



In-Line Sample Preparation Method Development

In-line sample preparation (ILSP) provides automated sample cleanup capabilities that are intended to replace time-consuming, manual processes, such as QuEChERS or SPE. When installed in an instrument configured for two-dimensional LC applications, a Restek Revive ILSP cartridge retains coextracted matrix components, which are subsequently removed by backflushing the cartridge with a wash solvent. Backflushing occurs during analysis, so the ILSP cartridge is ready to clean up a new sample before it is time for the next injection, creating a highly efficient workflow that can dramatically increase sample throughput. Revive ILSP cleanup is very effective and can be paired with a simple solid-liquid extraction to save time and money. This article details a simple procedure for in-line sample preparation method development so labs can realize the benefits of this powerful technique.

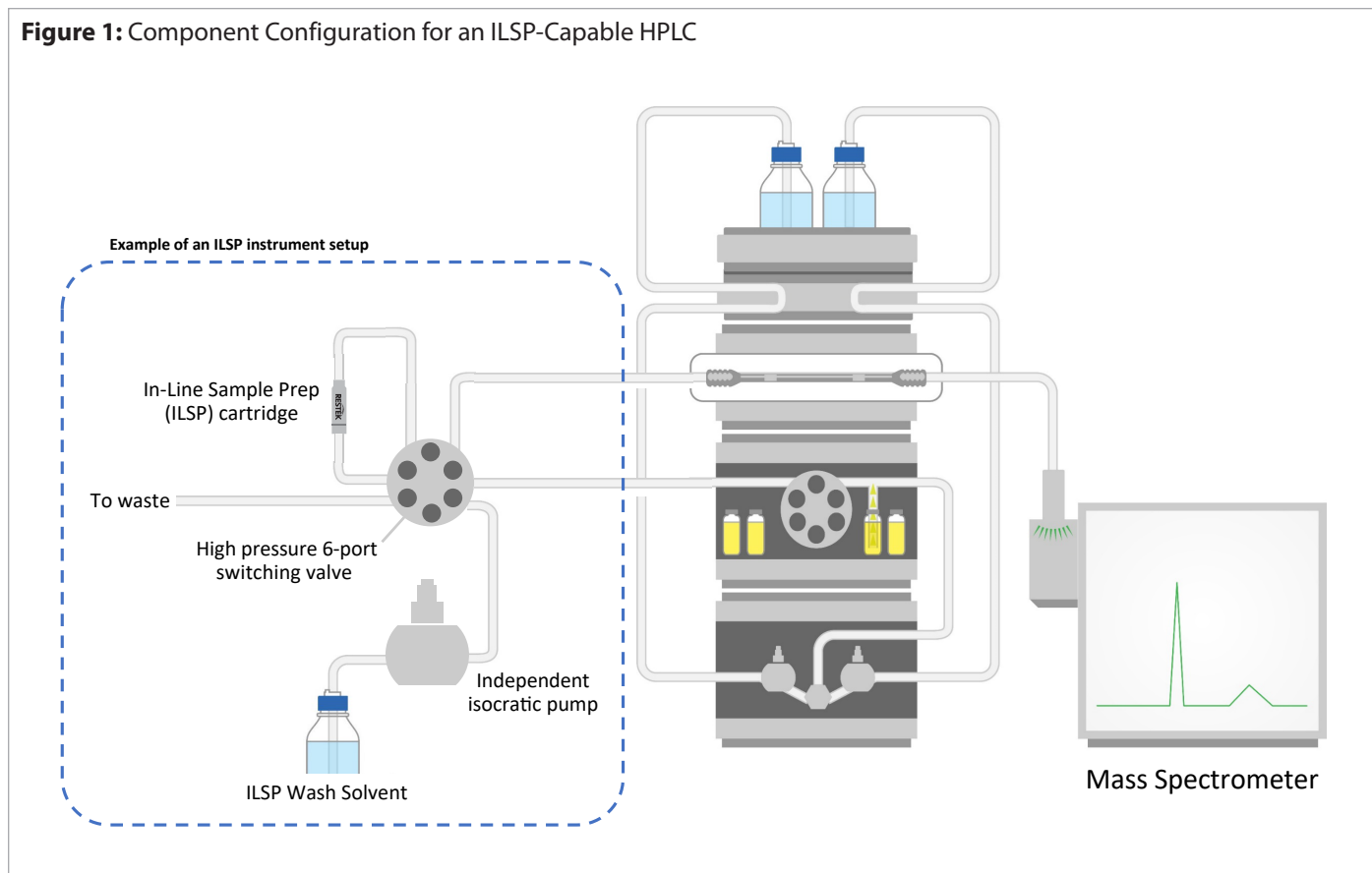
Instrument Configuration Requirements

To use the ILSP approach, you will need an HPLC equipped with a binary or quaternary pump, an autosampler capable of internal needle washing, a column compartment, and a detector. As shown in Figure 1, a compatible instrument must also include:

- An independent, isocratic pump that is capable of delivering wash solvent to the ILSP cartridge.
- A 6-port, high-pressure switching valve to control the flow to the ILSP cartridge during the wash step.

If you need assistance setting up your instrument, please consult your instrument manufacturer.

Figure 1: Component Configuration for an ILSP-Capable HPLC



Method Development

Just like any other sample preparation technique, it is necessary to evaluate ILSP conditions for each new list of target analytes and/or new sample matrix. Use the following “analyte pass-through” approach to in-line sample preparation method development to determine the optimal ILSP method conditions for your specific assay.

In the analyte pass-through approach, a Revive ILSP cartridge is used to elute target analytes while retaining interfering matrix components. Once all analytes have passed through the ILSP cartridge for separation on the analytical column, the 6-port, high-pressure valve switches position, and the matrix components that were retained on the ILSP cartridge are flushed to waste using the wash solvent on the independent pump. The following steps establish the appropriate valve timing and wash solvent conditions for a given method.

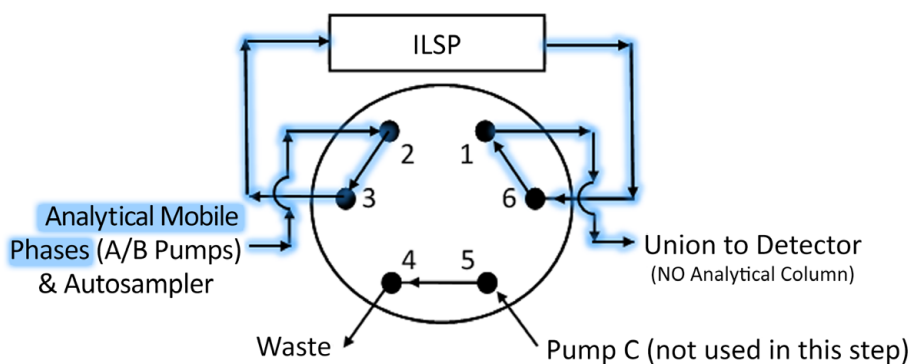
NOTE: The following assumes an appropriate chromatographic method for the matrix and target analytes has already been developed. If no analytical method exists, the analytical separation should be established before proceeding.

Step 1: Column Load - Establishing Analyte Retention on the ILSP Cartridge

This step is intended to monitor how long it takes for all of the target analytes to elute from the Revive ILSP cartridge, which will determine how long a sample is allowed to flow through the ILSP cartridge onto the analytical column before the first valve switching event.

- Configure the 6-port switching valve as shown in Figure 2 with a union in place of the analytical column.
- Inject a solvent standard (no matrix) of target analytes onto the ILSP cartridge using the preexisting mobile phase and gradient conditions for the analytical chromatographic method. Remember to adjust any MRM windows as needed.
- Locate the last eluting target analyte in the resulting chromatogram. The time at which this analyte completely elutes marks the earliest point when the valve can be programmed to switch from the analytical column load position to the ILSP wash position (which is described in Step 2 and Figure 3). To account for slight lot-to-lot variations in column retention, add 10-20 seconds to the time determined to completely elute the last target analyte.

Figure 2: Valve Position and Instrument Configuration for Step 1



Step 2: ILSP Wash – Establishing Wash Solvent Composition and Conditions

The second step of in-line sample preparation method development is to determine the appropriate rinse solvent composition and the best flow conditions and valve timing. This is to ensure that the retained matrix components from one injection are washed off the Revive ILSP cartridge prior to the next injection.

- Make an initial wash solvent comprised of 2 mM ammonium formate and 0.1% formic acid in methanol. This will be suitable for many applications, but as discussed below, other washes may need to be evaluated based on initial results.
- For ease, replace mobile phase B with the prepared wash solvent (Figure 3).
- Using a flow rate of 1 mL/min, inject a matrix blank onto the ILSP cartridge.
- Monitor for the presence of the matrix in full scan mode or, if specific matrix ions are known, in selected ion-monitoring (SIM) mode.
- Note the time required for a total ion chromatogram (TIC) to return to baseline, indicating that the observed matrix has been completely flushed from the ILSP cartridge.
- This is the time required to fully flush the ILSP cartridge of trapped matrix components, and it should be compared to the total analytical cycle time.
 - If the matrix is completely flushed from the ILSP cartridge before the end of the analytical run (Figure 4), the wash method likely does not need further optimization and can be programmed into the overall method, using a second independent pump to control the flow of the wash solvent (Pump C) through the ILSP cartridge during an actual analysis.
 - If the amount of time needed to fully flush the matrix from the ILSP cartridge is longer than desired, the wash solvent composition and/or the flow rate should be optimized to improve efficiency.
 - It is recommended to keep buffer concentrations between 2-10 mM and acid concentrations between 0.1-0.5% for best performance. Care should be taken to prevent buffer precipitation when equilibrating the ILSP cartridge back to initial conditions with mobile phase.
- *NOTE: Do not buffer the wash solvent outside of the pH 2-8 range.*

Figure 3: Valve Position and Instrument Configuration for Step 2

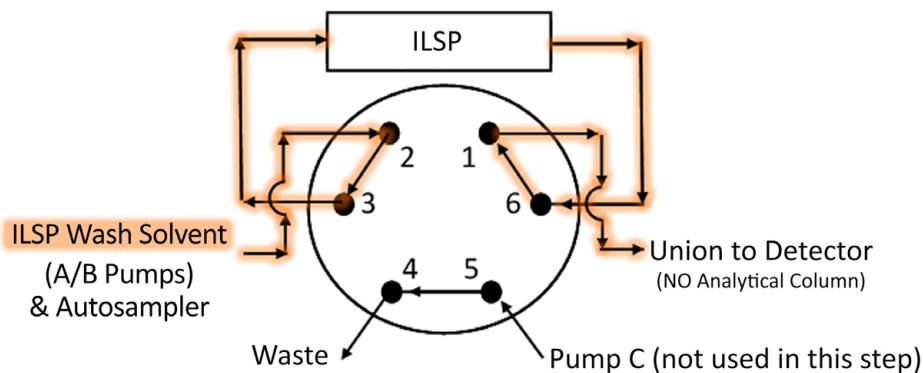
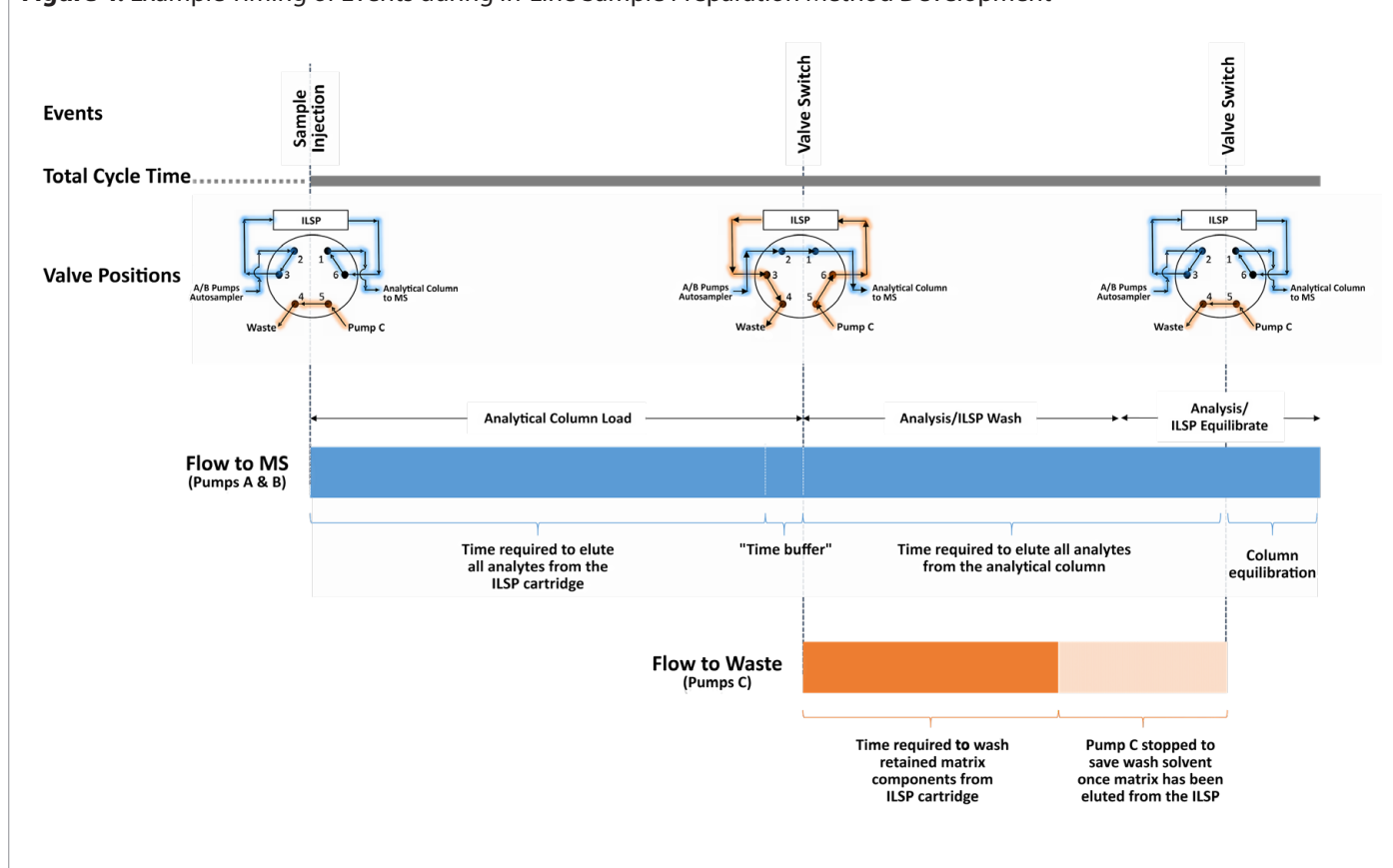


Figure 4: Example Timing of Events during In-Line Sample Preparation Method Development



Step 3: Method Updates – Adding ILSP Method Events to an Existing Analytical Method

This final step is intended to take the information gathered in steps 1 and 2 and use that to introduce the appropriate timed events into an existing analytical method. Figure 4 shows an example of where new events would be inserted into a typical HPLC gradient method. An example in-line sample preparation method development process for an existing assay of pesticides in food is shown in Table I.

Table I: Example Revive In-Line Sample Preparation Method Development Process for a Pesticides in Food Method

Original Chromatographic Events Table:		
Time (min)	%B Mobile Phase Composition	
0	5	
2	60	
4	75	
6	100	
7	100	
7.01	5	
9	Stop	
Values Determined During ILSP Method Development:		
Step 1:		
Time to elute the final analyte from ILSP = 4.6 min		
Time buffer added to ensure complete transfer of last eluting compound = 0.3 min		
Time to switch valve to ILSP rinse position (sum of the above) = 4.9 min		
Step 2:		
Rinse solvent composition = 2 mM ammonium formate & 0.1% formic acid in methanol		
Rinse solvent flow rate = 1 mL/min		
Time to elute last observable matrix components from the ILSP = 0.6 min		
Time buffer added to account for variations in severity of trapped matrix components = 0.4 min		
Total time to rinse ILSP cartridge = 1.0 min		
Note: No change to rinse solvent composition or flow rate necessary.		
Updated Chromatographic Events Table:		
Time (min)	%B Mobile Phase Composition	Event
0	5	
2	60	
4	75	
4.9	-	Valve switch to ILSP rinse position (see Figure 4).
4.92	-	Establish ILSP rinse solvent flow @ 1 mL/min (pump C).
5.92	-	Turn off ILSP rinse solvent flow to conserve solvent (pump C).
6	100	
7	100	All compounds have eluted from the analytical column at this point.
7.01	5	Return to initial mobile phase conditions to equilibrate column.
7.05	-	Valve switch to the "analytical column load" position to allow the ILSP cartridge to equilibrate.*
9	Stop	
*Waiting until this point for valve switching is recommended to avoid any possible baseline disturbances during the analytical separation as a result of valve switching.		

Maintenance and Troubleshooting

Most issues that arise with in-line sample preparation can be avoided or rectified with basic knowledge about the matrix and method. As a first step, matrix-specific ions should be identified by comparing precursor scan data of an extracted matrix blank and an extraction solvent blank. Once matrix-specific ions have been determined, they can be entered into the analytical method and monitored in SIM mode. Doing this provides a valuable diagnostic tool that can be used when troubleshooting performance issues to determine if there is a problem with persistent matrix contaminants being present. Solutions to common troubleshooting scenarios are presented in Table II.

By employing this approach to in-line sample preparation method development and being aware of how to address any issues that may arise, labs can add ILSP to existing methods with confidence and significantly increase sample throughput.

Table II: ILSP Troubleshooting Solutions

Symptom	Potential Cause	Remedy
Missing Late Eluting Peaks	Incorrect event time set for column loading	Lengthen the time required for column loading.
Missing Peaks	MRM windows incorrectly set	Addition of the ILSP cartridge may cause a slight change in retention time requiring MRM windows to be updated.
Broad/Distorted Peak Shapes for Early-Eluting Analytes	Needle rinse solvent mismatch	If using a strong solvent for internal needle rinsing, be sure to follow by rinsing with a weak solvent (initial mobile phase composition) to prevent band broadening.
	Sample diluent mismatch	Reduce the injection volume for samples dissolved primarily in acetonitrile or methanol solvents to prevent band broadening.
Peak Distortion/Baseline Disturbance	Internal needle rinsing can cause baseline disturbances which can affect peak shape	Initiate internal needle rinse once all analytes have eluted from the analytical column.
Poor Analyte Recovery	Matrix carryover from autosampler	Optimize internal needle rinsing with a solvent that will solubilize the matrix.
	Insufficient washing of the ILSP cartridge	Optimize the wash solvent to completely remove major matrix components.
Matrix Peaks in Solvent Blank Injections (when monitored)	Matrix carryover from autosampler	Optimize internal needle rinsing with a solvent that will solubilize the matrix.
	Insufficient washing of the ILSP cartridge	Optimize the wash solvent to completely remove major matrix components.

Revive In-Line Sample Preparation (ILSP)

- Automated, in-line sample extract cleanup dramatically reduces sample preparation time.
- Simultaneous analysis and ILSP cartridge wash eliminate downtime between samples.
- Fast, simple alternative to QuEChERS or SPE for multiresidue pesticides analysis in foods.
- Minimizes money spent on disposable sample preparation products and associated waste.
- Reduces sources of error and variability related to manual cleanup.

In-line sample preparation (ILSP) uses the power of an LC-MS/MS to streamline and automate sample extract cleanup. Restek's Revive ILSP Pesticides cartridges are an ideal sample preparation alternative for food safety labs seeking to spend less time and money on multiresidue pesticides samples without sacrificing performance. Revive ILSP Pesticides cartridges separate analytes from potentially interfering matrix components just like conventional QuEChERS and SPE methods do, but they replace time-consuming manual procedures with a faster automated cleanup process that occurs on the instrument concurrently with sample analysis.

With a Revive ILSP Pesticides cartridge, six-port valve, and an independent isocratic pump, your instrument can be transformed into an analytical workhorse, combining sample cleanup and analysis in a single, efficient method. Significantly decrease sample preparation time, cut the costs associated with disposable sample prep products, and reduce errors related to manual procedures by integrating Revive ILSP into your current methods for multiresidue pesticides analysis in foods.



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Description	qty.	cat.#
Revive ILSP holder for 5 mm cartridge	ea.	27880
Revive ILSP Pesticides cartridge (5 x 2.1 mm)	ea.	27881
Revive ILSP holder and Revive ILSP Pesticides cartridge (5 x 2.1 mm)	kit	27882
Revive ILSP Pesticides cartridges (5 x 2.1 mm)	3-pk.	27883