LEAP Technologies
Post Office Box 969, Carrboro, NC 27510
919 929-8814 or 800 229-8814 FAX 919 929-8956

LEAP Application Note

Title: Using the PAL Autosampler as a Syringe Pump for MS Tuning
Revision: 1.0
Date: 06-21-2004
Author: Thomas Tobien

Objective/Abstract
The PAL autosampler injection syringe is utilized for analyte infusion during mass spectrometer tuning. Infusion occurs either into an LC mobile phase stream (Option A) or directly into the LC interface of a mass spectrometer (Option B). After installation of a large volume syringe, a local firmware method and job is created that controls analyte infusion.

Option A - Infusion into an LC mobile phase stream

Setup
Any existing PAL autosampler can be configured to function as a syringe pump. Minor additional hardware is needed, e.g., large volume syringes and respective syringe holders. A list of part numbers is provided in the appendix. Below, a list of steps is given that describe how a regular PAL autosampler can be converted into a syringe pump.

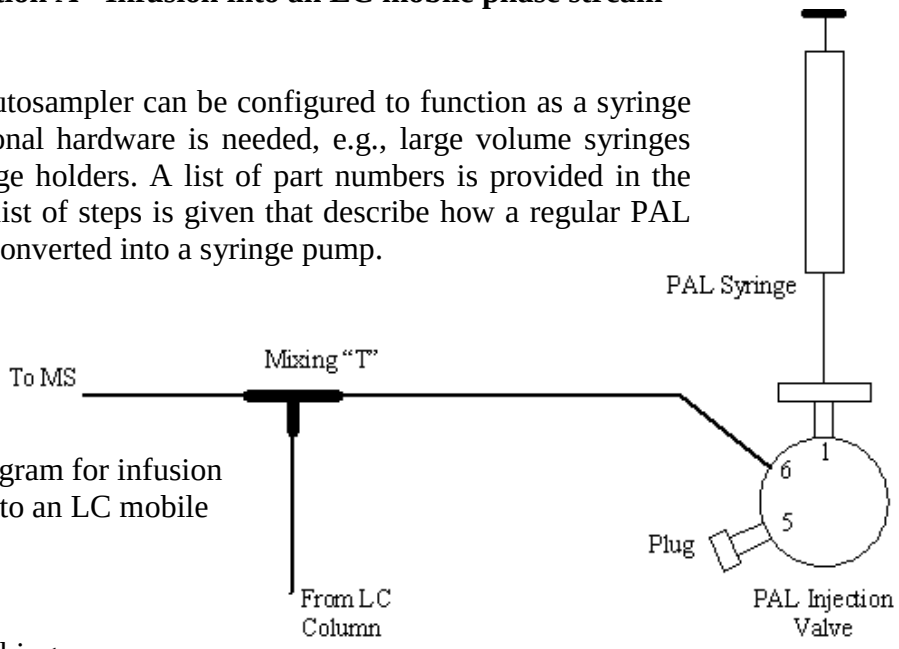

Figure 1:
Plumbing diagram for infusion of analytes into an LC mobile phase stream

1. Change Plumbing
   Place a mixing "T" between the LC column outlet and LC interface of the mass spectrometer. Connect the free mixing "T" inlet to either port #6 of a 6-port 2-position injection valve or port #10 of a 10-port 2-position injection valve. See figure 1.
2. Install Large Volume Syringe
   In order to use a large volume syringe, e.g., 1mL, 2.5mL or 5mL, the respective

object must be present in the PAL firmware. Using the PAL control terminal, check which syringes are available. The syringe sub-menu can be found at:
Menu->F3/Setup->Objects->Syringes
Missing syringe objects can be uploaded into the PAL firmware by utilizing the Object Manager software. If not already installed on the PC that controls the PAL, the Object Manager software can be found on a CD that was included with the original PAL manual. If missing, the software can also be downloaded from the support section of LEAP's webpage. (Note: The webpage address is www.leaptec.com. Registration to the website is required. Only LEAP customers will be granted access.) Once installed, the syringe objects "PAL SyrLC1000ul" (1mL syringe), "PAL SyrLC2500ul" (2.5mL syringe), or "PAL SyrLC5000ul" (5mL syringe) can be uploaded. After uploading, the respective syringe object needs to be configured via the PAL control terminal by lowering the parameter Min Speed to 10nL/sec. Example: The parameter Min Speed for the 1mL can be found syringe in the sub-menu:
Menu->F3/Setup->Objects->Syringes->1000uL
Finally, the syringe itself needs to be installed (path: Menu->F1/Chang Syr). Remove the existing blue syringe adapter and insert the corresponding large volume syringe adapter. Attach the syringe to the adapter and enter the syringe selection via the PAL control terminal.

3. Create a Local PAL Method
Via the PAL control terminal in the sub-menu Methods (path: Menu->Methods), create a new method (function key F2/Insrt Meth). Select "LC-Inj" as the local cycle and the newly installed syringe during method configuration. Once created, select the desired method parameters:

Method Parameter	Example	Explanation/Remarks
Sample Volume	500uL	volume of analyte solution to be infused
Fill Speed	100uL/sec	speed slow enough to fill a 1mL syringe without cavitation bubbles
Inject to	LC Vlv1	Choose your current injection valve.
Inject Speed	170nL/sec	Corresponds to 10uL/min
Vlv Cln Slv1	0	No valve cleaning should be selected since such cleaning would be performed using the slow Inject Speed. (Note: Valve cleaning can be performed after the infusion is done from within the Utilities menu.)
Vlv Cln Slv2	0	See above.

4. Create a Local PAL Job
Via the PAL control terminal in the topmost menu Job Queue, create a new job (function key F2/Add Job). During job configuration, select the tray that has the standard vial, its position, and the newly created method from step 3 above.

Example: In order to perform one infusion from a sample vial in position 46, the following parameters must be set in the job:

Job Parameter	Value
Tray	[Your tray name goes here.]
First	46
Last	46
Increment	1
Count	1
METHOD	[The name of your newly created infusion method goes here.]

5. Start flow of LC pump.
6. Select instrument tuning in MS operating software.
7. Execute local PAL job from PAL control terminal.
8. Clean valve after infusion job by
   A) re-connect normal injection valve plumbing
   B) start flow of LC pump through injection loop to clean port #5 (#9 for a 10-port injection valve)
   C) activate/deactivate valve with flow going through valve to clean valve; access path: Menu->Utilities->Injector->[injector name, e.g., LC Vlv1] ->F2/Act Valve (Deact Valve)
   D) wash the injection valve from the Utilities menu of the PAL control terminal
9. Exchange the infusion syringe with your regular injection syringe (including syringe adapter) and proceed with sample injections.

Helpful Hints/Remarks

The syringe plunger motion is controlled by a stepper motor like in the case of a syringe pump. The stepper motor "step height" of a regular PAL injection head is larger than that of a syringe pump. The result is pulsation which can be overcome by incorporation of sufficient mixing of infused analyte solution and mobile phase after the mixing "T" and before the mix enters the MS interface. Such sufficient mixing can be accomplished using larger ID tubing. An example is given in figure 2 below.

Figure 2: Influence of tubing ID on pulsation during analyte infusion; The tubing connects the mixing "T" and LC interface of MS

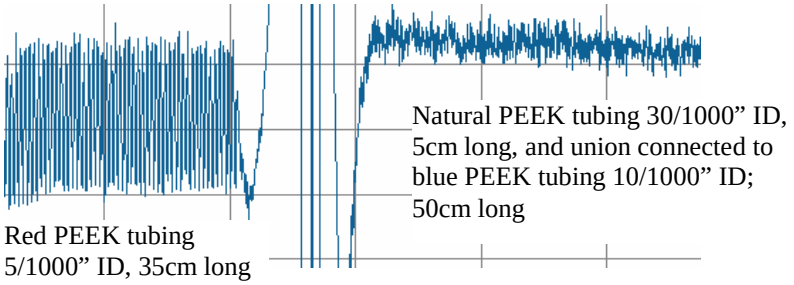

Figure 3 below shows flow rates and pressures obtained from a PAL that was setup to run analyte infusion into a mobile phase stream.

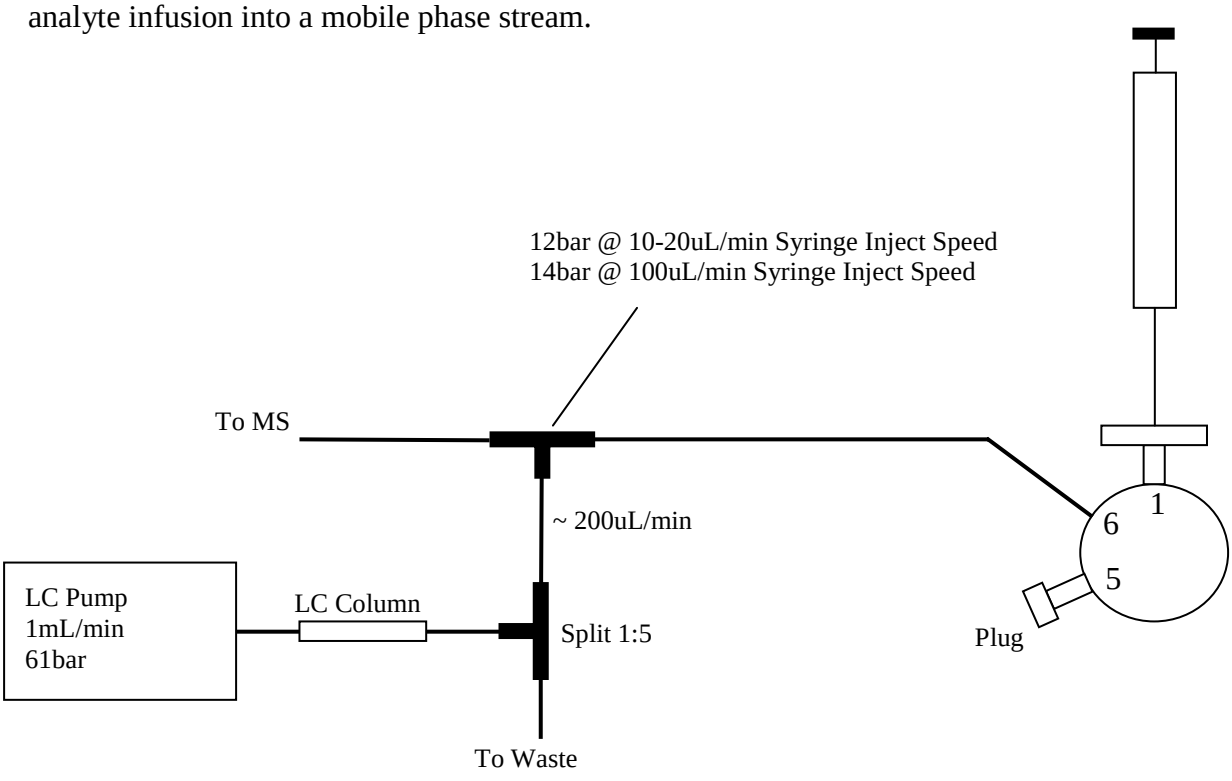

Figure 3: Flow rates and pressures for analyte infusion into a stream of mobile phase

Option B – Direct Analyte Infusion into an LC-MS Interface

Setup

The setup is identical to Option A except for step #5. See figure 4 for a plumbing diagram. Pulsation due to inhomogeneous mixing is not a problem anymore since analyte is directly infused into the LC interface of a mass spectrometer. Less severe pulsation might still be observed due to differences in flow rate. This residual pulsation does not interfere with instrument tuning in a significant way. See figure 5.

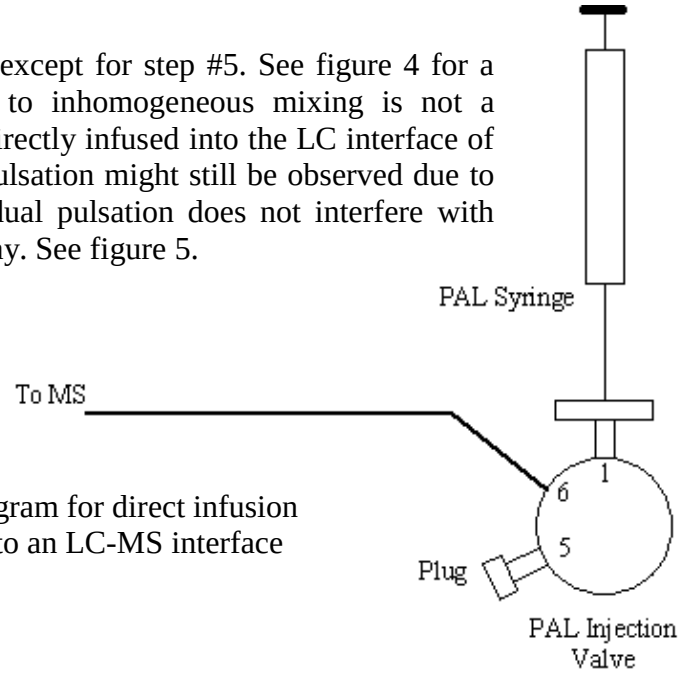

Figure 4:
Plumbing diagram for direct infusion of analytes into an LC-MS interface

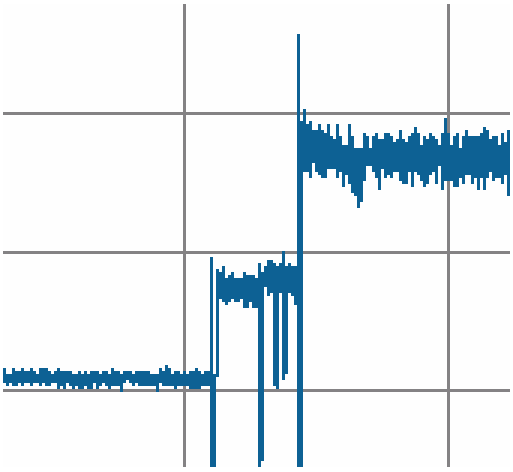

Figure 5:
Example of MS instrument tuning utilizing direct infusion @ 20uL/min syringe injection speed.

Appendix

A list of associated part numbers is included below. All parts are available from MicroLiter, Inc., Suwanee, GA. (Phone 888-232-7840 / Fax 888-232-7845 / www.microliter.com / info@microliter.com)

Item	Part Number	Comments
1mL syringe	L100.SYR1.0	
1mL syringe adapter	LPAL.SA1ML	
2.5mL syringe	L100.SYR2.5-3	3-pack
2.5mL syringe adapter	LPAL.SA2.5	
5mL syringe	L100.SYR5.0-3	3-pack
5mL syringe adapter	LPAL.SA5000	
Needle sleeve seal	L100.LCTEF	Use of a tight fitting needle sleeve seal is imperative. Otherwise, leakage around the inserted needle can occur. Replace needle sleeve seals on a regular basis and when worn.