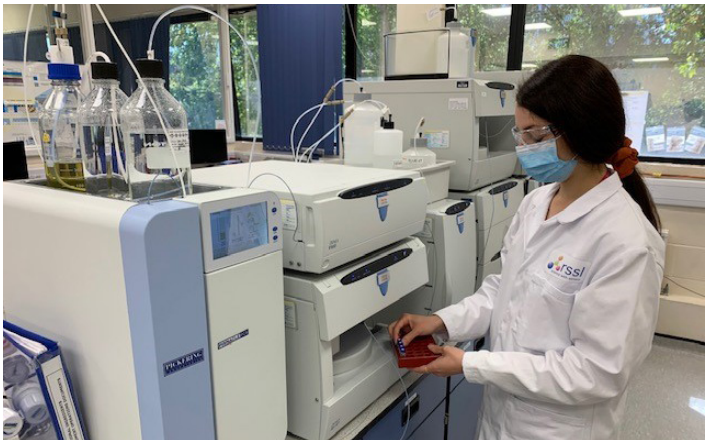
CASE STUDY 73754

RSSL expands GMP services for the pharmaceutical industry with combined Thermo Scientific Dionex ICS-6000 HPIC System—Pickering Pinnacle PCX amino acid analyzer solution

"Though we have a vast variety of testing that we do for clients, this IC solution is devoted to fast turnaround of raw material testing for amino acids per the EP monographs. Considering how much we have used the system so far, it has been very robust and a very good investment."

—Katie Reid
Scientist, Functional Ingredient Laboratory, RSSL



Dionex ICS-6000 standard bore ion chromatography (IC) system with the Pinnacle PCX post-column derivatization instrument. Image courtesy of Reading Scientific Services Ltd (RSSL).

Rugged IC system with post-column derivatization solution provides robust amino acid analysis

The pharmaceutical industry depends on contract research organizations like RSSL to ensure product safety and efficacy, and to reduce time and cost to market. Toward this need, RSSL's Functional Ingredients Laboratory provides a broad range of raw material and product testing services. This testing is governed—and the demand for it driven—by several regulations including the European Pharmacopoeia (EP) monographs that describe quality standards for ingredients, dosage forms, and methods of analysis.

With the initial release of EP 8.0 Amino Acid Analysis 2.2.56, Method 1—Post-Column Ninhydrin Derivatization, demand for Good Manufacturing Practice (GMP)-compliant amino acid testing has grown rapidly. Using the corrosion-resistant Thermo Scientific™ Dionex™ ICS-6000 standard bore ion chromatography system combined with the proven Pickering Pinnacle PCX post-column derivatization instrument, RSSL is able to meet this demand with fast turnaround of samples and GMP-compliant, high-confidence results. The system is now fully verified and in use for testing Serine, Valine, Proline, Leucine, Isoleucine, Histidine, Arginine, Lysine, Glycine, Alanine, Tyrosine, Cysteine, and Phenylalanine in client samples per the EP monographs. A separate optimized mobile phase and column temperature gradient method was developed by RSSL for Tryptophan that prevents co-elution with ammonium, which has also been fully verified to EP requirements.

"Having used the system almost every week for the past three to four months, it's clearly robust. We still get very good resolution between the difficult-to-separate isoleucine and leucine peaks, which was something we were very worried about. That is, I was worried we would not meet the EP separation criteria after a short time, and would have to buy new columns and possibly exchange instrument parts if they were disintegrating."

—Katie Reid, Scientist

The Dionex ICS-6000 system handles challenging post-column ninhydrin derivatization method

The presence of even minute levels of amino acid impurities can affect drug efficacy and cause undesirable side effects. In the past, these impurities were quantified by thin layer chromatography (TLC), a technique that involves a plate separation that is sprayed with ninhydrin. The disadvantage of this approach is that impurities are identified and quantities estimated by visual comparison with a reference solution, a process that is subjective and lacks accuracy.

EP 8.0 Amino Acid Analysis 2.2.56, Method 1, is now EP 10.2—Post-Column Ninhydrin Derivatization, transitions ninhydrin-positive amino acid testing to a more accurate approach using chromatographic separation with ion exchange columns, coupled to a post-column derivatization instrument that introduces ninhydrin to the sample after chromatographic separation is achieved, followed by Vis absorbance detection for quantification. Compared to TLC, the method provides more certainty in the analytical results.

Though high-performance liquid-chromatography (HPLC) is commonly used for separation, the reagents needed cause rapid degradation of liquid chromatography (LC) components and reduced column life. Robust and resistant to reagent corrosion (with a totally inert flow path), the Dionex ICS-6000 system avoids the problems associated with using HPLC for separation, while allowing use of Pickering's popular post-column derivatization solution. The IC instrument's quaternary gradient pump mixes and delivers the Pickering-supplied buffer solutions in defined, precise proportions. The regenerant buffer has a high pH to flush the analytical column, sustaining robust performance. According to Dr. Catherine Frankis, Senior Scientist, RSSL, "Because the eluents required have a very high salt content, precipitation forming blockages either in the column or reactor could occur, causing performance problems. Therefore we use a regeneration wash at the end of each injection and sequence."

The laboratory has fully verified the solution for amino acids testing per the EP monographs (Figure 1). Accurate and reliable, it's sensitive to impurities at a reporting threshold of 0.05% and can determine whether the overall impurity level exceeds EP-defined limits. The solution can also perform the EP ammonium limit test. Having these capabilities extends the range of GMP services that RSSL offers. The method can be applied to:

- Amino acid composition analysis of biopharmaceutical active ingredients
- Impurities and related substances in Active Pharmaceutical Ingredients (APIs) and intermediates such as free amino acids
- Single or total amino acid quantification in drug products, including markers in complex matrices

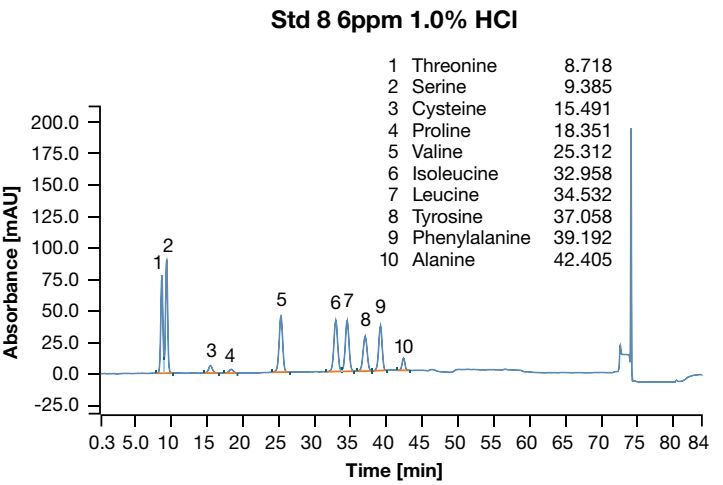


Figure 1. Ion chromatogram obtained from mixed standard solution during verification showing good resolution between the 10 analytes. Separation of isoleucine and leucine by a resolution factor of 1.6 exceeds the EP criteria which requires a value greater than 1.5.

"Together, the separation from Thermo Fisher Scientific and the complete kit from Pickering works very well for the EP monograph testing that it was purchased for. We are able to get very good separation of the required amino acids, so we actually find that very effective."

—Katie Reid, Scientist

Post-column derivatization increases sensitivity and selectivity

After separation, post-column derivatization renders otherwise invisible compounds visible by subjecting them to a chemical reaction that gives them an easily detectable physical property. The approach can increase the detection sensitivity by up to several orders of magnitude. Most reagents are selective for a particular class of substances, making analytes of that class more easily seen against a complex background. The most common reagent used for post-column derivatization of amino acids is ninhydrin, which when combined with primary amines, generates a colored compound.

Pickering Laboratories, Inc. offers the Pinnacle PCX instrument (as shown below), analytical columns and GARDs, buffers and Trione® Ninhydrin reagent as a complete kit for post-column derivatization of amino acids per the EP method. The Pinnacle PCX performs column temperature gradients that allow easily modified conditions and improved run times for amino acid separations. "The post-column derivatization system from Pickering is one of the most reliable on the market. Their application notes demonstrate that their methods and setup meet the EP criteria. When we found out it that could be coupled to the Thermo Scientific IC instrument controlled by Thermo Scientific™ Chromeleon™ Data System (CDS) software, both of which we were familiar with, it made sense even though it was coming from two vendors," said Dr. Frankis.

A scientist checks the Pinnacle PCX post-column derivatization instrument. Image courtesy of Reading Scientific Services Ltd (RSSL).

"Chromeleon CDS is really good for meeting regulatory requirements for data compliance and integrity. It allows for traceability, it has a full audit trail, and allows us to tailor privileges at each user level, or indeed tailor what actions require sign-offs or comment boxes."

—Dr. Catherine Frankis, Senior Scientist

Data integration and integrity, with ease of use

Chromeleon CDS provides control of the multi-vendor solution and consolidates all chromatography data, including data processing and results, on one platform. Chromeleon software is used to set up and manage sequences with start and stop control of the Pinnacle PCX pump, and then records and processes data in accordance with GMP requirements for data integrity (Figure 2). Dr. Frankis said, "I'm very impressed with Chromeleon CDS awareness of GMP requirements. However, Chromeleon CDS does not provide data integrity for the Pinnacle PCX instrument." Built with both laboratories and IT in mind, the software offers superior compliance tools, networking capabilities, instrument control, automation, and data processing. It is designed for easy-to-use tracking, accountability, and QA/QC.

"Catherine mentioned data integrity is a very important part of our quality requirements and that's a great aspect of the software, especially data processing, which is very intuitive. As well as providing data integrity, the software, especially data processing, is very intuitive. It's very easy to queue up sequences, or to add injections from different runs to overlay the chromatograms for review. Everything is consolidated in one place, making it easy to access the data from any run on any instrument, which we find limiting using other vendors' software," added Reid.

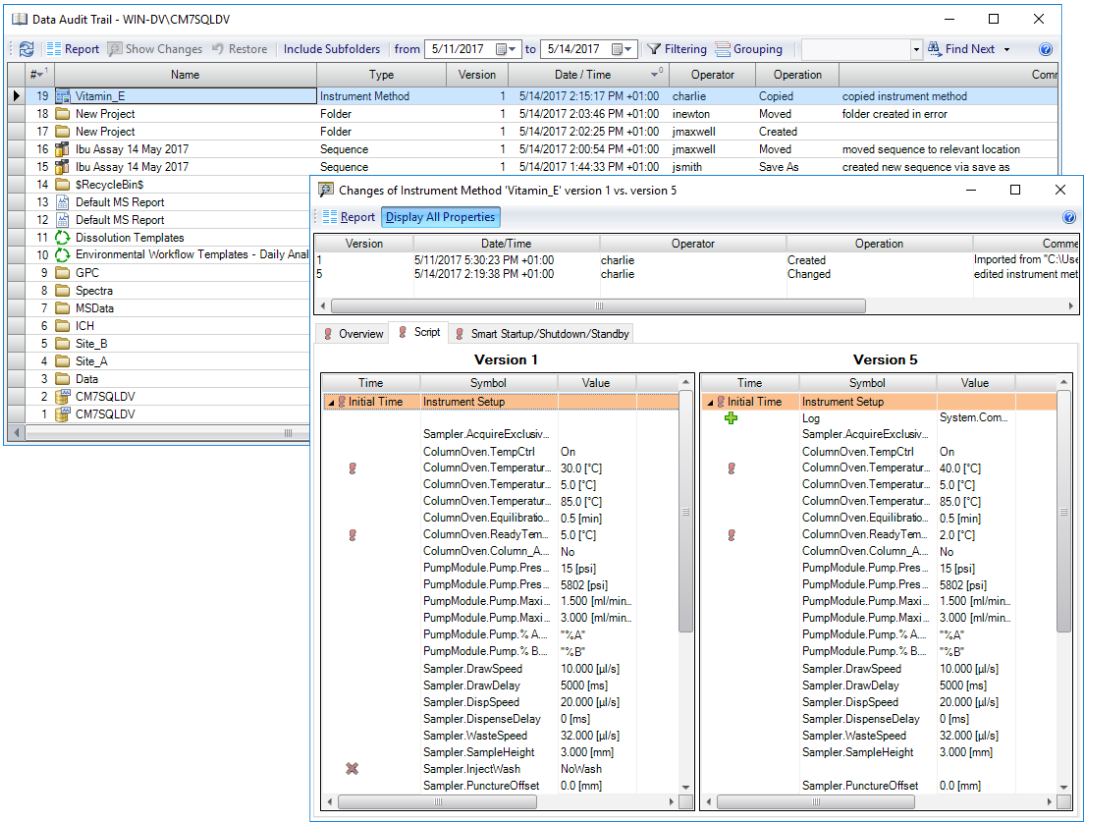


Figure 2. Chromeleon software's modification history tracks all changes to all data objects and lists the before and after state of each variable associated with each change.

"The main thing is that buying this equipment helps us better serve our clients. When we subcontract this testing, the lead-time for getting results back is much longer—up to two weeks longer—than if we were to do it in-house."

—Katie Reid, Scientist

Multivendor coalition consolidates amino acid testing in-house

Prior to investing in the Thermo Fisher Scientific-Pickering solution, RSSL had subcontracted out the analyses of ninhydrin-positive amino acids. However, with the increased demand generated by the EP and the longer turnaround times associated with samples sent out to subcontractors, RSSL needed to bring the analysis in-house. Reid explained, "Clients could be sending us samples, and we wouldn't be able to get results to them for as many as 20 days. When we have the test in-house we can offer as little as five days turnaround time if the client requires it. And, it brings all the client's results together under one quality system for their regulatory submissions. The solution is now in use quite a bit—we put on two batches per week, possibly even three, depending on how we can plan our time."

Teamwork and careful choreography managed by Thermo Fisher ensured the multivendor solution was successfully installed and qualified. After the IC system was installed and qualified by Thermo Fisher, LC Tech (who supports Pickering products in Europe) and Thermo Fisher worked together on-site to ensure the vendors' systems worked together reliably. Following the installation of the Pinnacle PCX onto the Thermo Scientific IC system, LC Tech ensured that the combined system was working as expected.

"I am always very impressed by the Thermo Fisher engineers and we have a very good relationship. They know what we need and what is expected of them, so I was really happy to see a good working relationship between the Thermo Fisher and the LC Tech engineers, which then resulted in a successful installation. It was quite a bit of scheduling for Thermo Fisher, but it worked out," said Dr. Frankis.

Conclusion

The robust and reliable Dionex ICS-6000 IC system combined with the EP-proven Pinnacle PCX post-column derivatization instrument has enabled RRSL to meet growing demands for GMP-compliant amino acid testing in-house and with faster sample turnaround times. Metal-free and resistant to degradation by corrosive reagents, the Thermo Scientific IC system avoids the performance drop-off associated with HPLC for the application. A multivendor coalition led by Thermo Fisher ensured the solution was successfully installed and qualified. RSSL has fully verified the solution for Serine, Valine, Proline, Leucine, Isoleucine, Histidine, Arginine, Lysine, Glycine, Alanine, Tyrosine, Cysteine, Phenylalanine and Tryptophan testing per the EP monographs, adding an important GMP-compliant service to RSSL's test offerings.

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About Katie Reid and Dr. Catherine Frankis

Katie Reid is a Scientist II in the Functional Ingredients Laboratory of RSSL, managing amino acid analysis. Reid joined RSSL in 2016 after completing her degree in chemistry (MSci) from the University of Glasgow. Reid began with RSSL as an analyst in the Pharmaceutical Development Laboratory doing analytical testing of pharmaceutical finished products using HPLC. In 2019, Reid moved to the Functional Ingredients Laboratory, where the analysis of food and pharmaceutical products, and method development and validation for a variety of different sample matrices is performed.

Dr. Catherine Frankis is a Senior Scientist at RSSL who works across the Functional Ingredients and Investigative Analysis teams, specializing in ion exchange chromatography and nuclear magnetic resonance spectroscopy. Frankis works on projects covering a wide variety of issues, including contamination, adulteration, and the development and validation of new analytical methods within the food, pharmaceutical, and cosmetic industries. In 2010, Frankis joined RSSL after completing her Ph. D. in the synthesis and characterization of stereoselective biopolymer initiators at the University of Bath, where she used a variety of analytical characterization and thermal property techniques.

About Reading Scientific Services Ltd (RSSL)

Based at Reading, United Kingdom, Reading Scientific Services Ltd is a global leader at the forefront of scientific analysis, consultancy, product development and training, serving the pharmaceutical, healthcare, biopharmaceutical, food, drink, and consumer goods industries. Whether for issues of quality, stability, safety, legality, or performance, RSSL can be relied on to deliver analytical services that provide clear, accurate, and actionable results. The company is highly experienced in developing, validating, and transferring new methods, and applying European Pharmacopoeia methods. Its scientists are practiced in working with APIs, excipients, and finished products, as well as with biomolecules, medical devices, herbal and natural products.

Reading Scientific Services Ltd in Berkshire, United Kingdom. Image courtesy of Reading Scientific Services Ltd (RSSL).

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