Steps to determine sources of carry over and potential remedial actions

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August 30th, 2007

Assessing carry-over is an issue that can cause frustration to both the user and the engineer.
When faced with this issue it is important to first identify the source/ sources of the carry over. This is most easily done by carrying out a systematic examination of the problem. (A flow chart for this process is shown below.) It is important to understand there can be multiple sources of carry-over within a system, and the carry-over characteristics of the system may well vary from compound to compound and matrix.
On many occasions the structure of compound that one is dealing with will not be available to you. To tackle the problem of carry-over any information about the chemical and physical properties of the molecule are useful in the designing a suitable washing protocol.

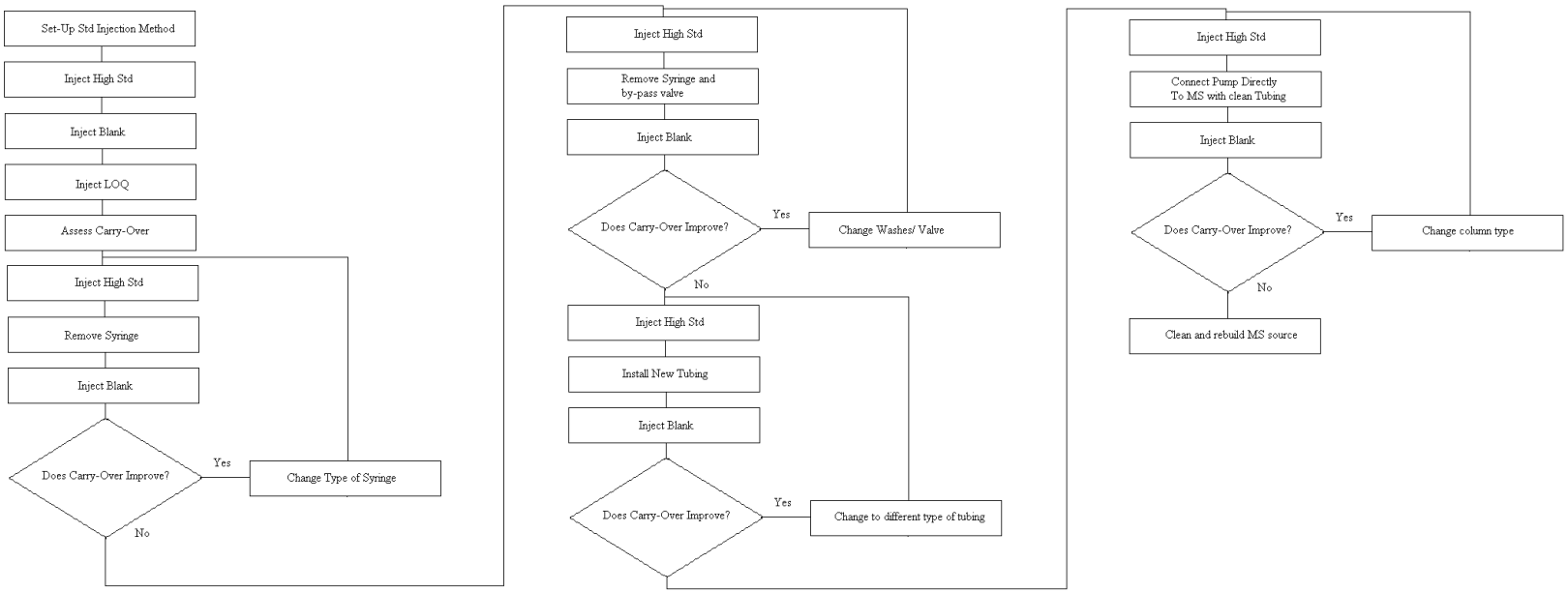
The questions that can be asked are:-

1 Is the molecule hydrophilic (water soluble) or hydrophobic (organic soluble)?

2 Does the molecule contain halide atoms (especially fluorine)?

3 Does the molecules contain acid or basic functional groups?

4 What solvents is the compound soluble in?


Set-Up Std Injection Method
Inject High Std
Inject Blank
Inject LOQ
Assess Carry-Over
Inject High Std
Remove Syringe
Inject Blank
Does Carry-Over Improve?
Yes
Change Type of Syringe
No
Inject High Std
Remove Syringe and by-pass valve
Inject Blank
Does Carry-Over Improve?
Yes
Change Washes/ Valve
No
Inject High Std
Install New Tubing
Inject Blank
Does Carry-Over Improve?
Yes
Change to different type of tubing
Inject High Std
Connect Pump Directly To MS with clean Tubing
Inject Blank
Does Carry-Over Improve?
Yes
Change column type
No
Clean and rebuild MS source

By getting answers to these questions and the principle that like attracts like, it is possible to understand some of the possible causes and solutions to the problem of carry-over.

Hydrophilic molecules will dissolve in water (aqueous solutions) (it is unlikely under normal circumstances that these molecules will give problems with carry-over in a standard reverse phase assay

Hydrophobic molecules will dissolve in organics (such as Methanol, Acetonitrile, Isopropyl Alcohol)

If the molecules contain halide atoms it is import to try to avoid materials in the system which contains fluorine atoms themselves. One example of such a material is PTFE (Teflon™) which can often be found in the syringes. The fluorine on the surface of these materials will act to trap fluorine containing molecules and cause carry over. The rotor materials within many of the valves contain fluoropolymers, therefore the correct choice of valve/ rotor is an important factor.

If the molecule has acid or basic functionality the use of acidic wash solvents for basic molecules or visa versa can improve the solubilities of these molecules by converting them into their ionic form.

Using a solvent that the molecule is known to be soluble in, as a wash solvent, is the easiest way of minimizing carry-over.

If using the VSW II along with choosing the correct valve, the following protocol is a good starting point for the development of a cleaning process

1ml @ 15ml/min of Solvent A (the initial starting HPLC solvent)
1ml @ 15ml/min of 3% Formic Acid + 0.1TFA in water
1ml @ 15ml/min of 1% EDTA in Water
1ml @ 15ml/min of 3% NH3OH in Acetonitrile/Methanol
1ml @ 15ml/min of Solvent A (the initial starting HPLC solvent)

The proceeding protocol can be used to wash the valve immediately following the injection. After the peak had been detected, the valve was switched to the load position and the wash sequence was repeated so that the loop was cleaned