

Highlighted Capillary IC Applications

Monika Verma and Mike Doyle
Thermo Fisher Scientific, Sunnyvale, CA, USA

Executive Summary

Capillary IC systems provide true on-request 24/7 uptime with no equilibration necessary, taking performance and ease-of-use to a whole new level. These *always ready* systems can be used for various applications, including, but not limited to, environmental, food & beverage, biopharmaceutical and pharmaceutical analysis.



Thermo Scientific™ Dionex™ ICS-4000 Capillary HPIC™ System

Keywords

Capillary IC, Reagent Free Ion Chromatography, High-Pressure Ion Chromatography, 4 μm Columns, HPAE-PAD

Benefits of Capillary Ion Chromatography (IC)

Column size, injection volumes, and flow rates are scaled down by a factor of 10 to 100 in capillary IC. A capillary IC system can be left *always ready* to run samples as soon as they are prepared. This helps improve system stability and reduces the need for recalibration as well. A continuous mode of operation is possible because capillary IC systems only consume 15 mL of water a day, translating into 5.2 L a year.

The waste produced in a capillary IC system is dramatically minimized which in turn reduces disposal costs. When operated as a Reagent-free™ Ion Chromatography (RFIC™) system, the Eluent Generation Cartridge lasts for 18 months under continuous operation. Using eluent generation, only water flows through the pumps, which greatly extends the life of seals and decreases the cost of maintenance.

Capillary RFIC systems provide trace level determinations using using sample volumes up to 250 μL . One approach is to perform a large-volume direct injection, which is suitable for samples with low levels of matrix ions. A 10 μL injection onto a 0.4 mm i.d. column in a capillary IC system is equivalent to a 1000 μL injection onto a 4 mm i.d. column. Similarly, an alternative approach would be loading a 250 μL sample onto a capillary concentrator rather than loading a 25 mL sample onto a 4 mm concentrator. Therefore, capillary IC systems can offer significant benefits in trace analysis where sample volumes might be limited.

The Dionex ICS-5000+ and the Dionex ICS-4000 IC systems represent our latest innovations in capillary ion chromatography. They are also high-pressure IC (HPIC) systems, which allow continuous operation up to 5000 psi when configured as an RFIC system.



Thermo Scientific Dionex ICS-5000+ HPIC System

These high-pressure capillary IC systems can support higher back pressure 4 μm particle ion exchange columns. The advantages of using 4 μm particle ion exchange columns include better performance due to their smaller particle size. The 150 mm length columns can be used at higher flow rates to increase productivity. The 250 mm length columns provide higher resolution separations of complex sample matrices.

Selected Capillary IC Applications

The application examples below have been selected to demonstrate the benefits of capillary IC as a technology in various markets. However, the benefits of capillary IC are not limited to only these applications.

Environmental

Perchlorate is identified as an environmental contaminant found in drinking, ground, and surface waters. By interfering with iodine uptake, perchlorate impairs normal thyroid function. Utilizing a 2D-Ion Chromatography (IC) system, trace concentrations of perchlorate in drinking waters can be determined by using capillary format in the second dimension.

2-D IC is significant for applications that include matrix diversion or matrix elimination prior to analysis of trace components. There are numerous advantages of the 2-D matrix diversion approach. Initial sample loading onto the 4 mm column allows a large amount of sample to be loaded due to the analytical column's high capacity. It also allows for higher selectivity of the analytes of interest relative to the matrix ions. Second, it is possible to focus the analyte peak that is partially resolved in the first dimension onto a concentrator column in the second dimension. Hence, by using a capillary format in the second dimension in the example below, there is an improvement in the detection sensitivity of perchlorate.

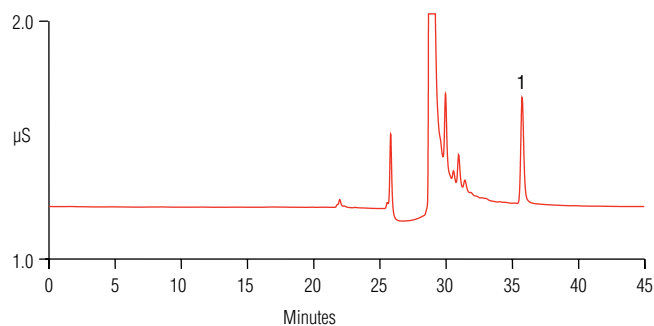


Figure 1. Chromatogram of a 2 $\mu\text{g}/\text{L}$ perchlorate standard in reagent water in the second dimension.

Food and Beverage

The beverage industry is required to meet product labeling requirements in 21 CFR part 101 by determining organic acids which are used as acidulants and flavoring agents. Organic acid determinations can be performed using higher flow rates on a high-resolution column and a capillary IC system capable of high system pressures, such as the high-pressure Dionex ICS-5000+ capillary HPIC system or Dionex ICS-4000 capillary HPIC system. Sample throughput is increased by just increasing the flow rate, thereby saving money and labor. Additional calibration time is eliminated with an *always ready* capillary IC system, as well.

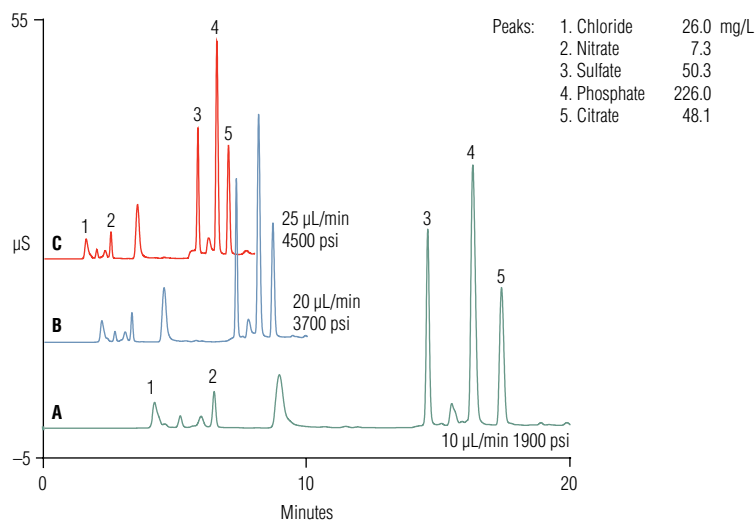


Figure 2. Fast separations of anions in a diet cola beverage by high-pressure capillary IC.

Biopharmaceuticals

Capillary HPAE-PAD

Carbohydrates are difficult to detect by UV absorption without lengthy and costly derivitization. However, carbohydrates can be determined directly by High Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD), a well-established technique that eliminates the need for derivitization.

Certain carbohydrates, such as mannitol, rhamnose, xylose, 3-*O*-methylglucose, and lactulose, are studied by researchers in both urine and serum samples to evaluate disease states. Capillary IC systems provide measurements using relatively small volumes with the benefits of an *always ready* system. As an example, mono- and disaccharides in synthetic urine can be determined on a capillary ion chromatography system as demonstrated below.

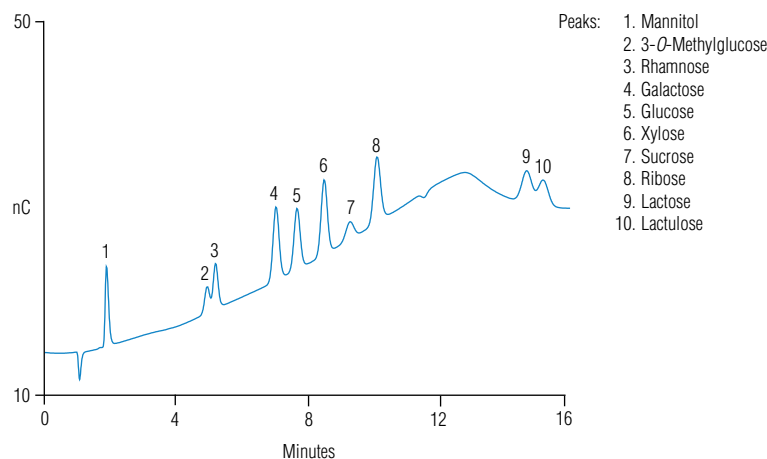


Figure 3. Carbohydrates of interest for urine analysis.

Capillary IC-MS

Therapeutic nucleosides and nucleotides are essential for determining drug efficacy and toxicity. Viral or cell proliferation is inhibited when these compounds are phosphorylated intracellularly to pharmacologically active nucleoside triphosphates. Nucleotides are commonly analyzed by techniques using ion-exchange chromatography or ion-pair reversed-phase high performance liquid chromatography due to their strong hydrophilic nature. Capillary IC-MS/MS offers unique chromatographic selectivity for polar metabolites and can be used as a complementary technique to reversed-phase LC-MS.

Table 1. Nucleotide pool content of methylglyoxal-treated and untreated extracts from 293T cells.

| | Treated (μM) | Untreated (μM) |
|------|---------------------------|-----------------------------|
| ADP | 13.8 | 21.2 |
| AMP | >100 | >100 |
| ATP | 0.524 | 2.53 |
| CDP | 5 | 8.66 |
| CMP | >40 | >40 |
| CTP | 0.351 | 1.17 |
| dATP | ND | ND |
| dCTP | ND | ND |
| dGTP | ND | ND |
| dTTP | 0.085 | 0.100 |
| GDP | 9.05 | 10.1 |
| GMP | >100 | >100 |
| GTP | 0.374 | 1.45 |
| IDP | ND | ND |
| IMP | 37.2 | 23.8 |
| ITP | ND | ND |
| UDP | 7.64 | 11.6 |
| UMP | >100 | >100 |
| UTP | 0.227 | 1.52 |

ND = Not detected

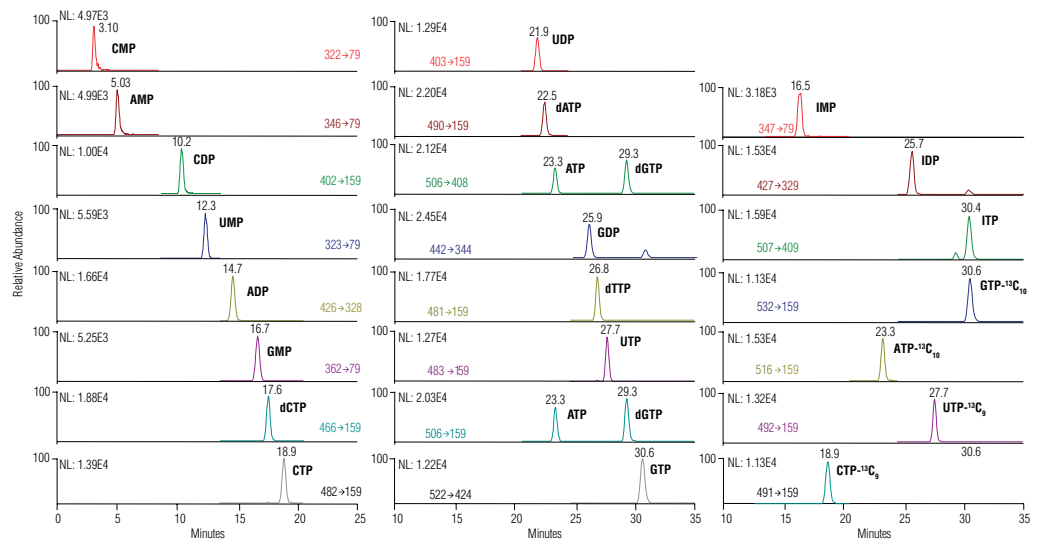


Figure 4. Chromatograms of nucleotide Q-SRM transitions.

Pharmaceuticals

Figure 5 shows the use of a capillary IC system to provide ultrafast separations of inorganic anions and cations relevant to the pharmaceutical industry. Counterions relevant to the pharmaceutical industry are separated by capillary ion-exchange chromatography and detected with high sensitivity by suppressed conductivity on a capillary IC system using Thermo Scientific™ Dionex™ CES™ Capillary Electrolytic Suppressors. The additional *always ready* feature of capillary ion-exchange chromatography systems benefits customers who require quick and flexible analysis.

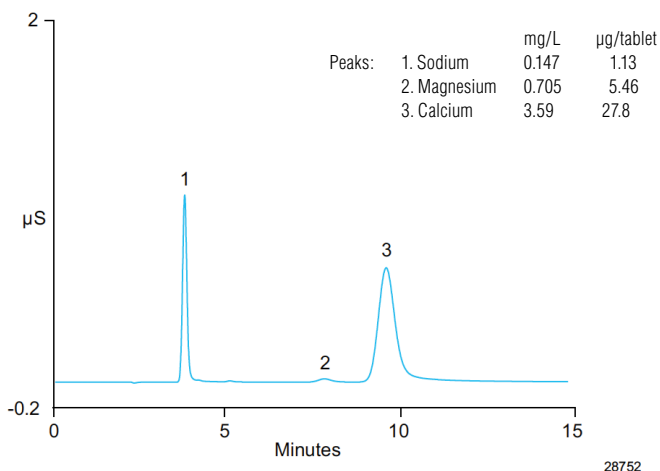


Figure 5. Counterion determinations in an atorvastatin calcium tablet by capillary IC.

References

- Capillary IC Technical Guide
- Whitepaper 70552
- Dionex Homepage (<http://www.dionex.com/en-us/products/ion-chromatography/ic-rfic-systems/ics-5000/lp-72594.html>)
- Dionex Homepage (<http://www.dionex.com/en-us/products/ion-chromatography/ic-rfic-solutions/capillary-ic/always-on-ready/lp-88647.html>)
- Dionex Homepage (<http://www.dionex.com/en-us/products/ion-chromatography/ic-rfic-solutions/capillary-ic/columns/lp-88588.html>)

www.thermoscientific.com/dionex

©2014 Thermo Fisher Scientific Inc. All rights reserved. ISO is a trademark of the International Standards Organization. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.



| | | | |
|--|---------------------------------------|--------------------------------------|-------------------------------------|
| Africa +43 1 333 50 34 0 | Denmark +45 70 23 62 60 | Japan +81 6 6885 1213 | Russia/CIS +43 1 333 50 34 0 |
| Australia +61 3 9757 4300 | Europe-Other +43 1 333 50 34 0 | Korea +82 2 3420 8600 | Singapore +65 6289 1190 |
| Austria +43 810 282 206 | Finland +358 9 3291 0200 | Latin America +1 561 688 8700 | Sweden +46 8 556 468 00 |
| Belgium +32 53 73 42 41 | France +33 1 60 92 48 00 | Middle East +43 1 333 50 34 0 | Switzerland +41 61 716 77 00 |
| Brazil +55 11 3731 5140 | Germany +49 6103 408 1014 | Netherlands +31 76 579 55 55 | Taiwan +886 2 8751 6655 |
| Canada +1 800 530 8447 | India +91 22 6742 9494 | New Zealand +64 9 980 6700 | UK/Ireland +44 1442 233555 |
| China 800 810 5118 (free call domestic) 400 650 5118 | Italy +39 02 950 591 | Norway +46 8 556 468 00 | USA +1 800 532 4752 |

Thermo
SCIENTIFIC

Part of Thermo Fisher Scientific