

## **A CASE STUDY OF THE TYPICAL ITSP SOLUTIONS, INC. DEMONSTRATION AND INTEGRATION OF AN ITSP SOLUTION FOR ONLINE SOLID PHASE EXTRACTION IN A PAIN MANAGEMENT/DRUGS OF ABUSE LAB.**

This case study is written based on an actual customer demonstration and consequent sale of two ITSP solutions for Pain Management and Drugs of Abuse for Quantum Analytics the financier of complete Agilent Mass Spectrometer Systems. Quantum is a valuable source for financing customers entering this fast-growth sector of LC/MS/MS instrumentation.

This event is typical when the customer is serviced properly and has hired competent staff members. The Lab Director was from a State Forensic Toxicology Lab and while he was familiar with GC/MS instruments as are typical in those labs he was less so with LC/MS/MS in Pain Management/Drugs of Abuse monitoring. Even so, he had three Agilent 6410 LC/MS/MS systems and one 6430 LC/MS/MS system. He had on order another 6430 and a 6460 Systems, both outfitted with the equivalent of the Agilent Infinity 1290 HTS but purchased from a third party reseller of PAL Systems.

The customer was competent in his current method using Dilute-And-Shoot, the typical approach that keeps labor for sample preparation to a minimum. He had also hired and trained two lab technicians who were also well trained to pipette and label samples ready for analysis. Beta-glucuronidase hydrolysis of urine was performed, samples were manually diluted and transferred to 2mL autosampler vials.

The customer was concerned about the operational costs that were confounding his daily work flow from fouled columns creating an increased number of repeats and maintenance that is a result of dirty samples in his flow path. Quantum and a third party PAL System provider had been in discussion with them regarding ITSP's capability to address these issues. As a result we were granted the opportunity to demonstrate ITSP and we focused on using the application note provided along with this document. The customer had purchased two Agilent 1200 HPLCs coupled with a 6430 and a 6460 QQQ. To keep costs down the third party PAL provider sold them legacy PAL-xt Sample Handlers. Our demo ran off-line on one of his 6410s.

Rick Youngblood, Technical Director for ITSP, Solutions, Inc., arrived at the customer site on Wednesday afternoon and set up the PAL to prep samples off-line. This was done so that the customer could run samples on his existing instruments to compare data against data he currently was generating. Included in the kit is everything needed to modify a PAL System including tray holders, solvent reservoirs, ITSP specific hardware and PAL System Software to develop a method and then convert files to the operating system (OS) of the analytical instrument. This allows the OS of the instrument to take full control of the ITSP extraction and sample injection. Rick Youngblood, in on-line installations, will install the method and test integration in the OS before the staff chemist arrives. In this demo, the PAL System was controlled using Cycle Composer with Macro Editor Software loaded using a demo license onto the computer controlling the PAL System.

Mark Hayward, Chief Science Officer of ITSP Solutions, Inc., came in on Thursday. Solvents were prepared in advance as was reasonable for the ITSP extraction from the details contained in the application note and placed on the deck in 100mL solvent reservoir vials provided in our hardware kit. Mark started with spiked urine to define the linearity. Then, using patient samples, the customer saw the advantages of ITSP after only four ITSP cartridge extractions through a 20-fold increase in recoveries. We ran samples overnight against a work list he had received that day and again we measured the

samples and created data beyond his expectations. There is some optimization still required but the advantages were obvious. This completed the chemistry / measurement phase of the demo.

To illustrate ITSP integration into MassHunter, we installed PAL System ICC-CE software (purchased with each PAL System). This illustrates how the PAL System allows MassHunter to control ITSP methods by taking advantage of the look-ahead feature to prep samples using an ITSP solution while the instrument analyzes the prior sample. We powered down the 1200 AutoInjector and MassHunter, then hard wired the PAL to the HPLC. Once MassHunter restarted the PAL System was recognized immediately. The customer expressed a satisfaction that the MassHunter screen on the computer only replaced the 1200 AutoInjector with a drawing in the same window with the PAL. He also expressed that he liked having access to the ITSP method in MassHunter and the ability to see everything as usual. Rick embedded the open ITSP method used in development but explained how he could lock down the ITSP method in MassHunter by making some of the variables invisible to the techs once validated. This is important because this also allows us to optimize the drug concentrations delivered to that particular LC/MS/MS to match its particular sensitivity capabilities (an ITSP solution is scalable to match all QQQs, [done empirically, it takes 2 injections]). This leads to higher success rates downstream in automatically processing results (big peaks = easy automatic peak integration and high precision too). Once done, the techs see absolutely no change in operations of the instrument other than those allowed by their superiors. We make the work as easy to perform as is currently possible.

**What was obvious to the customer was that ITSP added no additional labor, lab space or significant solvent/cartridge costs to add an ITSP solution to his work flow. With bulk preparation of solvents, work flow could possibly be reduced by eliminating the dilution required in dilute and shoot methodology of each patient sample.**

When the demo was complete, he asked that we leave the system set-up and on Monday he added two ITSP hardware kits to his lease with Quantum. All that is left for the system we set up is to mount and train the PAL System to recognize the valve, plumb it to the HPLC then train the technicians using ITSP Solutions, Inc. Certified Training Classes. His validation will be simplified as well because we were running known patient samples against his dilute and shoot data and he can use these comparisons to expedite the validation process.

It is important to understand that ITSP Solutions installation process does not include the analytical method development. Our warranty asks that the PAL System be running the customer's method using the LC-Inject cycle. This requirements separates ITSP related tasks from those required to operate the analytical instrument. ITSP when installed or demonstrated can then be more readily understood in both advanced chemistry, advanced lab productivity and ease of integration.

We have been asked and will modify the method to measure Oral Fluid (upfront dilution before the ITSP extraction using the PAL System) and add another method for Vitamin D. We will then return and add additional ITSP solutions for the Urine PM/DoA samples as these first two assays are his most labor intensive. The customer likes our short analytical column method and will validate it at the same time he validates ITSP. We have added a few days to the installation timeline to assist in this process. Depending on the instrumentation, this method for PM/DoA in urine could cut his run time as much as 33%. For PM/DoA in urine, we added nothing to his workflow except mixing of ITSP related solvents and placing of cartridges in a preassembled tray onto the deck of the PAL. As stated above no additional staff or lab space is required. It is presumed we will eliminate the upfront labor except for hydrolysis and centrifugation when required by the Urine, OF and Vit D methods.

The customer has plans for four additional LC/MS/MS systems that he wants to add as soon as a build-out is complete on new lab space and the sample quantities increase. We anticipate growing with him as he does. He is also Lab Director for two other labs and we see potential there as well.

We are excellent in this market (as well as others) and we hope that you will distribute the application note and this Case Study to your customers and/or field representatives and include us whenever possible. Thanks again for your efforts on our behalf.