

Solutions for Acetonitrile shortage - Downsizing 1

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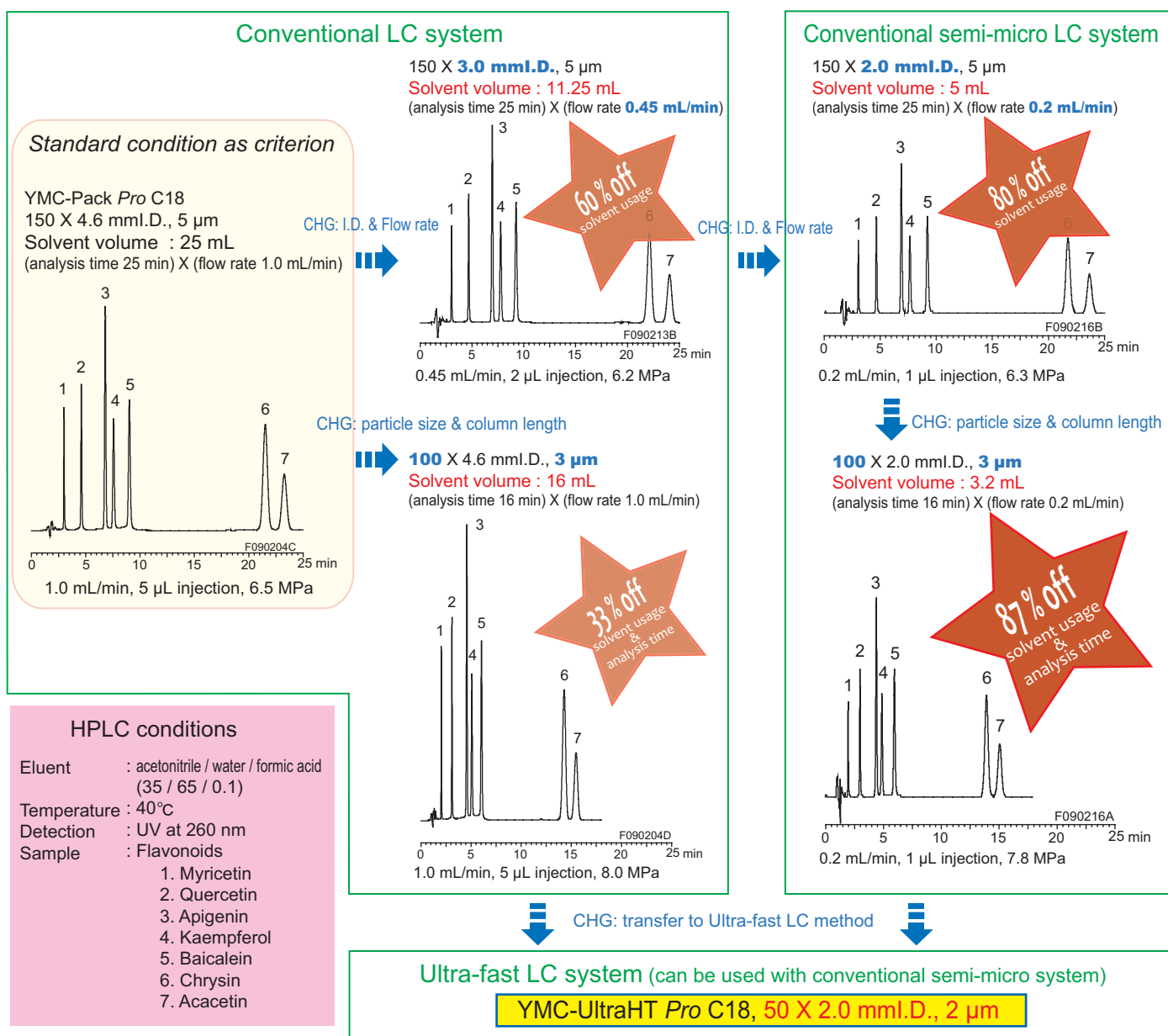
Acetonitrile (ACN) is an important solvent for HPLC. Due to the current shortage and increasing prices for ACN there is more interest in conservation and efficient utilization of ACN during chromatography. Changing from ACN to methanol usually changes retention and/or elution order for peaks of interest. A more simple method development approach is to "downsize" the separation by reducing column diameter and length while reducing the particle size of the stationary phase particle. As seen below, such an approach is effective when scaling down within YMC-Pack Pro C18 family of particles because selectivity and peak shape are consistent across all particle sizes. Such "scalability" allows for dramatic reductions in ACN usage while maintaining selectivity and adequate resolution and increasing the speed (productivity) of analyses.

correlation between I.D. of column / flow rate / sample volume / solvent volume

column I.D. (mm)	area ratio	flow ratio	sample ratio	solvent ratio	recommended system
4.6	1	1	1	1	conventional LC system
3	0.43	0.43	0.43	0.57	conventional LC system
2	0.19	0.19	0.19	0.81	conventional semi-micro LC system

correlation between column length and particle size

particle size	5 μ m	3 μ m	2 μ m
column length	150 mm	100 mm	50 mm



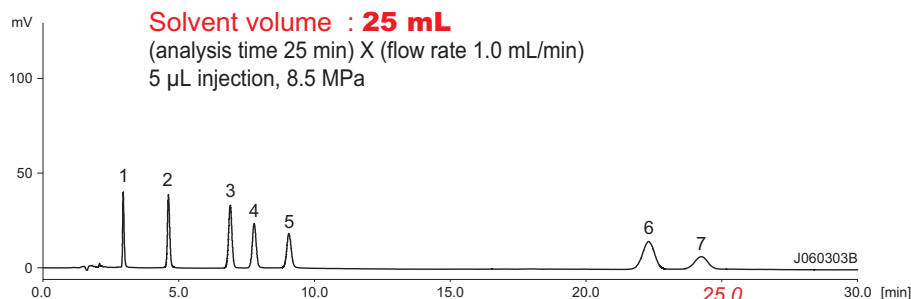
Solutions for Acetonitrile shortage - Downsizing 2

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Additional savings on Acetonitrile (ACN) usage can be realized by moving to a 2 micron particle. Moving from a 5 micron, 4.6 x 150 mm column to a 2 micron, 2.0 x 50 mm column within the YMC-Pack Pro C18 family of particles results in again maintaining selectivity and resolution across particle sizes while allowing for reductions in run time and ACN usage of 90% or better!

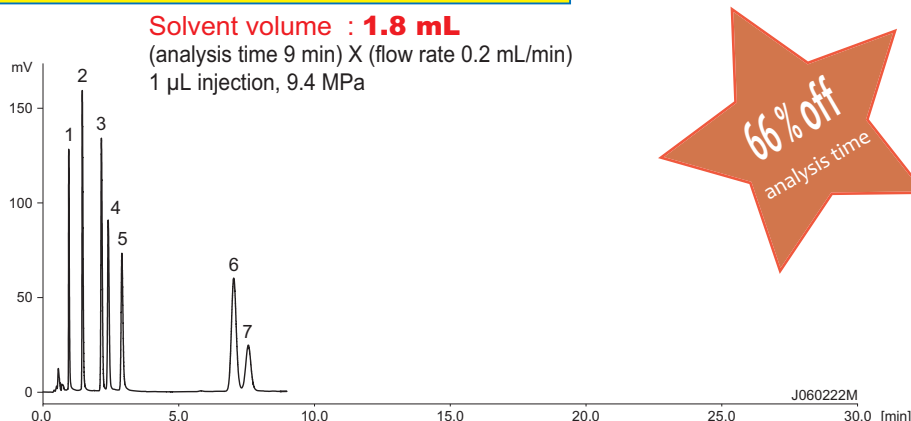
Standard condition as criterion

YMC-Pack Pro C18, 150 X 4.6 mm I.D., 5 μ m



CHG: column size for Ultra-fast LC

YMC-UltraHT Pro C18, 50 X 2.0 mm I.D., 2 μ m

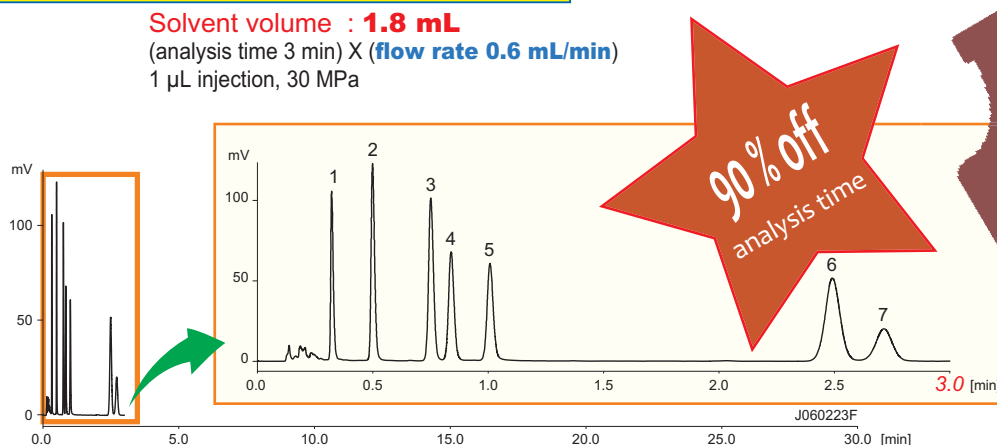


66% off
analysis time

93% off
solvent usage

CHG: flow rate = 3x, maintaining the resolutions and theoretical plate numbers

YMC-UltraHT Pro C18, 50 X 2.0 mm I.D., 2 μ m



90% off
analysis time

93% off
solvent usage

HPLC conditions : acetonitrile / water / formic acid (35/65/0.1), 40 $^{\circ}$ C, UV at 260 nm
Sample : Flavonoids (1. Myricetin, 2. Quercetin, 3. Apigenin, 4. Kaempferol, 5. Baicalein, 6. Chrysin, 7. Acacetin)

Solutions for Acetonitrile shortage - Solvent modification

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The current shortage and increasing price of acetonitrile (ACN) has generated interest in conservation and efficient utilization of ACN during chromatography. In some applications, changing the eluent from ACN to methanol is possible. The separation of flavonoids is just such an example.

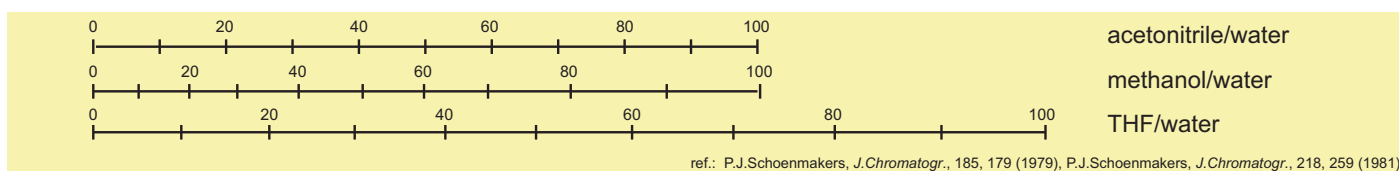
Indication for modifying of organic solvents

A table of relative elution power for solvents used in reverse phase chromatography can be viewed as: ^(*)

weak ← Eluent power → strong

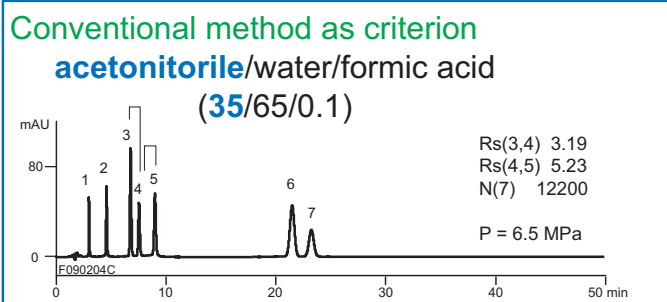
(water) < Methanol < Acetonitrile < DMF < Ethanol < Propanol < THF < Ethyl acetate ^(*)

The below chart illustrates the correlation between the solvent strength and the mix ratio (on a %v/v basis) of solvents mainly used in reversed-phase chromatography (e.g. 40% ACN = 50% MeOH = 32% THF). ^(*)



(*) It is not always the case. It is mainly affected by the mix rate of solvent or the solubility of samples. ^(*) Due to the compatibility, it cannot be made as AcOEt-aq without additives.

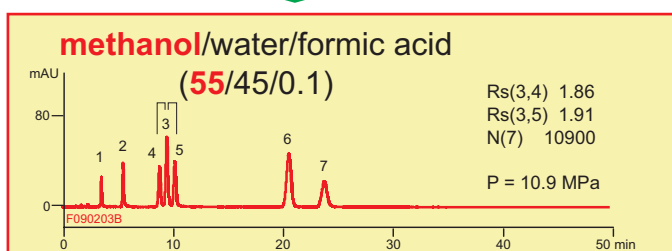
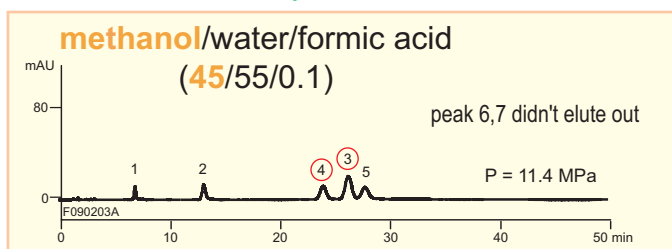
Solvent modification 1 : switch from acetonitrile to methanol



The application to the left is an example of solvent-switch from ACN to MeOH. The flavonoids were analyzed with 45%-MeOH, switched from 35%-ACN. The elution order of peaks 3 and 4 reversed as is often the case when substituting solvents in this manner. In addition to the elution order, the retention time and resolution between peaks may be changed when the eluent is modified in this manner. Thus re-confirmation of each peaks identity is required when changing solvents.

Additionally substituting MeOH for ACN will increase the viscosity of the eluent and the resulting system back pressure using MeOH will be higher than that of ACN. Reduction of the flow is often necessary to keep the system pressure at acceptable levels. In this application however, the flow rate was not adjusted. When using 45%-MeOH in this example, peaks 6 and 7 did not elute even though the column was run for more than 50min. The eluent was then modified in order to shorten analysis time.

The analysis time using 55%-MeOH was about the same time (25min) as the initial method (using ACN). Finally every peak was able to analyzed with baseline resolution.



Column	: YMC-Pack Pro C18 (5 μm, 12 nm) 150 X 4.6 mm I.D.	Sample : Flavonoids
Flow rate	: 1.0 mL/min	1. Myricetin 5. Baicalein
Temperature	: 40 °C	2. Quercetin 6. Chrysin
Detection	: UV at 260 nm	3. Apigenin 7. Acacetin
		4. Kaempferol

Solutions for Acetonitrile shortage - Adjustment by additives

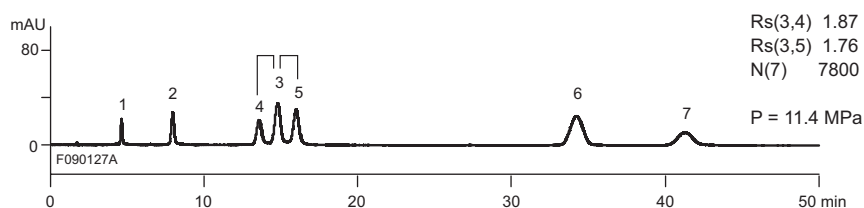
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Optimizing resolution and/or the analysis time by modifying the solvent from acetonitrile (ACN) to methanol is often a difficult task. Addition of a third solvent might provide the desired results, but method development requires more time and effort. The application below shows how addition of a 3rd solvent (additive) to optimize chromatography as a second step after switching from ACN to methanol can give good results.

Solvent modification 2 : Study of additives to methanol

Analysis of flavonoids with methanol as criterion

methanol/water/formic acid
(50/50/0.1)

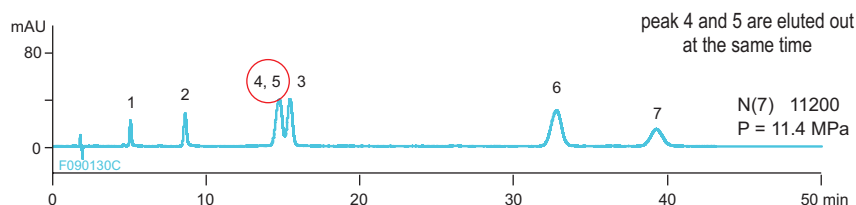


The chromatograms seen below are result of modifying the solvent eluent from 50%-methanol to 45%-methanol and 5%-additives.

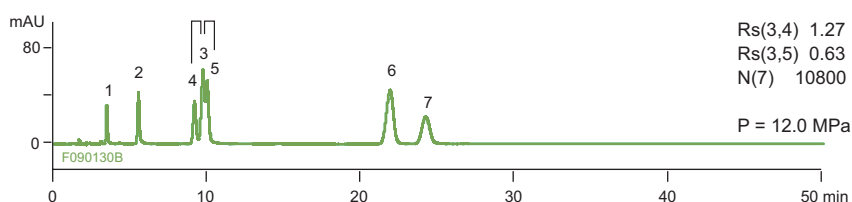
Eluent : methanol/additive/water/formic acid (45/5/50/0.1)

Additive

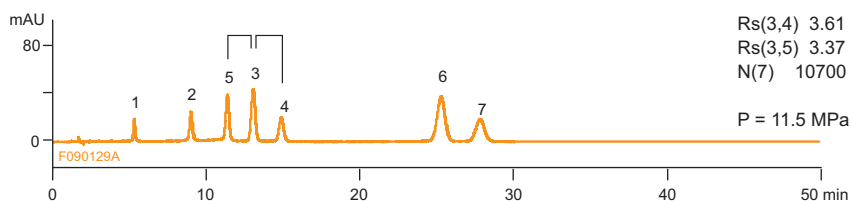
***N,N*-dimethylformamide (DMF)**



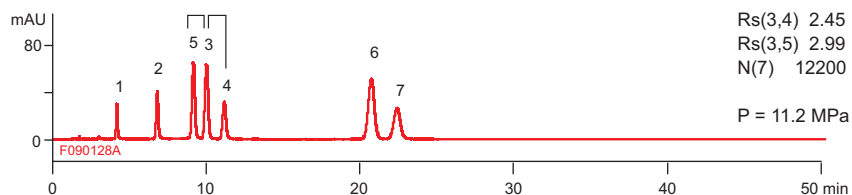
2-propanol



tetrahydrofran (THF)



ethyl acetate



Column : YMC-Pack Pro C18 (5 μm, 12 nm) 150 X 4.6 mm I.D.	Sample : Flavonoids
Flow rate : 1.0 mL/min	1. Myricetin
Temperature : 40°C	2. Quercetin
Detection : UV at 260 nm	3. Apigenin
	4. Kaempferol
	5. Baicalein
	6. Chrysin
	7. Acacetin

Adding ethyl acetate or THF to methanol is effective in this analysis. Good resolution between peaks and shortened analysis time (productivity increase) are obtained by substituting equivalent volumes of ethyl acetate or THF for the same volume of methanol.