

Prodigy ICP

Application Note: 1048




Application of the Prodigy ICP as a Multielement-Specific Detector in Chromatography

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Introduction

ICP-based techniques, such as ICP-AES and ICP-MS, are widely used in research and QA/QC laboratories owing to their ability to simultaneously quantify up to 60 elements in a sample within just a couple of minutes. Apart from the fundamentally different principals of operation between these techniques, ICP-AES carries with it the advantage of being able to handle sample solutions that contain up to 30% salt, whereas ICP-MS is inherently limited to the analysis of samples which contain less than about 0.5% salt [1] due to clogging of the ~1.0 mm I.D. sampler cone orifice. This versatility makes ICP-AES ideally suited for use as a detector for separation techniques such as liquid chromatography (LC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE), which all require mobile phases that can contain greater than 0.5% of various salts. This matrix tolerance of ICP-AES imposes fewer restrictions on the choice of the separation mechanism (*e.g.* size-exclusion, reversed-phase, and ion-exchange), and therefore allows this detector to be used in conjunction with more robust separations. In turn, this allows ICP-AES to be used for the direct analysis of real life samples which often contain complex matrices.

Triggered by the advent of environmental research and the realization that total element concentrations are often inadequate to characterize environmental and/or biological samples, instrumental techniques which allow identification of the molecular form of a metal/metalloid compound were developed. This approach typically required extraction of the analyte(s) of interest, followed by the subsequent chromatographic separation and identification/quantification of the separated analyte species in the column effluent by general detectors, such as UV/VIS or conductivity detectors. In the late '70's it was recognized that element-specific detectors, such as flame atomic absorption spectrometers, greatly facilitated the measurement of inorganic and organometallic species in environmental/biological samples [2] since these detectors respond only to the element of interest and generally offer lower detection limits when compared to less-specific general detectors. It follows from these two advantages that much less effort needs to be invested in the development of a separation method since only the analytes need to be separated from each other and not from the matrix constituents. This – at the time – novel analytical approach greatly improved our understanding of the global cycling of toxicologically relevant metal/metalloid compounds and was eventually referred to as “speciation analysis”, which was later defined by the International Union of Pure and Applied Chemistry as “the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample” [3].



The detector technology utilized in the Prodigy ICP-AES allows the system operator to simultaneously monitor multiple analytical wavelengths along with their spectral backgrounds and any internal standards of interest. This permits the instrument to also be used as a true simultaneous multielement detector when hyphenated to a separation technique. The ability to simultaneously measure peak and background emissions is critical to experiments where time varying signals are involved. The reason for this is the net emission intensity (peak minus background) that is used when relating intensity to analyte concentration; and many experiments that involve time resolution also involve changes in parameters (*e.g.* solvent composition) which can significantly alter background emission intensity. Without simultaneous peak and background measurement, these changes in background signal can mislead the operator into believing that an analytically significant event has occurred, when in fact it may have been a simple background shift. This advantage, together with the ability of ICP-AES to handle salt-containing solutions, makes the Prodigy ideally suited for the LC analysis¹ of solutions containing metals or metalloids in proteins (*e.g.* metalloproteins), in drugs, (*e.g.* cis-platin) and/or as impurities. Accordingly, LC-ICP-AES is expected to be applied much more frequently in the near future in applications ranging from analytical process control (*e.g.* biopharmaceutical production), to QA/QC, to clinical applications (*e.g.* blood plasma analysis in hospitals), as well as to bioanalytical chemistry research.

The following narrative describes some basic considerations to determine whether LC-ICP-AES is suitable for an intended analytical challenge. This is followed by a discussion of the requirements necessary to: (1) mechanically hyphenate the LC-system to the ICP-AES detector, (2) configure Prodigy's software for time resolved analysis (TRA) data acquisition, (3) initiate sample analysis and data acquisition, (4) view and export the data, and (5) process the data.

When Can/Should You Use LC-ICP-AES?

In order to determine whether ICP-AES is better suited than a conventional LC detector for a particular application, it is important to first ascertain whether the analyte(s) of interest contain(s) at least one element that is absent from the sample matrix and the mobile phase. If this basic stipulation is realized, then the minimum volume of sample that must be injected on the LC system in order to achieve a chromatographic peak must be determined and can be calculated based on the instrument detection limit of the analyte element(s). In general, if the required sample injection volume is less than 0.5 mL, a LC-ICP-AES method is feasible to separate and identify the analyte(s) of interest. Next, a LC separation method must be identified. Ideally, the scientific literature (*e.g.* Journal of Chromatography A or B) can be accessed to identify or modify an existing LC separation method. In the event that an identified LC method employs a mobile phase which contains high concentrations of organic solvent such as acetonitrile or methanol, it is recommended that you contact Teledyne Leeman Labs for assistance as high concentrations of these solvents may require specialized accessories such as cooled spray chambers [4,5]. In the event that an established separation method cannot be identified, a new method must be developed. This necessitates the identification of the mode of chromatography (*i.e.* nature of the stationary phase), the mobile phase composition (*i.e.* nature of buffer and its concentration, salt and organic solvent content), and the flow rate. An excellent resource for method development questions is a text entitled "Practical High-Performance Liquid Chromatography" [6].

¹ Throughout this application note, all liquid chromatographic separation techniques will be referred to as LC.

Preparation of LC and ICP-AES for Hyphenation

Prior to mechanical hyphenation of the LC to the Prodigy ICP, the LC must be assembled. LC assembly is achieved by connecting the mobile phase reservoir, HPLC pump (commonly used flow rate: 1.0-1.5 mL/min), sample injector valve (with a fixed volume sample loop of 20 to 500 μ L), and analytical separation column (up to 25 cm in length and 4.6 mm in internal diameter) with tubing, ferrules, and nuts [6]. With regard to the ICP-AES, a low dead volume Seaspray concentric nebulizer is inserted into a cyclonic spray chamber which is in turn connected to a standard torch. A low dead volume sample introduction system is necessary to attain sharp chromatographic peaks. Since the operation and maintenance of the ICP-AES are described in detail in the instruction manual [7], no summary of these procedures will be discussed in this application note.

Mechanical Hyphenation of the LC to the ICP-AES

Following basic LC best practices to minimize void volume and chromatographic band broadening [6], hyphenation of the LC to the ICP-AES is achieved by simply connecting the LC column to the ICP-AES nebulizer (Figure 1a).

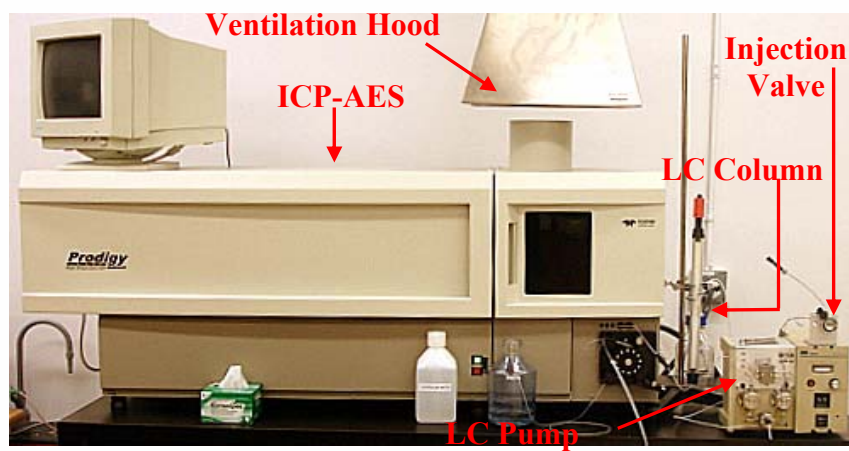


Figure 1a. LC-ICP-AES experimental set-up.

This is accomplished by coupling the PEEK (or steel) tubing of the LC column exit into the PVC tubing inlet of the nebulizer with the shortest possible length of flexible tubing, such as pharmaceutical grade polypropylene tubing (0.50 mm I.D., Gilson, Middleton, MI, USA), as illustrated in Figure 1b.

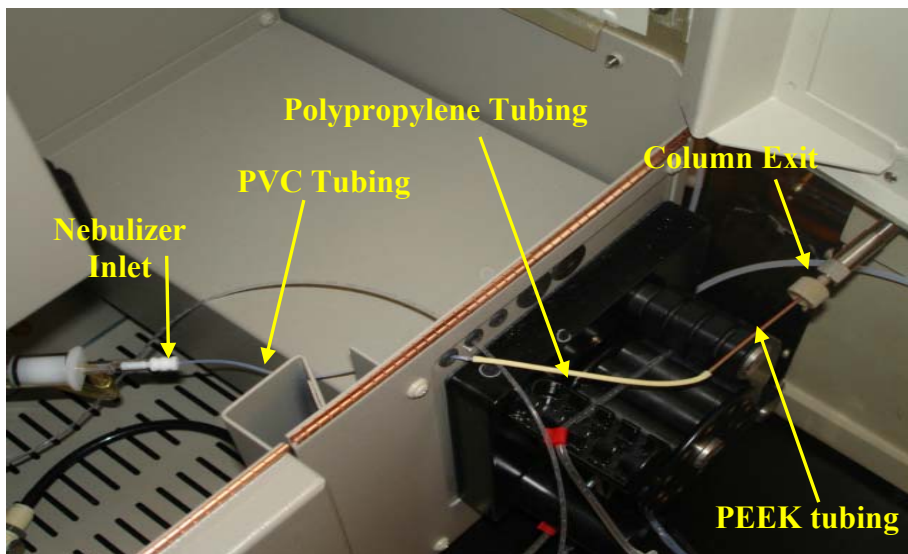


Figure 1b. LC-ICP-AES interface.

Preparation of Prodigy's Software for Chromatographic Analysis

With the mechanical hyphenation of the LC-ICP-AES completed, Prodigy's software can be configured for time resolved data acquisition (records emission intensity as a function of time). This is accomplished by selecting the TRA mode (tick the "Time-Resolved" box in the TRA information inset of Figure 2), the plasma viewing mode (radial or axial), the total analysis time and the time slice per data point. These parameters are located in the "Analytical Parameters" section under the **Method** tab in the navigation panel (Figure 2). In addition, the uptake box in the "Pump" section (Figure 2) must be set to 0 s.

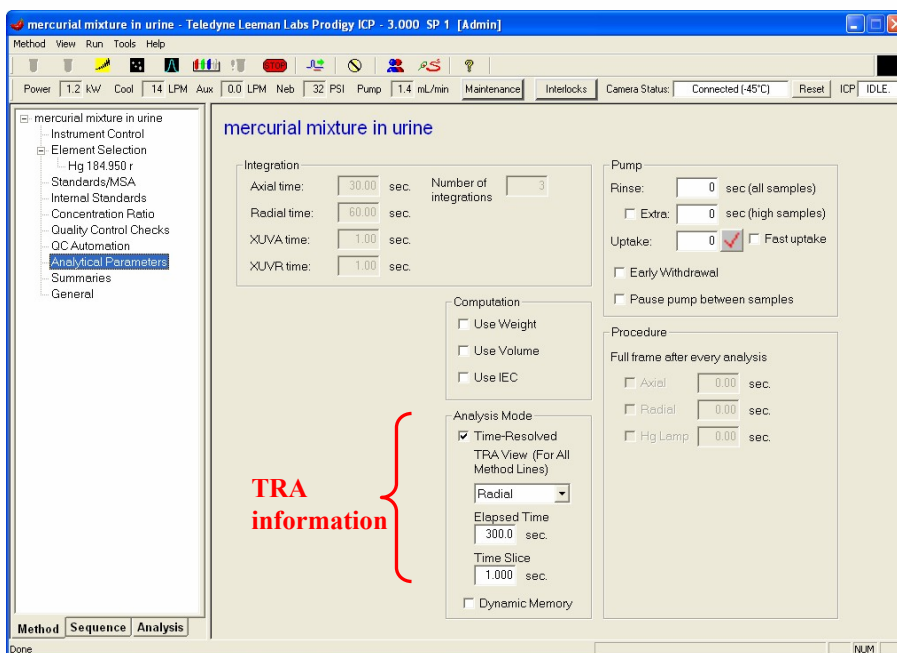


Figure 2. Selection of parameters for the acquisition of a TRA chromatogram.

Time slices of one data point/s are generally sufficient for LC-based separations since HPLC peaks are rarely shorter than ~15 s. This parameter should be adjusted if sharper or wider peaks are expected in order to capture a minimum of 10 data points per analytical peak.

Initiate Sample Analysis and TRA Data Acquisition

After selecting the **Analysis tab** in the navigation panel and the TRA tab in the detail panel (Figure 3), a TRA run is initiated by selecting the TRA icon on the tool bar (Figure 3), entering the sample title, and then clicking OK in the pop-up box whilst simultaneously injecting the sample onto the column by switching the injection valve to the inject position (the previous selection of 0 s uptake ensures that data acquisition coincides with the injection of the sample). Automated synchronization of sample injection and data collection can be accomplished by using Prodigy's "SampSynch" feature if the LC has a suitable input for a trigger signal.

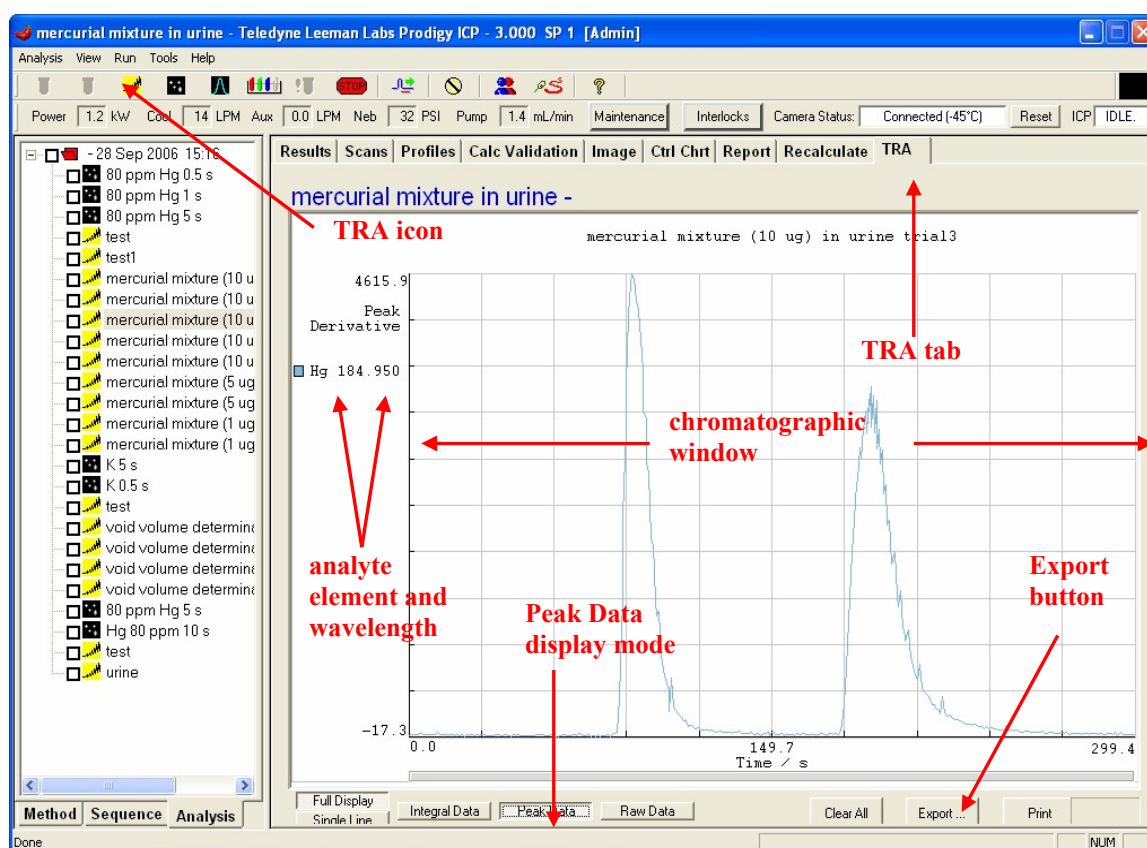


Figure 3a. LC-ICP-AES chromatogram of human urine spiked with mercuric mercury (Hg^{2+} , first eluting peak) and methylmercury (CH_3Hg^+ , second eluting peak).

Data Export and Post-Run Processing

As the sample data are being collected, the background corrected emission intensity of the analyte element(s) is graphically displayed on the screen in real time as shown in Figure 3a. Once the chromatographic analysis is completed, the TRA data can be exported as a comma delimited file by clicking the Export button (Figure 3a) then selecting the peak export data format from the pop-up menu (Figure 3b).

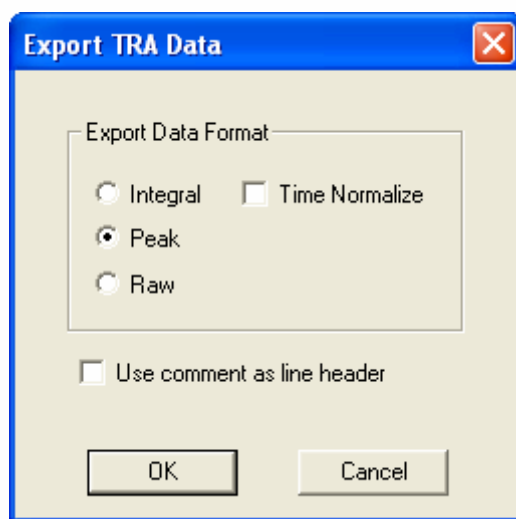


Figure 3b. TRA Data export.

To process the data and generate a figure, the exported data are opened as a Microsoft Excel file wherein the time (expressed in s) and the emission intensity of the analyte element(s) (counts/s) are recorded in consecutive columns. This information can be plotted directly in Microsoft Excel or pasted into an alternative graphical program, such as SigmaPlot or Origin Professional. The chromatogram indicated in Figure 4 was obtained by using SigmaPlot 10, which served as a tool for the visual representation of the TRA chromatogram generated in Figure 3a.

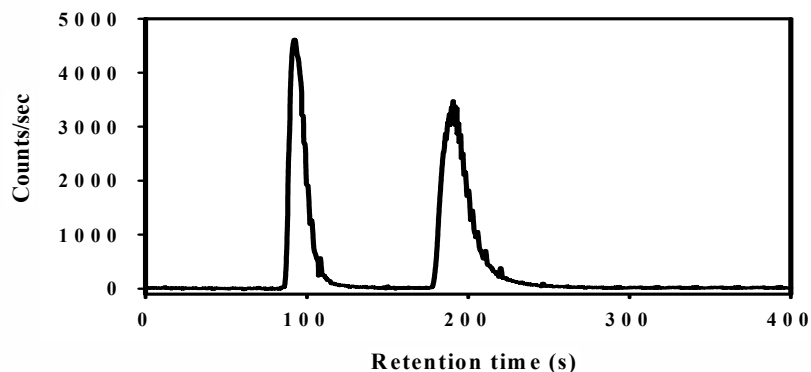


Figure 4. LC-ICP-AES chromatogram of human urine spiked with mercuric mercury (Hg^{2+} , first eluting peak) and methylmercury (CH_3Hg^+ , second eluting peak) [8].

In order to augment the presentation of a chromatogram (e.g. remove electronic spikes), data smoothing can be utilized. This can be achieved by bisquares weighting (polynomial regression of one) and is an available option in SigmaPlot.

To learn more about how the Prodigy ICP can be used to solve your elemental speciation needs, please visit www.LeemanLabs.com or contact your local Leeman Labs representative.



References

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