiked injection product and powder; recovery of 96%–103%. Analyzed products versus label claim; good agreement obtained.	%RSD of 11 standard injection < 2%.	Linear ranges as follows (µg/mL): EPH: 0.08–50; PEPH: 0.08–40; NEPH: 0.06–40.	No interference from 500-fold excess of Na, K, NH_4 or 100-fold excess of Ca and Mg.	LOD = 0.02 µg/mL	Not reported; method optimization described.	72
Quantiation of methenamines in tablets	Analysis of placebo tablets spiked with 60–140% of label; recovery = 100 ± 1%.	% RSD < 1% for 3 or 6 injections.	r^2 > 0.9999 over range of 0.035–7.01 mg/mL.	Analysis of placebos and forced degradation samples produced no interfering peaks.	LOD = 0.02 mg/mL; LOQ = 0.03 mg/mL.	Standard solutions stable for at least 48 hours at RT. Intra-day (*n* = 6) and inter-day (*n* = 12) %RSD < 2%.	73
Quantitation of B2-agonists (salbutamol, SAL; fenoterol, FEN; clorprenaline, CLO; clenbuterol, CLE) in tablets	Analysis of spiked tablet preparations; % recovery ranged from 97% to 104%.	% RSD of *n* = 11 ranged from 1.1% to 2.8% RSD.	Linear ranges as follows (ng/mL): SAL: 7–1400; FEN: 34–7800; CLO: 8–1600; CLE; 25–7500.	Not reported.	LODs as follows (ng/mL): SAL, 2; CLO, 3; FEN, CLE, 10.	Method optimization discussed. Effect of various operating parameters noted.	74
Quantitation of common anions in dentifrices for QC	Analysis of spiked toothpaste samples (spike level from 0.5 to 1.0 mg/L); % recovery rnaged from 96% to 104%.	% RSD form 10 replicate injections ranged from 0.15% RSD to 1.8% RSD.	Linear ranges as follows (mg/L): F: 0.05–20; Cl: 0.1–50; NO_2: 0.5–60; NO_3, GPO4: 0.5–40; Oxalate: 0.1–30; $MGPO_4$, SO4, PO_4: 0.5–50.	Not reported.	LODs ranged from 0.001 mg/L (F) to 0.013 mg/L (GPO4)	Method optimization is discussed. Interday %RSD of 2.8% RSD were reported.	75
Quantitation of Bisphosphonates (etidronate, ET; clodronate, CL; pamidronate, PA; alendronate, AL) in bulk materials or tablets.	Recovery study for AL. Spike range = 100–550 µg/mL, recoveries ranged from 97% to 103%.	Within-day precisions (*n* = 3) reported as < 2% RSD.	Linear range from 50–400 µg/mL for ET and CL; 100–500 µg/mL for PA and AL.	All peaks examined for peak purity via spectral analysis.	LOD (µg/mL): ET, 5.3; CL, 5.1; PA, AL, 10. LOQ (µg/mL): ET, CL, 50; PA, AL, 100.	Method optimization is discussed. Inter-day precision (*n* = 6) reported as < 2% RSD.	76
Quantitation of ascorbic acid in vitamins	Recoveries of spiked samples ranged from 94.2% to 96.1% and analysis of commercially available preparations gave results that agreed with the labeled values.	%RSD = 2.5% (*n* = 5)	r^2 = 0.9982 over the range of 1×10^{-6} to 1×10^{-2} M.	No interferences from common anions; no interferences noted in analysis of pharmaceutical samples.	LOD = 7.3 x 10^{-5} M.	Detector response and solutions noted to be stable for 30 days at RT. Optimization of the detector is discussed.	79

Table VI B1. Examples of Validation Data For Quantitative Ion Chromatography Methods

Application	Performance Parameters: Accuracy	Precision	Linearity	Specificity	LOQ/LOD	Ruggedness	Ref.
B. Excipients and Inactive Ingredients (continued).							
Alkyl sulfonic acids (for example, methansulfonic acid, MSA) by suppressed conductivity, total method performance (sample extraction)	Spiked samples at 6 levels between 80 and 120% of allowable limit, mean recovery (n = 6) = 102.9%.	Injection precision (n = 5) at the specification limit was 5% RSD.	r^2 = 0.9999 over application range.	Resolution demonstrated versus other sulfonic acids and chloride.	LOQ of 40 ppm (mg/g) in sample for MSA	Stock solutions of analytes stable for 1 week at RT. Analyzed 4 samples across 2 days, two columns and fresh mobile phase preparations. % RSD = 7.5%.	36
Methane sulfonic acid in intermediates and drug substances by direct conductivity	Determined by mass balance calculations in batch release applications.	Injection precision, 1–2% RSD. Method precision, 1–3% RSD	r^2 = 0.9997 over the range of 0.1–5 mg/mL.	Extensive investigation of elution characteristics.	Not evaluated.	Not evaluated.	37
Acetic and lactic acids in LVP i.v. solutions by ion exclusion with direct conductivity	Autoclaved formulation blanks spiked with 80, 100 or 120% of product specification levels. Acetate recovery 99–101%, 1500–7900 mg/L. Lactate, 99–103%, 680–3800 mg/L.	For 5 preparations in 4 formulations, 0.35–1.2% RSD at levels from 840–6600 mg/L.	Examined at 50 to 150% of sample dilution target with 5 standards, triplicate injections each (150–450 mg/L for sodium acetate trihydrate, 50 to 150 mg/L for sodium lactate). r2 = 0.9997, other data provided.	Autoclaved formulation blanks examined. Examined a test sample cocktail containing 13 dextrose impurities, related substances, "foreign sugars" and decomposition products.	LOQ = 9.9 mg/L for sodium acetate trihydrate and 3.6 mg/L for sodium lactate	Performed accuracy assessment with two runs (different analysts and columns). No difference in performance noted. Also examined robustness and response stability.	45
EDTA in contact lens care solutions and liquid injections	Spiked contact lens care solutions and a simulated drug injection solution; % recoveries ranged from 96% to 105%.	%RSD (n = 9) at 5 µg/mL = 1.5%.	r > 0.998 over range of 2.7 to 100 µg/mL.	Good resolution reported for commonly co-existing anions and other aminopolycarboxylic acids. Potential interference from other pharmaceutical substances was examined.	LOD = 0.87 µg/mL.	Method optimization is discussed.	80
Sulfate in liposome formulation	Standard additions of ammonium sulfate; recoveries ranged from 91% to 108%.	% RSD (n = 3) of 1% or less	r^2 > 0.999 over range of 0.075 to 0.35 mM.	No interfering peaks in placebo chromatograms.	LOD = 0.0006 mM. LOQ = 0.026 mM.	Studied day-to-day and analyst-to-analyst intermediate precision. %RSD < 2%. Samples stable refrigerated for 3 days.	82
Inorganic anions in seawater used as diluent in nasal sprays	Analysis of laboratory preparations; recoveries of 99% to 101%.	% RSD (n = 5) of 2% RSD or less.	r^2 > 0.999 over the following ranges: F: 0.02–0.4 mg/L; Cl: 40–160 mg/L; Br: 0.1–0.8 mg/L; SO4: 4–16 mg/L.	Baseline resolution of the analytes obtained.	Not reported.	Not reported.	83
Citrate and acetate in medical fluids	Analysis of laboratory preparations at 80–120% of nominal; recoveries of 99% to 101%.	Multiple preparations, % RSD = 0.3% or less.	r^2 > 0.999 over the range of 80–120% of nominal (2 to 6 g/L).	No interfering peaks noted in placebo samples.	Not reported.	Precision for multiple analysts, %RSD = 1% or less. Prepared samples stable for 48 h at RT.	84
Citrate and phosphate in injection solutions (USP Monograph)	Analysis of laboratory preparations at 80–120% of nominal; recoveries of 98% to 103%.	Multiple dilutions (n = 3 or 6), %RSD = 1.5% or less.	r^2 > 0.999 over the range of 50–150% of nominal (10 to 30 mg/L for citrate, 6 to 18 mg/L for phosphate).	No interfering peaks noted in matrix blanks.	Not reported.	Considered robustness in terms of mobile phase preparation, effect of diluents and effect of salts used for standards.	85
Citric acid/citrate and phosphate in dosage forms	Analysis of spiked samples of commercial products; recoveris of 95–105% reported. Generally close agreement between obtained results and labeled amounts.	6 Injections of 3 days; interday precision (% RSD) < 1% and intraday precision < 2%	r^2 > 0.999 over the range of 0.2–100 µg/mL for citrate, 0.2–60 µg/mL for phosphate).	Demonstrated by similarity in peaks shape and retention time for analytes in standards and samples.	LOQ = 0.2 µg/mL for both analytes	Demonstrated considering the impact of analyst, column lot, equipment and eluent preparation.	86

Table VI C1. Examples of Validation Data For Quantitative Ion Chromatography Methods

Application	Performance Parameters: Accuracy	Precision	Linearity	Specificity	LOQ/LOD	Ruggedness	Ref.
C. Impurities and/or Degradation Products.							
Cyanamide as a synthesis residual via PAD	Bulk drug substance spiked with 3 to 25 ppm additional analyte, % recovery from 89–106%.	Ten injection of sample at 5.4 ppm, % RSD = 4.6%. Method precision tested with 8 preparations of one lot, % RSD = 6.4%.	Assessed over the range of 15–150 ng/mL (7 levels, duplicate injections per level), r^2 = 0.9982.	Absence of response noted in degraded bulk drug substance.	Minimum quantifiable limit = 3 ppm.	Sample solutions stable for 53 h at RT.	49
Methanesulfonic acid as a synthesis impurity with suppressed conductivity	Batch of drug spiked with MSA levels of 0.1–2.0% (by weight), duplicate injections at 7 levels, % recovery 98–107%.	Six preparations of bulk drug, % RSD = 0.51%. Day to day reproducibility of 2.9–4.0% RSD.	Assessed over a range of 1–20 ppm, $r^2 > 0.999$ (n = 10)	Absence of response noted in formulation placebos.	LOD = 0.3 ppm.	Assessed as day to day reproducibility.	52
Oxalic acid, oxamic acid and oxamide as synthetic impurities by ion exclusion with UV detection	Examined by comparing calibration curves obtained in water matrix versus API. Less than 5% difference in slope, water versus API.	For six replicate preparations, % RSD = 9.3% at 2 ppm for oxalic acid; 4.1% at 1 ppm for oxamic acid and 3.4% at 0.6 ppm for oxamide.	r^2 = 0.9999 over range of 0.4–24 ppm for oxalic and oxalic acids; 0.1 to 6.2 ppm for oxamide. Six concentration, replicate injections.	Blank API matrix repetitively injected with no interfering peaks.	LOQ of 0.2 to 0.6 ppm.	Standards and samples stable for 24 h at RT. Method tested in two different laboratories with new and aged columns.	57
Sulfate and sulfamate, decomposition products of topiramate, by suppressed conductivity	Examined by analyzing tablets spiked with the analytes over the range of 0.24–1.0 mol%. Mean recovery of 103%.	1.1% RSD (10 injections) for sulfamate and 1.5% RSD for sulfate at 0.5 mol%.	0.04 to 27 mol% for sulfamate, 0.1 to 30 mol% for sulfate, $r^2 > 0.999$ but systematic skew in calibration curve noted.	Formulation placebo (degraded and fresh) examined for absence of interfering peaks.	LOQ = 0.05 mol% for sulfamide, 0.1 mol% for sulfate.	Samples and standards stable for at least 6 days at RT. Performed robustness assessment.	65
Trifluoroacetate in injections solutions and peptides	Recovery at 300 ng/mL was ~ 98% in several sample types.	Not reported.	r^2 = 0.998 over range of 100–300 ng/mL.	Separation of TFA from other interfering anions (CL, PO4) in high excesses.	LOD = 100 ng/mL	Not reported.	88
Azide in protein samples	Recovery in 9 protein samples spiked with 0.08–0.80 mg/L was 95 ± 8%.	%RSD of 6 replicate preparations was 5.7% RSD at 0.13 mg/L.	r^2 = 0.999 over range of 0.02–0.80 mg/L.	Separation between analyte and other common anions (e.g., Cl)	LOQ ranged from 0.02 to 0.06 mg/L	Robustness assessed by considering impact of small changes in operating conditions. Standards stable for 295 h at RT, samples for 46 h.	89
Monoethylsulfate in Indinavir drug substance	Recovery over range of 250–600 µg/g ranged from 94–100%.	Injection and method precision < 5% RSD at 1000 ng/mL.	r^2 = 0.9997 over range of 75–1200 ng/mL.	No interferences in the matrix blank.	LOQ = 75 ng/mL	Precision using 2 analysts and systems was < 5% RSD. Samples stable for 22 h at RT.	90
N-methylpyrrolodine in cefepime hydrochloride	Recovery in samples spiked with 0.3% NMP, ranged from 99–101%.	Triplicate sample preparations and analyses, %RSD = 1.2%. Injection precision, *n* = 6, RSD = 1.0%	r^2 = 0.9990 over range of 5–50 µg/mL.	No interferences from matrix blank and from mixed cation solutions containing common cations and amines.	LOD = 0.15 µg/mL; LOQ = 0.4 µg/mL	Method optimization discussed	92
Azide in Irbesartan	Recovery in samples spiked with 5 µg/L and 30 µg/L; recoveries ranged from 99–104%.	%RSD of triplicate preparations = 3.9%. Injection precision, *n* = 6, = 4.0%.	r^2 = 0.9990 over range of 5–100 µg/L.	No interferences from matrix blank and from mixed anion solution containing common inorganic anions.	LOD = 2 µg/L; LOQ = 6 µg/L	Robustness assessed by considering the impact of small changes in operating conditions. Samples stable for 40 h at RT.	93

Table VI C2. Examples of Validation Data For Quantitative Ion Chromatography Methods

Application	Performance Parameters: Accuracy	Precision	Linearity	Specificity	LOQ/LOD	Ruggedness	Ref.
D. Process Streams.							
Tetrabutylammonium bromide in Levetiracetam	Recovery in samples spiked with 0.1 µg/mL and 0.2 µgm/L; recoveries ranged from 98–102%.	%RSD of triplicate preparations < 1%.	r^2 = 0.9990 over range of 0.05–2.0 µg/mL.	No interferences from matrix blank and from mixed cation solutions containing common cations and aliphatic amines.	LOD = 39 ng (1.6 µg/g); LOQ = 120 ng (4.8 µg/g) 36 h at RT.	Separation optimization discussed. Sample solutions stable for	94
Formic acid in ceftazidime	Provided in the literature	Provided in the literature	r^2 > 0.98 over range of 0.25–25 µg/mL.	Provided in the literature	Provided in the literature	Not provided	95
Inorganic cations in culture media by suppressed conductivity	Spike recoveries determined in chemically defined and complex media formulations.	Inter-day (n = 6) and intra-day (n = 12) precision assessed at concentrations ranging from 0.5 to 50 ppm. At > 1 ppm, inter-day %RSD < 1%, intra-day < 2%.	r^2 > 0.9999 using polynomial model, 0.5 to 25 ppm for Na and K, 1 to 50 ppm for ammonium, Ca, Mg.	Specificity considered from a theoretical perspective based on the separation and detection methods.	LOD 0.5 to 1 ppm.	Assessed via intra-day precision.	59
Hydroxylamine in waste streams by PAD	Mean recoveries of analyte spiked into waste water of 69.4% at 0.05 ppm and 93.3% at 0.5 ppm.	Triplicate injections at 0.05 ppm and 0.5 ppm had %RSD of 2.8% and 1.5%.	r^2 = 0.999 over range of 0.01 to 2.0 ppm.	Examined versus a mixture of *n*-methylhydroxy-amine analogs at 1 ppm.	LOQ = 0.015 ppm.	Standards stable for 12 h at RT.	63
Chloride, nitrate, sulfate in pharmacopoeial grades of water with suppressed conductivity	Recovery in solutions at 75%, 100%, and 125% of nominal standard concentration. Mean accuracy = 105.1% for Cl; 104.5% for nitrate; 105.3% for sulfate.	Method Repeatability assessed by six injections on one day at the specification limit. %RSD = 5.0% for Cl, 3.3% for nitrate, 0.7% for sulfate. Method reproducibility assessed by duplicate injections on six different days by different analysts. % RSD for Cl = 12.4%, 7.8% for nitrate, 4.0% for sulfate.	Duplicate injections for 5 standards over range of 25–150% of the pharmacopoeial limit (0.1 ppm Cl, 0.2 ppm nitrate, 1 ppm sulfate); r2 > 0.99.	Tested against other common inorganic anions.	LOQ = 0.05 ppm, Cl; 0.004 ppm, nitrate; 0.04 ppm sulfate.	Standards and samples stable for > 7 days at RT. See method reproducibility.	64
Ammonium, magnesium, calcium in pharmacopoeial grades of water with suppressed conductivity	Recovery in solutions at 75%, 100%, and 125% of nominal standard concentration. Mean accuracy = 97.9% for ammonium; 98.5% for magnesium; 98.4% for sulfate.	Method Repeatability assessed by six injections on one day at the specification limit. %RSD = 2.7% for ammonium, 1.7% for magnesium, 0.4% for calcium. Method reproducibility assessed by duplicate injections on six different days by different analysts. % RSD for ammonium = 5.4%, 1.7% for magnesium, 2.6% for calcium.	Duplicate injections for 5 standards over range of 25–150% of the pharmacopoeial limit (0.2 ppm ammonium, 1.0 ppm magnesium, 2 ppm calcium); r^2 > 0.99.	Tested against other common inorganic cations.	LOQ = 0.02 ppm, ammonium; 0.25 ppm, magnesium; 0.35 ppm, calcium.	Standards and samples stable for > 7 days at RT. See method reproducibility.	64
Measurement of trace amounts of hydroxylamine in waste streams	Recovery samples prepared by spiking actual process effluent with 0.05 µg/mL and 0.5 µg/mL. Recovery at 0.05 µg/mL = 69%; recovery at 0.5 µg/mL = 93%.	Injection to injection precision (n = 3) at 0.05 µg/mL = 2.8% RSD; at 0.5 µg/mL = 1.5% RSD.	r^2 = 0.9988 over the range of 0.1–2.0 µg/mL.	Not reported.	LOD = 0.002 µg/mL; LOQ = 0.05 µg/mL	Method optimization (especially detector waveform) is discussed.	96
Measurement of residual CIP-100 detergent (EDTA as the target analyte) in cleaning validation	In Method #1, swabs spiked with CIP were extracted in water. In Method #2, metal plates were spiked with CIP-containing solutions. The plates were then swabbed and the CIP recovered by water extraction of the swab. Method #1 recoveries averaged 101%. Method #2 recoveries ranged from 63% to 72%.	Injection to injection precision (n = 3) was % RSD ~ 2.2%. Intermediate precision, multiple determinations by one analyst on multiple days and multiple analysts on a single day were on the order of 2.8% RSD.	r^2 = 0.99999 over the range of 1.3–21 mg/L.	Not reported.	LOD = 0.13 mg/L; LOQ = 0.39 mg/L.	See Intermediate precision.	97