University of Applied Sciences and Arts Northwestern Switzerland

PAL SYSTEM

Minimizing Carry-over for High Throughput Analysis

Christian Berchtold[1], Reto Bolliger[2], Guenter Boehm[2], Götz Schlotterbeck[1]

[1]FHNW Fachhochschule Nordwestschweiz, Hochschule für Life Sciences Gründenstrasse 40 CH-4132 Muttenz

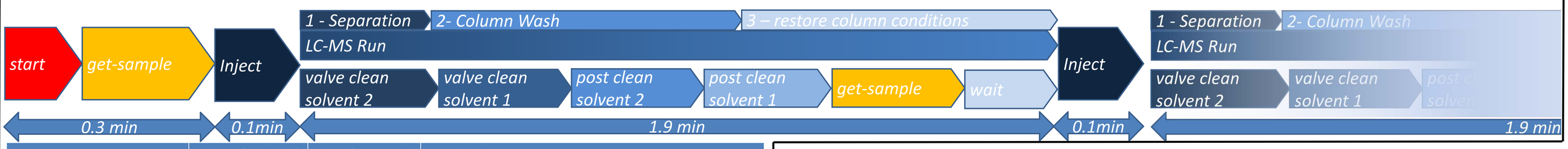
[2]CTC Analytics AG Industriestrasse 20 CH-4222 Zwingen

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.

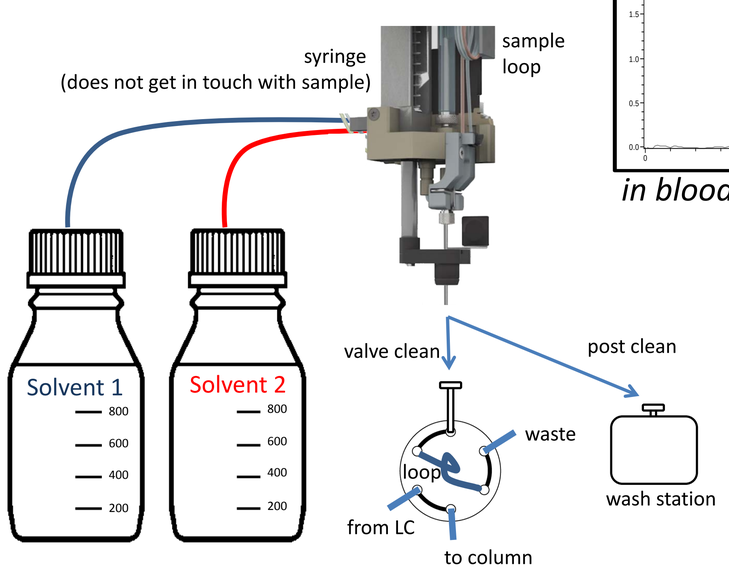
HT-Analysis schedule (examples)

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.

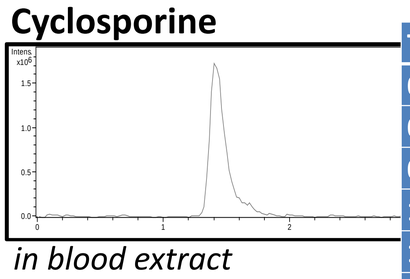


Wash-Parameter	Values (Default)	Time (per Task)	Description
Valve clean with solvent 1	0 – 200 (90) µL	~ 7 s	Restore start conditions in the valve (use Eluent A)
Valve clean with solvent 2	0 – 200 (90) µL	~ 7 s	clean the valve (use strong wash solution)
Post clean with solvent 1	0 – 5 (2) strokes	~ 3 s	Restore start conditions in the tool(use Eluent A)
Post clean with solvent 2	0 – 5 (2) strokes	~ 3 s	Restore the starting conditions in the tool (wash solution)
Stator wash	0 – 180 s	(depends)	Wash the valve with eluent (switch during the run)

Schematic PAL LC-MS tool

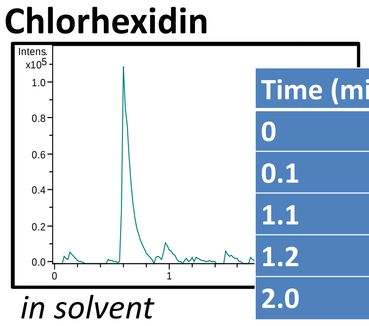


Cyclosporine



Time (min)	% B
0	0
0.1	0
0.2	100
1.3	100
1.4	0
3.0	0

Chlorhexidin



Time (min)	% B
0	0
0.1	100
1.1	100
1.2	0
2.0	0

Methods and Instrumentation

Agilent 1100 HPLC
MSD 3000 Trap;
PAL RTC equipped with LC/MS-Tool, high pressure injection valve (VICI C72VC-6676D-CTC), 2 µL loop.

MS Parameter	A	B
Capillary [kV]	3500	3500
Gas-Flow [l/min]	12	10
Gas pressure [psi]	70	50
Temperature [°C]	350	350
Accumulation [ms]	5	50

	Chlorhexidin[A]	Cyclosporin[b]
Eluent A	Water/MeCN 80:20; 0.1 % FA	Water/MeOH 20:80; 0.1 % FA
Eluent B	MeCN; 0.1% FA	MeOH; 0.1% FA
Flowrate	1.5 ml/min	0.7 ml/min
Column	Agilent Zorbax SB-C18 (3 µm, 2.1x50mm)	HALO peptide ES-C18 (1.7 µm,2.1x50mm)
Solvent 1	Eluent A	
Solvent 2	magic mix H_2O/ACN/MeOH/2-propanol (25/25/25/25) + 1% formic acid)	

Strategy of HTS – LC-MS Method development

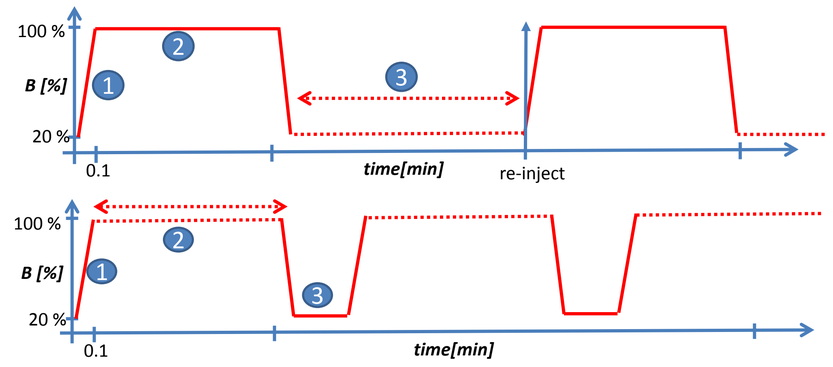
1. HPLC-Method development

Evaluate MS-Parameters (Spray, parent-mass, fragments, MS/MS), and HPLC separation method.

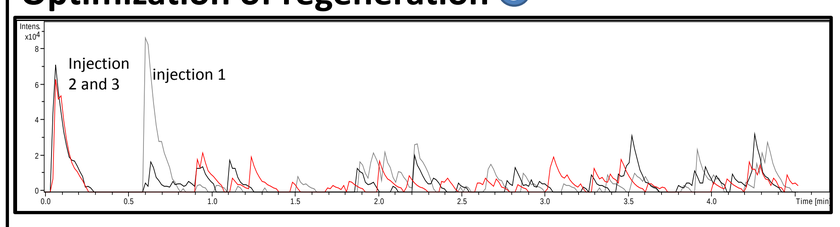
2. Optimize HTS

Minimize the time for flushing and the time to restore and maintain starting conditions

1. Separation - gradient
2. Column flush
3. Column regeneration

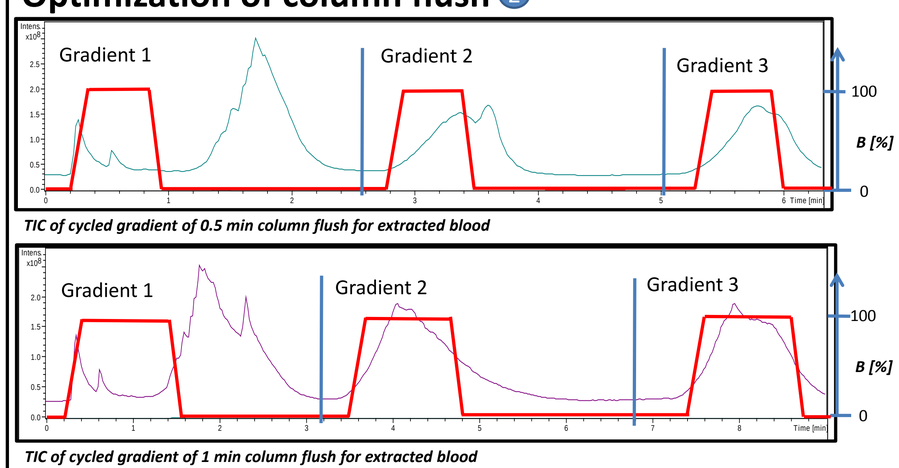


Optimization of regeneration 3



The regeneration time is evaluated by comparing 3 consecutive injections. The regeneration is too short as soon as the signals don't overlap. This is buffer, column and flow dependent.
(Example chlorhexidin)

Optimization of column flush 2



TIC of cycled gradient of 0.5 min column flush for extracted blood

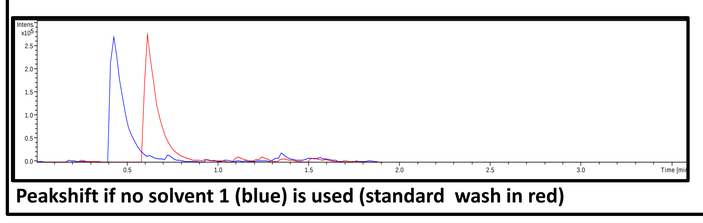
TIC of cycled gradient of 1 min column flush for extracted blood

Matrix is injected and the gradient is run in cycles. Several runs are carried out with shorter flushing times. As soon as significant effects are observed in the 2nd and 3rd gradient, the method is considered as too short.

(Example cyclosporine in MeCN crashed blood)

3. Optimize sampler wash steps

The wash steps (see schedule above) are optimized to reduce wash time, solvent consumption and to maintain reproducible retention times.



Peakshift if no solvent 1 (blue) is used (standard wash in red)

Solvent 1 equals eluent A to avoid any effect on the retention (e.g. shorter retention-time).
Solvent 2 must solve the analyte and matrix perfectly.
(Example chlorhexidin)

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)

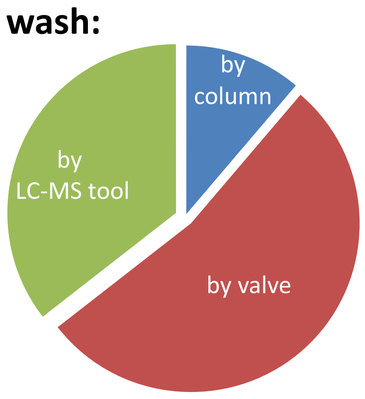
Sorptive carryover:

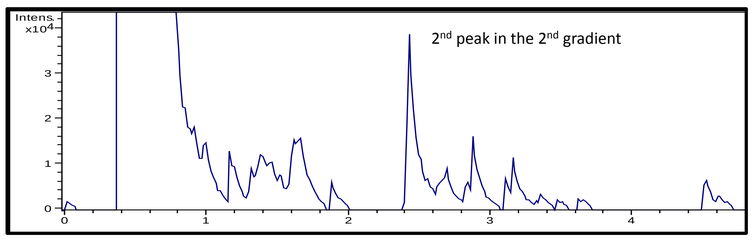
- Chemical adsorption of molecules to surfaces of tubings, loops, injection needles, or valves
- Sample adsorption to the column's stationary phase or inner surfaces
- Solvent contaminants concentrated on and released from the column during a gradient run

Distribution of carry-over

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

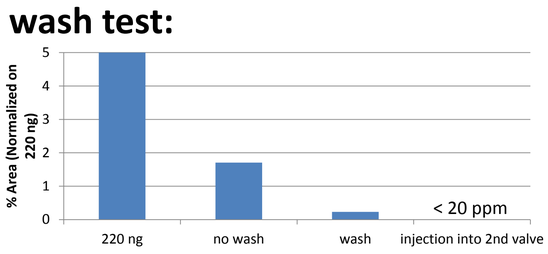
no wash:



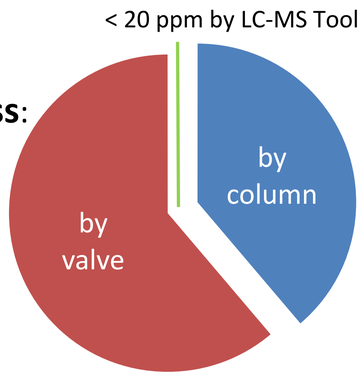


carry-over caused by the column (into the next run)

wash test:



wash success:



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

References

[1] Dolan, JW; LCGC 19, Feb 2001, 164-68
[2] Dolan, JW; LCGC 19, Oct 2001, 1050-54
[3] Determination of carry-over and contamination for MS – Based chrom sssays: Hughes, NC et al. AAPS Journal 2007; 9 (3) Article 42
[4] EMA Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009)
[5] Carryover, and how to minimize it http://www.palsystem.com/index.php?id=280

Tips to aviod 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