Minimizing Carry-over for High Throughput Analysis

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Introduction

Carry-over is the appearance of an analyte signal in a blank after the analysis of samples with higher analyte concentrations. It is compound and method dependent. Minimal carry-over is an important quality criterion of modern auto sampler technology and critical in HT-Analysis. In this study a strategy to minimize carry-over for HT-analysis was developed. The sources and relative contributions of carry-over were evaluated.

HT-Analysis schedule (examples)

A total cycle time of < 2 min is achieved by overlapped wash and cleaning steps. Wash efficiency/low carry-over and cycle time/ compete against each other.

<table>
<thead>
<tr>
<th>Wash-Parameter</th>
<th>Values (Default)</th>
<th>Time (per Task)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valve clean with solvent 1</td>
<td>0–200 (90) µL</td>
<td>7 s</td>
<td>Restore start conditions in the valve (use Eluent A)</td>
</tr>
<tr>
<td>Valve clean with solvent 2</td>
<td>0–200 (90) µL</td>
<td>7 s</td>
<td>Clean the valve (use strong wash solution)</td>
</tr>
<tr>
<td>Post clean with solvent 1</td>
<td>0–5 (2) strokes</td>
<td>3 s</td>
<td>Restore start conditions in the tool (use solvent 1)</td>
</tr>
<tr>
<td>Post clean with solvent 2</td>
<td>0–5 (2) strokes</td>
<td>3 s</td>
<td>Restore the starting conditions in the tool (wash solution)</td>
</tr>
<tr>
<td>Station wash</td>
<td>0–180 s</td>
<td>(depends)</td>
<td>Wash the valve with wash (switch during the run)</td>
</tr>
</tbody>
</table>

Schematic PAL LC-MS tool

Cyclosporine

in blood extract

Chlorhexidin

in solvent

Contributions of different sources of carry-over

Physical carryover:
- Dead volumes caused by bad connections between tubing and fittings
- Scratches on rotor/stator of valves
- Generally badly flushed volumes (cavities)
- ESI/APCI source components (needle, spray shield)

Sorptive carryover:
- Chemical adsorption of molecules to surfaces of tubings, loops, injection needles, or valves
- Sample adsorption to the column's stationary phase or inner surfaces
- Solvent contaminants concentrated on and released from the column during a gradient run

Distribution of carry-over

Carry-over, if no wash step is applied (injection of 220 ng chlorhexidin)

no wash:

wash test:

> The carry-over of the tool is not detectable!

References

[7] Determination of carry-over and

Tips to avoid carry-over

- Always check your carry-over with an non-selective method (TIC)
- Always check the solubility of your analyte in the wash solutions
- Reduce dwell volume and check for cavities (bad connectors)
- Reduce matrix by good sample preparation and dilution if possible