Speciation Applications Summary

Ion Chromatography

Trace Elemental Species Separation and Detection
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The Ion Chromatography System

Thermo Scientific™ Dionex™ IC Systems have led the analytical instrument industry for over 30 years with solutions that represent state-of-the-art technological advancements and patented technologies. Our products have evolved over many generations, with each new product providing enhanced performance, greater reliability, and easier operation.

Our High-Pressure Ion Chromatography HPIC™ Systems, including the Thermo Scientific Dionex ICS-5000+ system, are optimized for flexibility, modularity, and ease-of-use, combining the highest chromatographic resolution with convenience. Additionally, Capillary IC takes convenience to a whole new level.

The Thermos Scientific Dionex ICS-4000 is the world's first dedicated capillary high-pressure Reagent-Free™ (RFIC™) IC system, delivering high-pressure IC on demand with a system that is always ready for the next analysis.

Reagent-Free IC systems eliminate daily tasks of eluent and regenerant preparation in turn saving time, preventing errors, and increasing convenience. RFIC-EG systems use electrolytic technologies to generate eluent on demand from deionized water, and to suppress the eluent back to pure water to deliver unmatched sensitivity.

At the heart of our ion chromatography is a unique set of column chemistries that provide high selectivities and efficiencies with excellent peak shape and resolution. Thermo Scientific™ Dionex™ IonPac™ chromatography columns address a variety of chromatographic separation modes including ion exchange, ion exclusion, reversed-phase ion pairing, and ion suppression. Our column chemistries are designed to solve specific applications, and we offer a variety of selectivities and capacities for simple and complex samples. Additionally, our Dionex IonPac column line is available in standard bore, microbore and capillary formats for the ultimate application flexibility.

Learn more about our IC systems and consumables at www.thermoscientific.com/dionex.
Speciation Analysis

Speciation Analysis

Separate and Quantify Different Element Chemical Forms

The need to distinguish between chemical forms of an element has become critical for multiple industries, including the food, environmental, and pharmaceutical sectors. In the past, measuring the total amount of an element was sufficient. Unfortunately, the effects of an element extend far beyond its absolute amount. Different forms of an element can exhibit very different physiochemical properties, including varying toxicities. The process of separation and quantification of different chemical forms of an element, more specifically termed speciation analysis, can determine an element’s various chemical forms, and thus deliver a better understanding of the environmental or health related impact associated with a particular sample.

Speciation analysis can be split into two components: separation of individual ionic species by ion chromatography followed by trace elemental detection and quantification using ICP-MS. This combined method is termed Ion Chromatography-Inductively Coupled Plasma Mass Spectrometry (IC-ICP-MS).

ICP-MS is a multi-element spectrometry method to determine total elemental concentrations without bias towards metal species. The technique provides rapid and robust total element concentration determinations in various types of samples with high sensitivity (sub-part per thousand [ppt] detection). This technique leverages the combination of an ICP source with a mass spectrometer. The ICP is a high temperature source that decomposes and atomizes molecules, then ionizes the atoms. The mass spectrometer separates and detects these ions.

The process of ion chromatography allows ions and polar molecules to be separated on the basis of their charge, size, and polarizability. This specific method determines ionic species mainly with a conductivity detector, but can also be used with other types of detectors. With its metal-free fluidic flow path, ion chromatography is ideally suited to elemental speciation analysis. Furthermore, the system can analyze a range of compounds, from anionic and cationic contaminants to disinfection by-products. These are all important indicators of quality in environmental waters, the pharmaceutical industry, and food applications; many of which are toxic and need to be regulated.

The Thermo Scientific™ iCAP Q™ ICP-MS system represents a unique platform to determine the total elemental concentration of a sample. The iCAP Q allows for high sensitivity that can provide single figure ppt detection limits for many elements. As a result, a full mass range analysis can be carried out for routine samples. Additionally, the iCap Q series houses a proprietary collision/reaction cell with low mass cut-off so unwanted species do not pass through the quadrupole mass filter. When coupled with Dionex IC systems, these techniques successfully provide a complete picture for analyzing both total elemental concentration as well as chemical form of the element of interest.

Learn more about speciation analysis at www.thermoscientific.com/speciation.
Complete Inorganic Analysis

Periodic Table of Total Inorganic Analysis

- **AA/ICP/ICP-MS**
- **IC**
- **ICP/ICP-MS only**
- **Unstable elements**
- **Not measurable**
Introduction
Recent media reports in the US have claimed that some apple juices may contain high levels of arsenic. However, in these determination studies, only the total arsenic concentration was assessed; no detailed investigation of the chemical form of the element was carried out. This is an important distinction since the inorganic forms of arsenic (As (III) and As (V)) are highly toxic, while the organic forms (e.g., arsenobetaine) are not considered to be toxic. Typical levels of total arsenic found in apple juice are lower than the US EPA drinking maximum contaminant level (MCL) of 10 ng/g so apple juice is generally considered safe and is currently not regulated. However, as a consequence, the FDA is currently reviewing data, and may eventually lower its current guidelines.

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Arsenic Speciation in Apple Juice

Equipment
- Dionex ICS-5000 IC System*
- Dionex IonPac AS7 column, 2 mm i.d. × 250 mm
- iCAP Q ICP-MS

*Dionex ICS-5000+ HPIC system can be used for equivalent results

iCAP Q Operating Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Power</td>
<td>1550 W</td>
</tr>
<tr>
<td>Nebulizer Gas</td>
<td>0.80 L/min</td>
</tr>
<tr>
<td>Injector</td>
<td>2 mm i.D., quartz</td>
</tr>
<tr>
<td>Interface</td>
<td>Ni sampler and skimmer</td>
</tr>
<tr>
<td>QCell He Gas Flow</td>
<td>4.8 mL/min</td>
</tr>
<tr>
<td>QCell KED</td>
<td>2 V</td>
</tr>
<tr>
<td>75As Dwell Time</td>
<td>100 ms</td>
</tr>
</tbody>
</table>

As species concentrations, method detection limits (MDLs) and total arsenic concentrations in two of the apple juice samples analyzed

<table>
<thead>
<tr>
<th>Species</th>
<th>Expected (ng/g)</th>
<th>Found (ng/g)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsB</td>
<td>2.19</td>
<td>2.27</td>
<td>104</td>
</tr>
<tr>
<td>DMA</td>
<td>1.40</td>
<td>1.15</td>
<td>82</td>
</tr>
<tr>
<td>As (III)</td>
<td>1.35</td>
<td>1.38</td>
<td>102</td>
</tr>
<tr>
<td>AsC</td>
<td>1.94</td>
<td>1.87</td>
<td>94</td>
</tr>
<tr>
<td>MMA</td>
<td>1.09</td>
<td>1.13</td>
<td>104</td>
</tr>
<tr>
<td>As (V)</td>
<td>1.10</td>
<td>1.07</td>
<td>98</td>
</tr>
</tbody>
</table>

Spike recovery for six arsenic species in apple juice

Dionex ICS-5000 IC Operating Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution</td>
<td>Gradient</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>A: 20 mmol/L Ammonium carbonate</td>
</tr>
<tr>
<td></td>
<td>B: 200 mmol/L Ammonium carbonate</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>20 µL</td>
</tr>
<tr>
<td>Duration</td>
<td>15 min</td>
</tr>
</tbody>
</table>

Analysis
IC-ICP-MS
Results
See tables below.
Introduction

Media reports and scientific publications on the determination of arsenic (As) in foodstuffs have sparked renewed interest from consumer groups and politicians leading to responses from national regulatory bodies. Based on recent reports, the FDA began carrying out a study on As in rice and rice products, including organic brown rice syrup, OBRS, an ingredient in a variety of organic foods. In this study, OBRS samples were analyzed for their total arsenic content by ICP-MS and then subsequently by IC-ICP-MS to determine the concentration of six arsenic species: the two toxic inorganic species As (III) and As (V), and four organic species that are considered harmless.

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IC-ICP-MS Speciation Analysis of As in Organic Brown Rice Syrup (ORBS) using the Thermo Scientific iCAP Q ICP-MS

Arsenic Speciation in Organic Brown Rice Syrup

Equipment
- Dionex ICS-5000*
- Dionex IonPac AS7 column, 2 mm i.d. × 250 mm
- iCAP Q ICP-MS

*iCAP Q-5000* HPLC system can be used for equivalent results

**iCAP Q Operating Parameters**
- Forward Power: 1550 W
- Nebulizer Gas: 0.80 L/min
- Injector: 2 mm I.D., quartz
- QCell He Gas Flow: 4.8 mL/min
- QCell KED: 2 V
- Dwell Time: 100 ms

Dionex ICS-5000 IC System Operating Parameters**
- Elution: Gradient
- Mobile Phase: A: 20 mmol/L Ammonium carbonate
  B: 200 mmol/L Ammonium carbonate
- Injection Volume: 20 µL
- Duration: 15 min

**Operating parameters used in analysis based on configurations in AN 43126**

Analysis

IC-ICP-MS

Results

See table and figure below.

<table>
<thead>
<tr>
<th>75As (ng/g)</th>
<th>75As (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBRS Sample #1</td>
<td>118 ± 7</td>
</tr>
<tr>
<td>OBRS Sample #2</td>
<td>136 ± 7</td>
</tr>
<tr>
<td>OBRS Sample #3</td>
<td>107 ± 11</td>
</tr>
</tbody>
</table>

Total As concentrations of >100 ng/g were found in three analyzed samples.

IC-ICP-MS chromatogram of arsenic species found in ORBS sample. As (III) was the most abundant species detected.
Chromium Speciation in Drinking Water

Equipment
- Dionex ICS-5000 IC system*
- Dionex IonPac AG7 column, 2 mm I.D. × 50 mm
- iCAP Qc ICP-MS
* Dionex ICS-5000+ HIPEC system can be used for equivalent results

iCAP Q Operating Parameters
- Forward Power: 1550 W
- Nebulizer Gas: 0.80 L/min
- Injector: 2 mm I.D.
- QCell He Gas Flow: 4.8 mL/min
- QCell KED: 2 V
- Dwell Time: 100 ms

Dionex ICS-5000 IC System Operating Parameters
- Elution: Isocratic
- Mobile Phase: 0.4 mol/L HNO₃
- Flow Rate: 400 µL/min
- Injection Volume: 20 µL
- Duration: 150 s

Analysis
- IC-ICP-MS

Results
- See figures and table below.

Recovery of Cr (VI) and (III) species from drinking water samples

<table>
<thead>
<tr>
<th>Conc. spiked (ng/g)</th>
<th>Cr (VI)</th>
<th>Cr (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found (ng/g)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>2.34 of each</td>
<td>2.31 ± 0.01</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>6.03 Cr (VI); 1.90 Cr (III)</td>
<td>6.01 ± 0.02</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>1.87 Cr (VI); 6.20 Cr (III)</td>
<td>1.85 ± 0.01</td>
<td>99 ± 1</td>
</tr>
</tbody>
</table>

Cr(III) and Cr(VI) chromatograms obtained using 0.2 (A), 0.3 (B) and 0.4 (C) mol/L nitric acid as mobile phase. Note that the x-axis in (C) has been shortened to 300 s.
Determination of Iodide and Iodate in Soy- and Milk-Based Infant Formulas

**Introduction**

Accurate measurement of iodine in food matrices requires a robust iodine extraction method and a sensitive analytical method for iodine quantification. This application note includes the acetic acid digestion method for iodide extraction, coupled with an IC-Pulsed Amperometric Detection (PAD) method for iodide detection first developed in an archived version of this application note. The IC method coupled with electrochemical detection allows for selective and sensitive determination of iodide in complex matrices. The acid digestion procedure to extract iodide was optimized for milk- and soy-based infant formulas. In addition, sample preparation conditions to convert iodate to iodide for determining total iodine (i.e., iodide and iodate) are presented.

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**Iodide and Iodate Speciation in Infant Formula**

**Equipment**

- Dionex ICS-5000 IC system* including:
  - Gradient pump
  - DC Detector Chromatography Compartment
  - ED Electrochemical Detector without cell (P/N 079830)
  - ED Electrode, Ag, with gasket and polishing kit (P/N 079836)
  - Ag/AgCl reference electrode (P/N 061879)
  - Dionex AS or AS-AP Autosampler
- Dionex IonPac AG11 guard, 4 × 50 mm (P/N 44078)
- Dionex IonPac AS11 analytical, 4 × 250 mm (P/N 44076)
- Thermo Scientific™ Dionex™ OnGuard™ II RP Cartridges, 2.5 cc (P/N 057084)
- EO Eluent Organizer with two 2 L plastic bottles and pressure regulator
- Vial Kit, 0.3 mL polyprop with caps and septa (P/N 055428)
- Micro Tubes 1.5 mL, type D, without skirted base, screw cap assembled, sterile (Sarsted™ P/N 72.692.005 or equivalent)
- Thermo Scientific™ Nalgene™ narrow-mouth bottle, HDPE/PP, 1000 mL (P/N 2002-0032)
- Nalgene polystyrene lab filter unit, 500 mL upper capacity with 1000 mL receiver capacity 0.2 micron, 75 mm membrane diameter (P/N 154-0020)

* Dionex ICS-5000+ HPIC system can be used for equivalent results

**Reagents and Standards**

- Deionized (DI) water, type I reagent grade, 18 MΩ-cm resistivity or better filtered through a 0.2 μm filter immediately before use
- Nitric acid
- Sodium iodide
- Sodium iodate
- Ascorbic acid
- Acetic acid

**Dionex ICS-5000 IC System Operating Conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>1.5 mL/min</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>100 μL</td>
</tr>
<tr>
<td>Column Temp</td>
<td>30°C</td>
</tr>
<tr>
<td>Backpressure</td>
<td>1000 psi</td>
</tr>
<tr>
<td>Flush Volume</td>
<td>1000 μL</td>
</tr>
<tr>
<td>Detection</td>
<td>PAD</td>
</tr>
<tr>
<td>Cell Temp</td>
<td>30°C</td>
</tr>
<tr>
<td>Background</td>
<td>2–10 nC</td>
</tr>
<tr>
<td>Working Electrode</td>
<td>Silver working</td>
</tr>
<tr>
<td></td>
<td>electrode</td>
</tr>
<tr>
<td>Reference Electrode</td>
<td>Mode: Ag/AgCl</td>
</tr>
<tr>
<td></td>
<td>Noise: 3–5 pC</td>
</tr>
</tbody>
</table>

**Analysis**

**IC-PAD**

**Results**

See chromatograms below.

Left: Determination of iodide in DI water (A), milk-based infant formulas (B–E), and soy-based infant formula (F). Right: Chromatogram of iodide in infant formula (A) and infant formula spiked with iodate (B).
Mercury Speciation in Herbal Medicines

Equipment
- Dionex ICS-1600 IC System including:
  - Dionex AS-AP Autosampler (P/N 074921)
  - VWD Variable Wavelength Detector 3400 (P/N 070221)
- Dionex IonPac CS5A mixed cation/anion exchange column set
- Inductively Coupled Plasma Mass Specrometer (ICP-MS)

Standards and Reagents
- Acetic acid
- Sodium perchlorate
- Cystine

Analysis
IC-ICP-MS

Results
In this mercury poisoning cluster example, the IC analysis provided mercury speciation which defines to potential toxicity based on the toxicity of each species, whereas the ICP-MS analysis provided a fast multi-element screening. This revealed that mercury caused the clinical symptoms, and determined the total mercury contamination. Inorganic mercury determinations are shown in the table below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured (mg/L)</th>
<th>Calculated (wt %)</th>
<th>Calculated (μg/tablet)</th>
<th>Methylmercury / Ethylmercury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>3.69</td>
<td>0.0586</td>
<td>52</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>15.6</td>
<td>0.2265</td>
<td>387</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>4.82</td>
<td>0.0437</td>
<td>117</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>5.31</td>
<td>0.0473</td>
<td>123</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>11.3</td>
<td>0.0708</td>
<td>243</td>
<td>ND</td>
</tr>
</tbody>
</table>
Mercury Speciation in Contact Lens Solutions

Equipment
- Dionex ICS-5000 IC System*
- Dionex IonPac CS5A column, 2 mm I.D. × 250 mm
- iCAP Qc ICP-MS
  *Dionex ICS-5000’ HPIC System can be used for equivalent results

iCAP Q Operating Parameters
Forward Power: 1550 W
Nebulizer Gas: 1.05 L/min
Injector: 2 mm I.D., quartz
Interface: Ni sampler and skimmer
Dwell Time: 10 ms, 100 ms for speciation analysis
Analysis Mode: Standard (no cell gas)

Dionex ICS-5000 IC System Operating Parameters
Elution: Isocratic
Mobile Phase: 10 mmol/L NaClO₄, 10 mmol/L acetic acid, 10 mmol/L cystine
Flow Rate: 0.5 mL/min
Injection Volume: 20 µL
Duration: 5 min

Analysis
IC-ICP-MS
Results
See table and chromatogram below.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Amount Spiked (mg/kg)</th>
<th>Amount Recovered (mg/kg)</th>
<th>Spike Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.2</td>
<td>10.9 ± 0.04</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>18.1</td>
<td>18.8 ± 0.07</td>
<td>104</td>
</tr>
</tbody>
</table>

Chromatographic separation of EtHg⁺ derived from thimerosal hydrolysis

Spike recovery of thimersal in contact lens solution

Introduction
While there is continual awareness regarding exposure to mercury (Hg) sources in general and MeHg⁺ in particular due to its presence in food samples such as fish, less interest is paid to the potential risk from ethylmercury (EtHg⁺ or EtHgX). One of the main reasons for this is the faster degradation and consequently excretion of EtHg⁺ in the human body that results in considerably lower chronic toxicity. There remains however potential sources where acute intake of EtHg⁺ can occur, for example as a consequence of exposure to thiomersal. Thiomersal is used as a bactericide in multi-dose and in other health related products such as eye drops or contact lens solutions. The compound hydrolyzes in aqueous solution to form EtHg⁺ and thiosalycilate which is an effective bactericide.
Literature References

References for Application Note 43099

• Letters from the FDA to the Dr. Oz Show Regarding Apple Juice and Arsenic (http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm271746.htm)
• FDA arsenic in apple juice resources site: http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm271595.htm
• FDA arsenic in apple juice results: http://www.fda.gov/Food/FoodSafety/FoodContaminants/Adulteration/Metals/ucm272705.htm

References for Application Note 43126

• Arsenic, Organic Foods, and Brown Rice Syrup. Brian P. Jackson¹, Vivien F. Taylor¹, Margaret R. Karagas², Tracy Punshon¹, Kathryn L. Cottingham¹, ¹Trace Element Analysis Laboratory, Department of Earth Sciences, Dartmouth College, Hanover, NH, 03755, USA, ²Dartmouth Medical School, Section of Biostatistics and Epidemiology, Department of Community and Family Medicine, One Medical Center Drive, Lebanon, NH 03756, ³Department of Biological Sciences, Dartmouth College, Hanover, NH, 03755, USA. Environmental Health Perspectives, 2012, http://dx.doi.org/10.1289/ehp.1104619
• FDA Statement on Arsenic in Brown Rice Syrup, February 2012: http://www.fda.gov/Food/FoodSafety/FoodContaminants/Adulteration/Metals/ucm292531.htm
• IC-ICP-MS speciation analysis of As in apple juice using the Thermo Scientific iCAP Q ICP-MS, Application Note AN43099.

References for Application Note 43098


References for Application Note 437


• Yang, S.X.; Fu, S.J.; Wang, M.L. Determination of Trace Iodine in Food and Biological Samples by Cathodic Stripping Voltammetry. Anal. Chem. 1991, 63, 2970–2973.


References for Application Note 43130


References for Application Note 43141

• Thimerosal in Vaccines Questions and Answers, US Food and Drug Administration; http://www.fda.gov/BiologicsBloodVaccines/Vaccines/QuestionsaboutVaccines/UCM070430


• Thimerosal in Vaccines, US Food and Drug Administration; http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/VaccineSafety/UCM096228
<table>
<thead>
<tr>
<th>Country</th>
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<tbody>
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<td>Australia</td>
<td>+61 3 9757 4486</td>
</tr>
<tr>
<td>Austria</td>
<td>+43 1 333 50 34 0</td>
</tr>
<tr>
<td>Belgium</td>
<td>+32 53 73 42 41</td>
</tr>
<tr>
<td>Brazil</td>
<td>+55 11 3731 5140</td>
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<tr>
<td>China</td>
<td>+852 2428 3282</td>
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<tr>
<td>Denmark</td>
<td>+45 70 23 62 60</td>
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<tr>
<td>France</td>
<td>+33 1 60 92 48 00</td>
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<tr>
<td>Germany</td>
<td>+49 6126 991 0</td>
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<tr>
<td>India</td>
<td>+91 22 6742 9404</td>
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<tr>
<td>Italy</td>
<td>+39 02 51 62 1267</td>
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<td>Japan</td>
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<tr>
<td>Korea</td>
<td>+82 2 3420 8600</td>
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<tr>
<td>Netherlands</td>
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<tr>
<td>Singapore</td>
<td>+65 6289 1190</td>
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<tr>
<td>Sweden</td>
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<td>Switzerland</td>
<td>+41 62 205 9966</td>
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<tr>
<td>Taiwan</td>
<td>+886 2 8751 6655</td>
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<tr>
<td>UK/Ireland</td>
<td>+44 1442 233555</td>
</tr>
<tr>
<td>USA and Canada</td>
<td>+847 295 7500</td>
</tr>
</tbody>
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