# When HPLC fails: IC in food, water, and pharmaceutical analysis

Stephanie Kappes

**High-Performance Liquid Chromatography (HPLC) and Ion Chromatography (IC) are commonly used in the pharma, food, and environmental sectors to analyze samples for specific components and to verify compliance with norms and standards. However, users of HPLC may run into the limitations of this technique, e.g., when analyzing standard anions or certain pharmaceutical impurities. This white paper outlines how such challenges can be overcome with IC.**

Metrohm International Headquarters; Herisau, Switzerland

## What is liquid chromatography?

The term liquid chromatography (LC) denotes a class of physicochemical techniques to separate and quantify compounds in a sample. LC involves a liquid mobile phase, which carries the sample, and a stationary phase at which the separation takes place. The stationary phase consists of small particles in the single µm range packed in a separation column.

The separation of sample compounds is based on their relative strength of interaction with the mobile and stationary phases: compounds with a stronger relative affinity for the stationary phase will experience a stronger retention on the separation column and will therefore elute after compounds with a weaker relative affinity for the stationary phase. Following the separation, the compounds can be quantified using a variety of different detection techniques.

Ion chromatography (IC) and high-performance liquid chromatography (HPLC) are among the most commonly used liquid chromatography techniques in analytical chemistry. For various industries, they are essential tools, which are used from raw material inspection to analysis of additives to quality control of the final product. Even thermally sensitive samples can be analyzed without any problems, as both techniques can be performed at room temperature.

LC has become indispensable in the production of pharmaceuticals as well as foods and beverages and in the analysis of water – from environmental waters to process water to sewage water. In these strongly regulated sectors, IC and HPLC provide sensitive and selective analyses that ensure the compliance with norms and standards.

# HPLC vs. IC: The differences

**In the literature, the line between HPLC and IC often becomes blurred. In fact, the two techniques are closely related. So, what are the differences between HPLC and IC?**

## Separation mechanisms

In HPLC, separation is based on hydrophilic interactions (normal-phase HPLC) or hydrophobic interactions (reversed-phase HPLC) of the analytes with the stationary phase. This makes the technique suitable for the separation of polar and nonpolar, organic compounds. Reversed-phase HPLC is the most commonly used mode of HPLC with the most applications. The separation relies on organic solvents as eluents.

In IC, the most important separation mechanism is ion exchange. Other mechanisms, such as ion exclusion and ion pairing are also used depending on the application. Separation by ion exchange relies on chemical reactions of the ionic analytes with the ion exchanger groups of the stationary phase rather than physical interactions as in HPLC. However, the separation of non-ionic, polar compounds is equally possible using IC equipment when the right column and mobile phase are used. This makes IC suitable for the separation of organic and inorganic ions and polar substances.

## Eluents

HPLC separations are done using organic solvents as eluents, in general using gradient elution to obtain good resolution for the weakly retained analytes and speed up the elution of strongly retained analytes. In order to be miscible with the eluent, the samples are typically dissolved in organic solvents as well. Contrary to HPLC, the separation in ion chromatography takes place in the aqueous phase. Typical eluents are made up of ultrapure water with dissolved salts or acids. The vast majority of IC separations can be done by isocratic elution, i.e., without a gradient.

## Separation columns

Separation columns in IC and HPLC are both based on surface-modified particles as stationary phases that interact with the analytes to be separated. But the surface functionalization differs: the stationary phase in IC is characterized by anion or cation exchanger groups, whereas HPLC relies on hydrophobic (reversed-phase HPLC) or polar (normal-phase HPLC) functional groups.

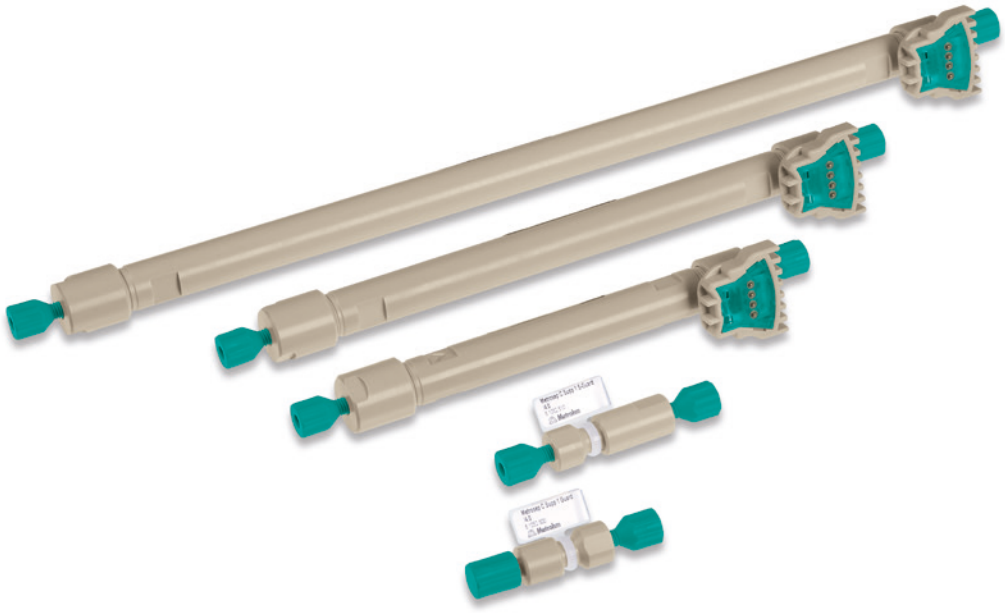


**Figure 1.** IC columns differ from HPLC columns not only on the inside: IC column housings are made of chemically inert PEEK rather than stainless steel, which is susceptible to corrosion when exposed to the eluents used in IC.

## Detection

The primary detector used in HPLC is the UV detector. UV detectors are capable of detecting light-absorbing substances: the eluate leaving the separation column is irradiated with UV light. The analyte is quantified from the difference in absorbance measured between the mobile phase without sample and eluate containing analyte.

In IC, a conductivity detector is classically used. This detects and quantifies the analyte by measuring the changes in the conductivity of the eluate. Conductivity detection is a nonselective but sensitive detection technique.

| | HPLC | IC |
|---|---|---|
| **Analytes** | Polar and non-polar organic molecules | Ionic and polar inorganic and organic molecules |
| **Injection volume** | 50–20 µL | Variable volume up to 1000 µL |
| **System** | Stainless steel, 0–50 (100*) MPa, typically 15–25 (90*) MPa | PEEK, 0–35 MPa, typically 10–20 MPa |
| **Eluent** | Organic solvents, typical gradient profile | Ultrapure water with salt, typically isocratic method |
| **Gradient** | High and low pressure gradients | High and low pressure gradients |
| **Separation** | Adsorptive effects, → fast with very sharp peaks | Chemical reactions, → typically 15 min for 7 peaks |
| **Detection** | Primarily UV | Primarily conductivity |
| **Special features** | | Suppression |

*Maximum pressure in Ultra-High-Performance Liquid Chromatography (UHPLC)

**Table 1.** Comparison of ion chromatography and high-performance liquid chromatography

# When HPLC Fails

### Limitations of HPLC

There are limitations to what HPLC can do, which are primarily due to the columns and the detector used. For instance, it is not possible to separate most ionic analytes on HPLC columns. This means that HPLC is not suitable for analyses of standard anions or cations in water and in various foods. The same holds for organic acids, e.g., in wine, beer, or dairy products.

In other cases, HPLC may reach its limits in the detection step: UV detection requires that the analytes absorb UV light. Certain analytes that are important in the analysis of water, food, and pharmaceuticals, including fluoride and chloride, do not absorb UV light, so they cannot be determined by HPLC.

### Overcoming the limitations of HPLC – with IC

When HPLC fails, ion chromatography can often provide a solution. Using ion exchange as separation mechanism, IC enables separation of many analytes that cannot be separated on HPLC columns. IC is ideal for the separation and determination of ions and polar molecules, both organic and inorganic.

Furthermore, analytes that do not absorb UV light, such as fluoride and chloride, can be determined by IC thanks to conductivity detection. Using conductivity detection with suppression, which will be explained in the following, a very high sensitivity can be achieved.

# Suppression

### Suppression: Increase measuring sensitivity

Ion chromatographic separation requires conductive buffers in the mobile phase. Therefore, the eluate leaving the separation column is highly conductive. Conductivity detection of the analyte ions against this high background conductivity is not ideal. The solution to this problem is called suppression: suppression brings down the background conductivity. Ion chromatographic analyses with conductivity detection, in particular of anions, virtually always involve suppression.

Salts of weakly dissociated acids, e.g., sodium carbonate or sodium hydrogen carbonate, are used as eluents in ion exchange chromatography. During suppression, the positively charged ions of the eluent are replaced with protons by means of ion exchange. The carbonic acid formed as a result is very weakly dissociated and thus exhibits a very low residual conductivity. Likewise, the positively charged counterions of the analyte anions are replaced with protons. As a result, the conductivity of the analyte-containing eluate increases substantially.

To sum up, suppression decreases the background conductivity of the eluent, minimizes baseline noise, optimizes the signal-to-noise ratio, and increases the sensitivity of the measuring system.

### The Metrohm Suppressor Module

The patented Metrohm Suppressor Module, or MSM for short, consists of a small rotor containing three cartridges filled with cation exchanger resin (**Figure 2**). While the first cartridge is used for suppression, a regeneration step is performed on the second cartridge. Meanwhile, the third cartridge undergoes a rinsing step. Thanks to the three-cartridge system, a freshly regenerated cartridge is always available for every new sample – there is no idle time.

Compared to membrane suppressors, which are also commonly used in IC, the MSM has several benefits for the user, including exceptional back pressure resistance, compatibility with organic solvents and the like as well as a lifespan of 10+ years and a 10-year warranty to guarantee this.

In the MSM, the regenerated suppressor unit is rinsed using the suppressed eluent after detection. This process is called STREAM (Suppressor Treatment Reusing Eluent After Measurement). STREAM has a number of advantages: there's no need for ultrapure water for rinsing, the regenerant consumption is reduced by a factor of three, and the demand for consumables is considerably reduced, which ultimately results in less waste, lower costs, and an eco-friendly analysis.

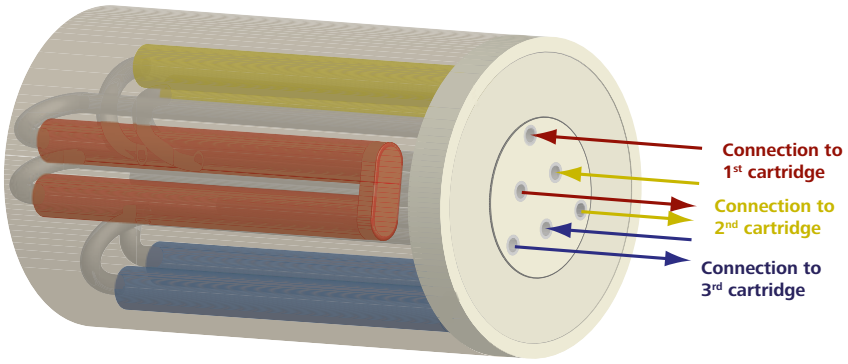



**Figure 2.** The Metrohm Suppressor Module contains three suppressor cartridges. A cartridge is always available for suppression while the other two undergo regeneration and rinsng, respectively.

# IC analysis of water, food, and pharmaceuticals

### Benefits for IC users

Ion chromatography covers a wide range of applications in the analysis of water, food, and pharmaceuticals. Typical samples range from ultrapure water and drinking water to strongly polluted wastewater and from environmental samples to food and beverage samples. IC can handle the strongly varying analyte concentrations of these samples, from parts per trillion to the percent range.

Ion chromatography comes with a wide array of sample preparation options, many of which can be performed inline, fully automatically. These include ultrafiltration, dialysis, and dilution. With the Metrohm intelligent Preconcentration Technique with Matrix Elimination (MiPCT-ME), accurate determinations down to the µg/L and even ng/L ranges are enabled. In addition, Metrohm IC can be combined with «intelligent» sample injection techniques such as the Metrohm intelligent Partial Loop Technique (MiPT, **Figure 3**), which enables the system to measure samples across a wide concentration range by adapting the injection volume of the undiluted sample.

Users of IC furthermore benefit from large calibration ranges, which allow them to measure samples with widely varying analyte concentrations with a single calibration.

Compared to HPLC, IC has the advantage that analysis takes place in the aqueous phase, which means that organic solvents aren't required in the mobile phase. This saves users costs both for purchase and for the proper disposal of the solvents.

In the following, three example applications of IC are presented, one each for the sectors water, pharmaceuticals as well and food and beverages.

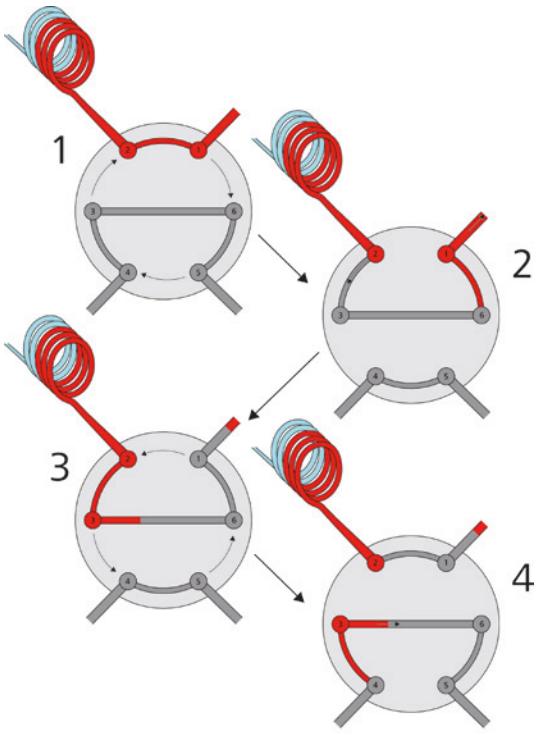



**Figure 3.** With the Metrohm intelligent Partial Loop Technique, it is possible to inject different sample volumes without any changes to the IC system. If a sample exceeds the calibration range, the injection volume is automatically adapted.

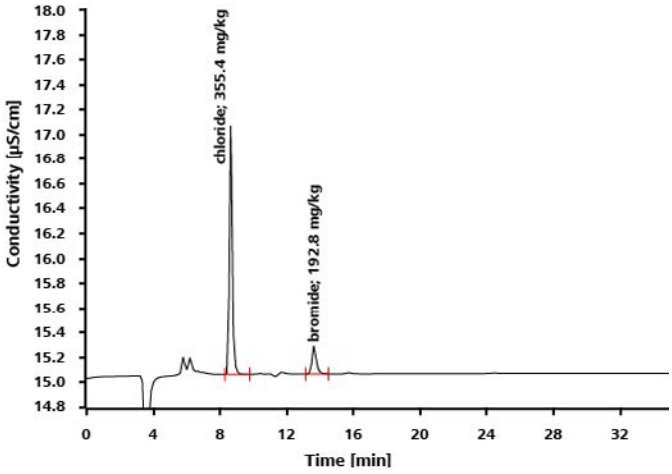
# Application: Determination of chloride and bromide in levetiracetam

### Scope
Chloride and bromide occur as impurities in the drug levetiracetam, which is used to treat epilepsy. While HPLC cannot separate these anionic impurities, chloride and bromide are separated on IC columns and determined with high sensitivity and precision using suppressed conductivity detection.

### Sample preparation
The sample is dissolved and diluted with ultrapure water followed by 5 minutes of sonication. Before injection, the sample is filtered through a 0.2 µm membrane. Dilution and filtration can be done automatically, inline (see «Instrumentation»).

### Instrumentation
As dilution and filtration are required to determine chloride and bromide in levetiracetam, it is recommended to use an IC system with a Dosino to handle the liquids and an ultrafiltration cell. This way, both steps can be carried out inline and fully automatically. Furthermore, a guard column is required before the separation column to protect the latter from contamination. Separation of the analytes takes place on an anion exchange column. The system is further equipped with an anion suppressor and a conductivity detector.

A sample processor allows further automation of the analyses and increases sample throughput.

### Results



### Further information
Find more applications for the pharmaceutical industry in our brochure «Pharmaceutical analysis», available for download at **www.metrohm.com/documents/80005139**.

# Application: Inorganic anions in drinking water according to EPA 300.1 Parts A and B

## Scope

EPA Method 300.1 describes the determination of inorganic anions in drinking water by ion chromatography. The anions covered by the norm include seven common anions (Part A) as well as three oxyhalides (Part B). The latter occur in drinking water as disinfection byproducts. For details, see Table 2. With IC, it is possible to determine both anions and oxyhalides in a single analysis run.

| Common anions | Oxyhalides |
|---|---|
| Fluoride | Chlorate |
| Chloride | Chlorite |
| Nitrite | Bromate |
| Nitrate | |
| Bromide | |
| Phosphate | |
| Sulfate | |

**Table 2.** Analytes described in EPA Method 300.1 Part A (common anions) and B (oxyhalides)

## Sample preparation

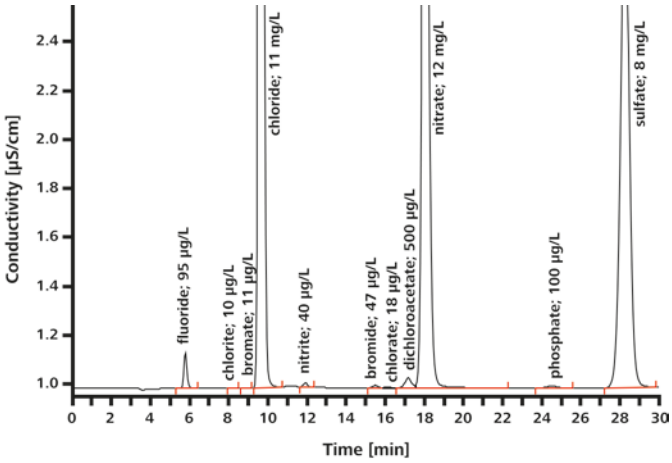
If the samples contain particles larger than 0.45 microns or if reagents contain particles larger than 0.20 microns, a filtration step should precede the injection to protect the column. This step can be automated (see «Instrumentation»).

## Instrumentation

If filtration is needed, this can be done inline and fully automatically by adding an ultrafiltration cell to the IC system. EPA 300.1 stipulates the use of a guard column upstream of the separation column to protect the latter. Detection is done following suppression, using a conductivity cell.

A sample processor allows further automation of the analyses and increases sample throughput as well as reproducibility.

## Results




## Further information

For more detailed information on this application, please visit **www.metrohm.com/applications** and download application note AN-S-312.

More applications for the field of water analysis can be found in our brochure «Water analysis» available for download at **www.metrohm.com/documents/80005141**.

# Application: Anions in beer

## Scope

When brewing beer, impurities in the water used for brewing end up in the finished product and can affect the flavor if they exceed the defined limits. Therefore, anions such as chloride, phosphate, nitrate, and sulfate need to be determined in the scope of quality control of beer.

## Sample preparation

Dilution of samples may be necessary. In addition, beer samples should be filtered before analysis. Both steps can be done inline, automatically (see «Instrumentation»).

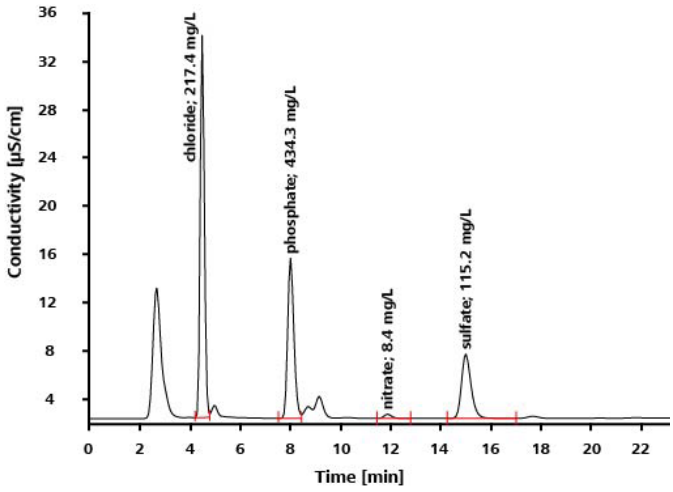
## Instrumentation

It is recommended to use an IC system equipped with Metrohm Inline Dilution Technique (MIDT) and Metrohm Inline Ultrafiltration. With this setup, all necessary sample preparation steps are perfeormed fully automatically and inline. MIDT gives the user the flexibility to inject samples diluted or undiluted and the possibility of spiking samples automatically. Logical dilutions are also possible: if a sample exceeds the calibration range, the MagIC Net software will trigger a new dilution, followed by an injection with an adjusted dilution factor.

Furthermore, a guard column is required upstream of the separation column to protect the latter from contamination. Separation of the analytes takes place on an anion exchange column. The system is further equipped with an anion suppressor and a conductivity detector.

A sample processor allows further automation of the analyses and increases sample throughput.

## Results




## Further information

More applications for the food and beverage industry can be found in our brochure «Food analysis», available for download at **www.metrohm.com/documents/80005257**.

WP-045EN by Metrohm, published May 2, 2019

**Contact:** info@metrohm.com

Metrohm