Combi*Flash*® EZ Prep & NextGen Normal Phase/Reverse Phase Flash Changeover

TELEDYNE ISCO
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Chromatography Application Note AN28

Abstract

This application note describes a general method for changing the flash column portion of the Teledyne ISCO Combi*Flash* EZ Prep system between normal phase (NP) and reverse phase (RP). A specialized procedure is needed because normal phase and reverse phase solvents are not miscible. These instruction will provide improved chromatography. The procedure will flush the pumps, injector valve, solid load line, and detector flow paths. The Combi*Flash* NextGen 300 and EZ Prep systems allow automatic methods to change the solvent without the need for intermediate solvents such as 2-propanol or acetone, while the NextGen 100 does require an intermediate solvent.

This application note makes the following assumptions:

- The pumps are primed.
- The reverse phase solvents do not contain mineral acid buffers such as phosphate, sulfate, salts such as sodium citrate, or other buffer that may precipitate in organic solvent.

Overview

Incomplete solvent changes cause unpredictable purification of compounds (Figure 1).

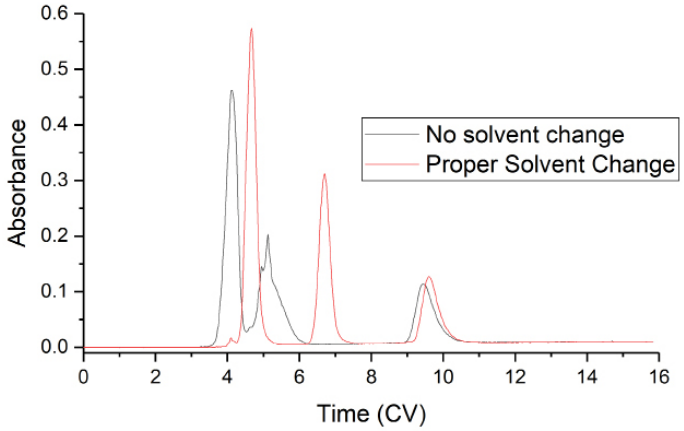

Figure 1: Normal phase purification after reverse phase purification with and without proper solvent change.

Within this document "NP" stands for "normal phase" and "RP" means "reverse phase."

NextGen 300/300+ and EZ Prep

Although the EZ Prep has an automatic phase change between normal phase flash and reverse phase preparative HPLC, there is no automatic phase change for flash chromatography. The method below can be used for both the NextGen 300 and EZ Prep systems. Instructions for the NextGen 100 are given later.

The solvents are assumed to be installed as per our recommendations, where Line 1 = weak NP (i.e. Hexane, heptane, petroleum ether, dichloromethane); Line 2 = strong NP (i.e. ethyl acetate, methanol); Line 3 = weak RP (i.e. water); and Line 4 = strong RP (i.e. acetonitrile, methanol).

Changeover to RP

General settings in method editor

- Peak Collection = NONE
- Column = Silica 4g
- FLOW RATE = 100 ml/min
- EQUILIBR. VOL. = 0
- AIR PURGE = 0.5 Min.
- TIME TO CV set for "Time"

Gradient Table:

Note: in the table below solvent line numbers are used rather than solvent names, as solvent names may vary by user preference.

SOLVENT A	SOLVENT B	LENGTH, min	%B
1	2	0.0	100.
1	2	1.0	100.
3	4	1.0	100.
3	4	0.0	0.0
3	4	1.0	0.0

Create the method listed above and save it for future use.

Changeover to NP

General settings in method editor

- Peak Collection = NONE
- Column = Silica 4g
- FLOW RATE = 100 ml/min
- EQUILIBR. VOL. = 0
- AIR PURGE = 0.5 Min.
- TIME TO CV set for time

Gradient Table:

Note: in the table below solvent line numbers are used rather than solvent names, as solvent names may vary by user preference.

SOLVENT A	SOLVENT B	LENGTH, min	%B
3	4	0.0	100.
3	4	1.0	100.
1	2	1.0	100.
1	2	0.0	0.0
1	2	1.0	0.0

Create the above methods and save them for future use.

Running the methods

1. Put the priming tube in place of a flash column.
2. If the solid load cartridge line is to be flushed, install an empty solid load cartridge which may be reused for this procedure.[1]
3. Open the desired method.
4. Enter a SAMPLE NAME, such as “wash.” This sample name merely avoids cluttering the hard drive with wash runs. Allow the system to overwrite this file or delete the file after the run.
5. Place a rack in the system; if the method was set up as described above, no samples will actually be collected.
6. Press the Play button.
7. Set the SAMPLE LOADING to SOLID when washing the solid load lines, otherwise use None (on column).
8. Press OK.

NextGen 100

The NextGen 100 only has two solvent inlet lines, and so needs an intermediate solvent such as acetone or 2-propanol to allow changes between normal and reverse phase. Acetone and 2-propanol are miscible with most solvents used for normal and reverse phase. The “A” line is the weak chromatography solvent, and the “B” line is the strong solvent.

1. Put the priming tube in place of the column and connect the bulkhead-to-column-block-tubing.
2. Place both solvent inlet lines into the intermediate solvent, prime the pumps, and use manual control to pump 100 mL solvent.
3. Remove the solvent lines from the intermediate solvent, dry the sinkers, and place in the correct solvent bottles.
4. Using manual control, pump 100 mL of the B solvent, followed by 100 mL of the A solvent. This will leave the system filled with the weak chromatography solvent.

Conclusion

Errors can occur any time a system is reconfigured for different experiment. After changing the solvents, it is a good idea to run Universal Test Mix, or another sample with known behavior for the column and solvent system to verify all solvents are correctly set up on the system. One common error is solvent lines placed in the wrong bottles. Sometimes, users do not purge the solid load cartridge lines when changing to normal phase from reverse phase causing the first run in a series of injections the have early eluting peaks. Verify the correct solvents are chosen for the method.

[1] Alternatively, an adapter that replaces a solid load cartridge and cap for this application can be made from the following parts available from Idex Health and Science (formerly Upchurch Scientific): P-135 (1/4-28 to 5/16-24 flat bottom fitting adapter) and P-683 (1/4-28 to Luer adapter). These fittings are connected to create an adapter for connecting the solid load cartridge line to the inlet at the top of the CombiFlash injection valve.

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