Status: Currently Official on 12-Feb-2025
Official Date: Official as of 01-Aug-2016
Document Type: General Chapter
DocId: GUID-1E654232-BCFD-4855-8481-ACD22AB9A406_1_en-US
DOI: https://doi.org/10.31003/USPNF_M897_01_01
DOI Ref: 5zz9i

⟨1065⟩ ION CHROMATOGRAPHY

INTRODUCTION

Ion chromatography (IC) is a high-performance liquid chromatography (HPLC) instrumental technique used in USP test procedures such as identification tests, assays, and determination of impurities, including limit test and quantitative tests. IC is used to measure anions and cations derived from organic or inorganic molecules, from small molecules to larger biomolecules. This may include, but is not limited to, organic acids, carbohydrates, sugar alcohols, aminoglycosides, amino acids, amines, phosphonates, peptides, aminoglycosides, oligosaccharides, proteins, and glycoproteins.

IC has been applied to all aspects of the manufacturing and disposition of pharmaceutical products, including characterization of active ingredients, excipients, degradation products, impurities, and process streams. Raw materials, intermediates (including media and culture broths), bulk active ingredients, diluents, formulated products, production equipment cleaning solutions, process water, and waste streams may be analyzed using IC.

The majority of IC methods use either anion- or cation-exchange chromatography coupled with suppressed conductivity detection. IC is especially valuable for ionic or ionizable (in the mobile phase) analytes that have little or no native UV absorbance. In addition to suppressed conductivity detection, the ion-exchange separation can be coupled to other detection strategies, including pulsed amperometric detection (PAD), UV/Vis absorbance detection, inductively-coupled plasma mass spectrometric detection (ICPMS), and mass spectrometric detection, providing a wide range of analyte sensitivity and specificity. Ion-exclusion separations expand the range of IC applications to some nonionic analytes (e.g., alcohols) and provide a different selectivity for some analytes that can also be separated by ion exchange. The wide dynamic range of the majority of the IC detection methods makes IC applicable to the quantification of trace contaminants as well as major product components in the same run. IC typically uses dilute acids, alkalis, buffers, or salt solutions as the mobile phase, reducing solvent cost and simplifying disposal logistics. In most cases, the effluent can be disposed of after appropriate neutralization and, when necessary, after dilution with water.

IC is typically performed at or near ambient temperature. As with other forms of HPLC, IC separations are based on varying capacity factors and typically follow the Knox equation. IC is a technique often complementary to reversed-phase and normal-phase HPLC as well as atomic absorption and inductively-coupled plasma techniques in pharmaceutical analysis.

APPARATUS

IC instruments can be either conventional HPLC instruments or instruments specially designed to only perform IC. What type of instrument is chosen depends on the application. Typical components of an IC instrument may include an autosampler, a high pressure pump, a sample loop of suitable size, a guard column, an analytical column, an optional suppressor if conductivity detection is used, a flow-through detector, and a data system (*Figure 1*).

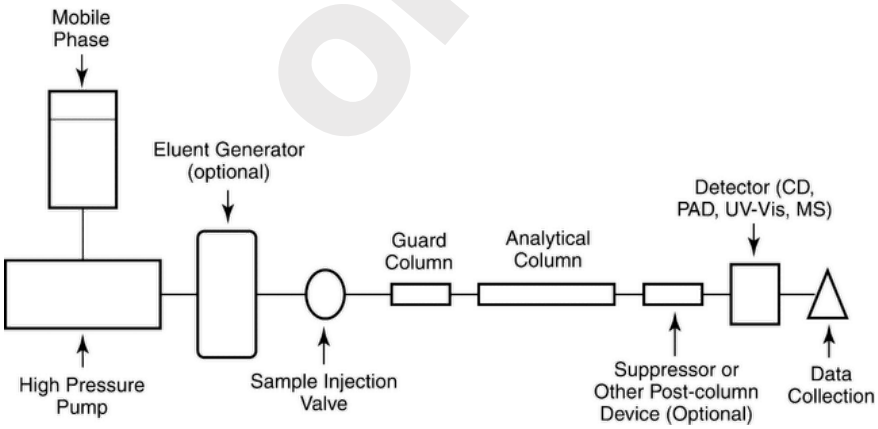

Figure 1. Components of a typical IC system illustrated schematically; CD = conductivity detector, PAD = pulsed amperometric detector, and MS = mass spectrometric detector. [NOTE—Mobile phase is water when the optional *Eluent Generator* is used.]

Because mobile phases generally consist of dilute acids, alkalis, or salt solutions, the components in contact with the mobile phase and the sample are typically made from inert materials, such as polyetheretherketone (PEEK). A conventional HPLC system can be used provided that its components are compatible with the mobile phase and injected sample solutions, though a metal-free system should be used for trace metal analysis. Following suitable preparation, if needed, the sample is introduced via the injection valve. Because IC uses a

predominantly ionic mobile phase, a suppressor is typically necessary prior to conductometric detection when high sensitivity is needed, although nonsuppressed conductometric detection has been successfully used in pharmaceutical analysis.

Mobile Phases

Nearly all IC separations require acids or bases diluted in high-purity water, generally with resistivity greater than 18 megohms-cm to prepare the mobile phases. As in every chromatographic method the sensitivity dictates the detection method, and the selectivity dictates the mobile phase and column selection.

For suppressed conductivity detection, the bases and acids used are suppressed to water or weakly dissociated species. If the analytes are anions, the mobile phase bases used as counterions by suppressed conductivity detection are sodium or potassium hydroxide, sodium carbonate, sodium bicarbonate, and less often sodium tetraborate. If the analytes are cations, the mobile phase acids used as counterions by suppressed conductivity detection are methanesulfonic acid, sulfuric acid, and less often hydrochloric acid. When determining low ion concentrations, appropriate trapping technology should be used to purify the mobile phase.

When the detection is based on UV or visible absorbance, then a wide variety of salt solutions may be used to prepare the mobile phase, including phthalic acid and *p*-hydroxybenzoic acid for the determination of anions, and methanesulfonic acid for the determination of cations.

Amperometric detection uses either a strong acid or base solution as the mobile phase, although some methods can use a salt solution near neutral pH or an acid or base solution containing a salt. When using alkaline mobile phases, carbon dioxide/carbonate contamination can be minimized through the use of high purity reagents and storage under nitrogen or helium headspace. Most IC-MS methods use the same mobile phases used for suppressed conductivity, though volatile amines and volatile salt solutions have also been used. IC methods that use an ion-exclusion column use solutions of strong acids such as sulfuric acid as mobile phases. Organic solvents commonly used for HPLC are sometimes used to prepare the mobile phase, typically at NMT 20% concentration. This addition is usually made to either modify selectivity or to enhance solubility of sample components that might otherwise contaminate the stationary phase.

Also, protein molecules may get a different surface charge distribution according to their structure and the pH of the mobile phase. In those cases, the pH, gradient of pH, ionic strength, or a combination of those parameters proved to be useful in the separation.

Stationary Phases

IC separations rely on ion exchange, and therefore stationary phases for IC are anion or cation exchangers, and less commonly phases containing both functionalities. Stationary phases may be silica-based or polymer-based materials supporting ionic functional groups. However, due to the solubility of silica gel in water, particularly at alkaline pHs, the use of silica-based support is limited when the mobile phase is alkaline. In those cases, a polymeric support for IC is useful over an extended pH range. Most of the phases used today are constructed with highly cross-linked polymers, increasing their compatibility with the organic solvents that sometimes are needed to prepare the mobile phase. Polymeric anion-exchange phases for IC are typically constructed from polystyrene/divinylbenzene, ethylvinylbenzene/divinylbenzene, or polyvinyl alcohol polymeric substrate and polymethacrylate supports, with particles sizes ranging from 4 to 15 µm in diameter either nonporous or with pores up to 2000 Å. To provide functionality to the anion exchanger, the anion-exchange groups are attached to the substrate typically in one of two manners, electrostatically or covalently by condensation polymerization (a.k.a. a step-growth polymerization). Cation-exchange phases for IC use the same polymeric substrates as the anion-exchange phases, but because the mobile phases for cation IC are acidic, silica can also be used. Ion-exclusion chromatography uses a porous strong cation-exchange stationary phase, while ion-pair chromatography typically uses a polymer reversed-phase stationary phase.

Detection

Conductivity detection is the most commonly employed mode of detection in IC, especially for suppressed conductivity detection.

In suppressed conductivity detection, the background conductance of the ionic mobile phase is significantly reduced as it flows through the suppression device (suppressor). For example, when diluted sodium hydroxide (10–50 mM) used as the mobile phase in IC of anions flows through the suppressor, it is converted to water (H_2O), which shows very poor conductivity. Analogous reactions occur using a suppressor for cations, where the acidic mobile phase is converted to water, and the analyte cations are converted to highly conducting hydroxide forms (due to higher equivalent conductance of hydroxide ions compared to other anions).

The reduced background conductance and the enhanced signal due to the ionic species result in a significantly enhanced signal-to-noise ratio for the conductometric detection of ions in suppressed IC. This results in reduced baseline noise while increasing the sensitivity and reproducibility of the analysis. Commonly used suppressors can be classified into two categories. In the first type, the reactions occur across an ion-exchange membrane with the regenerant ions furnished by either a chemical or as products of electrolysis of water. In the second type, the suppression reactions occur in a packed bed of high-exchange capacity resin or monolith material, with regeneration either by a chemical or by electrolysis of water.

Pulsed/Integrated Amperometric Detection (PAD/IPAD)

PAD and IPAD are modes of amperometric detection that apply more than one potential to the working electrode. These detection modes are commonly used for the detection of electroactive species, e.g., organic compounds such as carbohydrates, sugar alcohols, amino acids, amines, and organic sulfur species that can be easily oxidized. Analytes are detected by an oxidative desorption process at the surface of an electrode located in the column effluent stream. PAD uses one potential for detection while IPAD uses multiple potentials. The current generated during the fixed time periods these detection potentials are applied is integrated to yield charge. Following detection, a series of potentials are applied for fixed time periods to clean the electrode surface. Unlike conventional direct current amperometry that suffers

from electrode surface fouling, this rapidly repeating sequence of potentials for detection and electrode cleaning, referred to as a waveform, allows detection and removal of the products of redox reactions from the working electrode surface.

UV DETECTION

Direct UV detection is used for inorganic and organic ions that absorb UV light, typically at low wavelengths. These include organic acids, bromide, iodide, nitrate, nitrite, thiosulfate, and cyano-metal complexes. Indirect UV detection uses eluents that strongly absorb in the visible or ultraviolet spectral region. A wavelength is selected where the eluent absorbs but the sample ions do not, and then negative peaks proportional to the analyte concentration are detected. In spectral detection after post-column reaction, some analytes are detected after the column effluent is combined with a reagent resulting in the formation of a compound that absorbs light at either a UV or visible wavelength. A classic example is the determination of metal ions, where the metal ions in the column effluent are chelated with 4-(2-pyridylazo)-resorcinol followed by detection at 510 and 530 nm.

MASS SPECTROMETRY (MS)

Typically, analytes are detected after they have first passed through a suppressor to make the resulting effluent compatible with the mass spectrometer. Negative mode electrospray ionization is used for anions while the positive mode is used for cations. The suppressor effluent is sometimes augmented with an organic solvent to improve ionization for increased sensitivity. Certain metal ions can be determined by an ion-exchange separation followed by ICPMS.

SAMPLE PREPARATION

Sample preparation may range from simple sample dissolution or dilution to the proper concentration, often followed by filtration, and in other cases more complex preparations need solid-phase extraction (SPE). If the solution is cloudy and/or contains particulates, then filtration through a syringe filter of 0.45-µm pore size that is suitable for IC is needed. Samples containing a high concentration of ions of the same charge as the target analyte may require a sample pretreatment to selectively remove the high concentration ion.

PROCEDURE

The choice of mobile phase is typically dictated by the choice of column, which in turn is chosen based on the selectivity for the analyte(s) compared to other ions of the same charge known or likely to be present. In situations where the other ions are in high concentration, a column with higher capacity is chosen to prevent column overload. This is especially important for many limit tests, where low concentration of a target analyte is in the presence of a large concentration of another ion of the same charge state. For anion IC, some mobile phases can be prepared from the solid or from commercial concentrates or ready-to-use solutions, e.g., sodium bicarbonate/carbonate. Other mobile phases should be prepared and handled with care, e.g., sodium hydroxide solutions, minimizing air exposure, and prepared from 50% sodium hydroxide solutions. Sodium hydroxide pellets and commercial dilute solutions contain large amounts of carbonate, thus altering the desired composition of the mobile phase. The acid solutions for cation IC are prepared by diluting high-purity concentrated acids. Alternately, carbonate/bicarbonate, hydroxide, and methanesulfonic acid mobile phases can be produced by an eluent generator. Most analyses will require the injection of 5–50 µL of sample solution, but larger volumes may be required for the analysis of low concentration analytes. As in other LC techniques, quantification is made by either internal or external standardization procedures, where the concentration is calculated by interpolation of the sample response into a calibration curve. IC methods are validated according to the recommendations described in *Validation of Compendial Procedures* ⟨1225⟩.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

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Pharmacopeial Forum: Volume No. PF 41(3)

Current DocID: GUID-1E654232-BCFD-4855-8481-ACD22AB9A406_1_en-US
DOI: https://doi.org/10.31003/USPNF_M897_01_01
DOI ref: 5zz9i