LC × LC

- Multi-dimensional separations can be either heartcutting or comprehensive in approach. In heart-cutting separation a region of interest is excised from the first-dimension separation, and subjected to a further separation in the second-dimension.

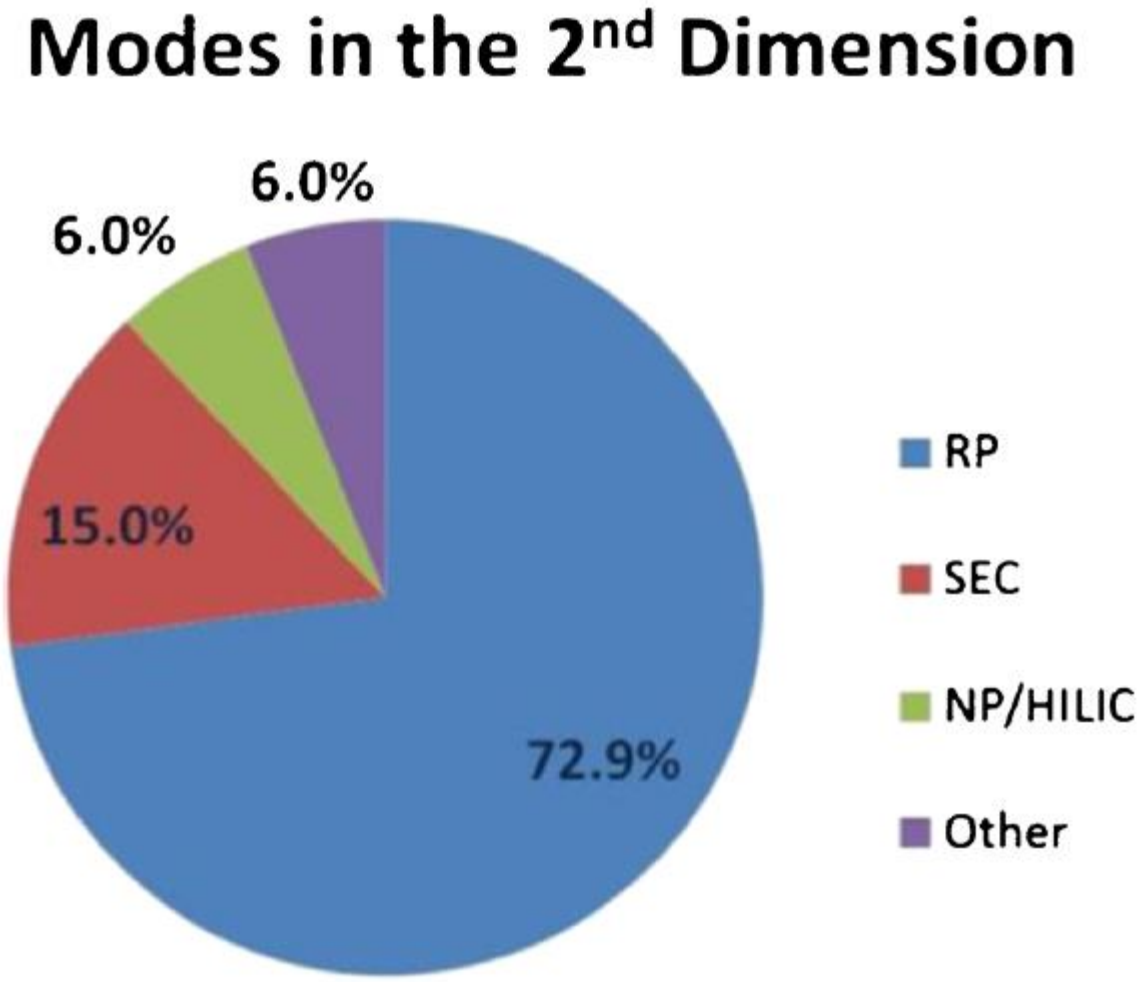
Multi-modal and mixed-bed separations are often confused.

- 2D LC or LC×LC method which either combination of two columns with different stationary phases or by use of two columns with the same stationary phase running under different mobile phases.

- *Mixed-bed stationary phases where a mixture of two* stationary phases are packed into a single column (or columns are coupled), or where the stationary phase shows multiple retention mechanisms.

Mode name		Mode description	Potential in on-line comprehensive orthogonal MDLC?
Multi-modal	Multi-modal	Sequential elution with different chromatographic modes e.g. NP then RP, or SFC then GC across the same stationary phase	—
	Multiple mechanism	Applying multiple mechanisms across a stationary phase, e.g. chromatophoresis, MEKC, pH and organic gradient simultaneously	*****
	Unified chromatography	Different physical states of mobile phase during a separation. If sequential then potentially multi-dimensional	—
Mixed-bed	Mixed-bed	Mixed mode of retention within the stationary phase using the same chromatographic mode	***
	Coupled stationary phases	As above but in two distinct columns or one biphasic column	***
	Coupled stationary phase with independent mobile phase conditions	As above but using independent mobile phase conditions between dimensions (commonly used in multi-dimensional HPLC)	*****

Term	Definition
Multi-dimensional	Separation from deliberately different mechanisms
Orthogonal	Independent mechanisms without retention correlation
Comprehensive	All analytes are subjected to all separation mechanism dimensions, ideally all will be differentially retained by these mechanisms, although this is not specified. Resolution is maintained between dimensions. All dimensions are independent separation mechanisms, and retention is linked only as is inevitable from an analyte having the same physico-chemical properties in any dimension
On-line	Sample transfer is automated between dimensions with uninterrupted flow



Modes in second dimension of comprehensive two-dimensional liquid chromatographic system (LCxLC). Literature (up to May 2014) summarized according to valve-based online comprehensive twodimensional liquid chromatography

Duxin Li & Cornelia Jakob & Oliver Schmitz, Anal Bioanal Chem (2015) 407:153–167

The main cause for the use of RP mode in LCxLC systems can be attributed to the following conditions:

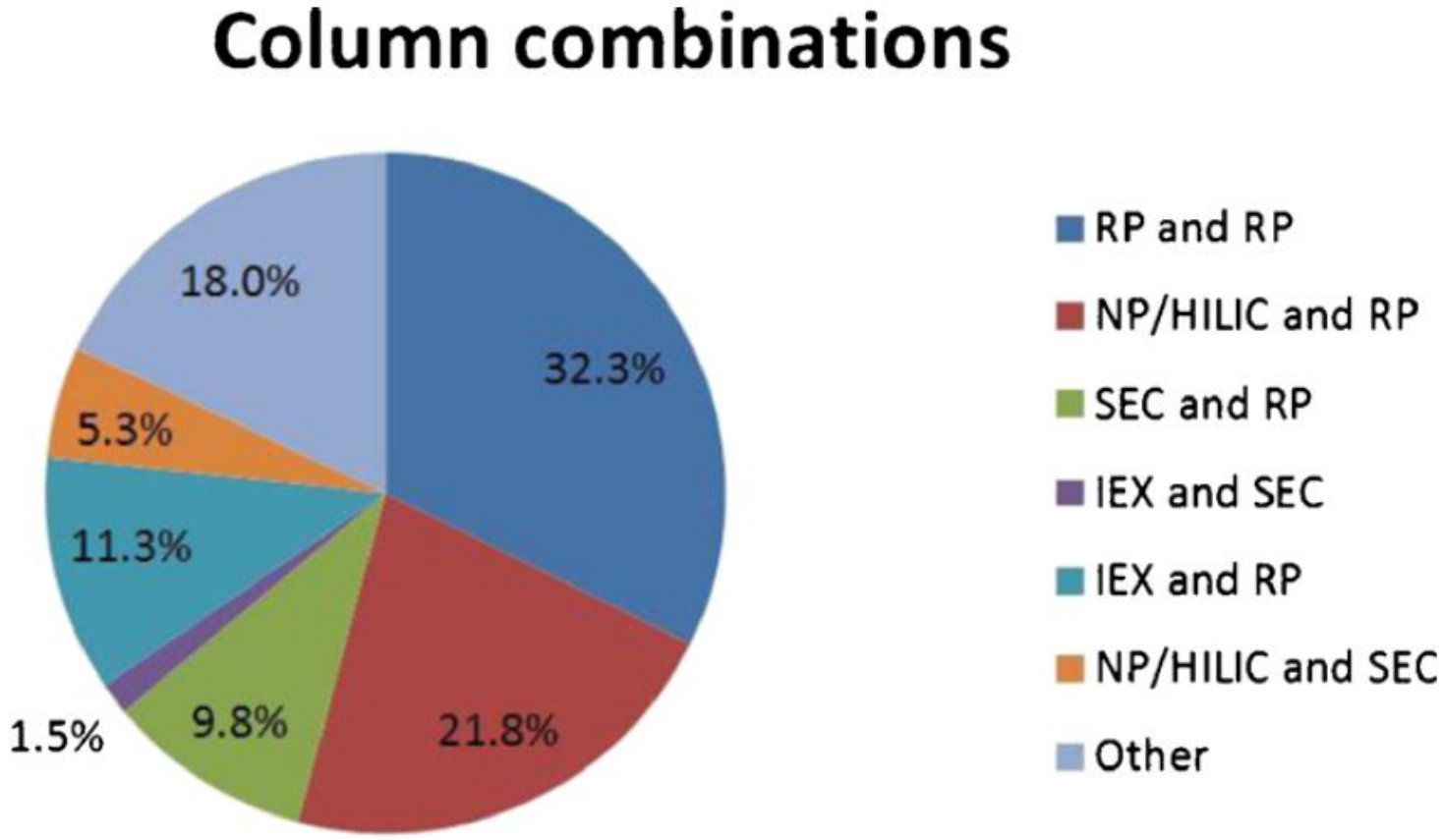
(1) ***Wide applicability:*** The applicability of RP conditions to a variety of samples, detailed knowledge of the RP retention mechanisms over 30 years, and the commercial availability of several hundred different stationary phases.

(2) ***Fast equilibration***: The equilibration time can be as short as one column volume. HILIC also has high performance as RPLC, however, the slow equilibration [9] is a challenge for the fast second-dimensional separation.

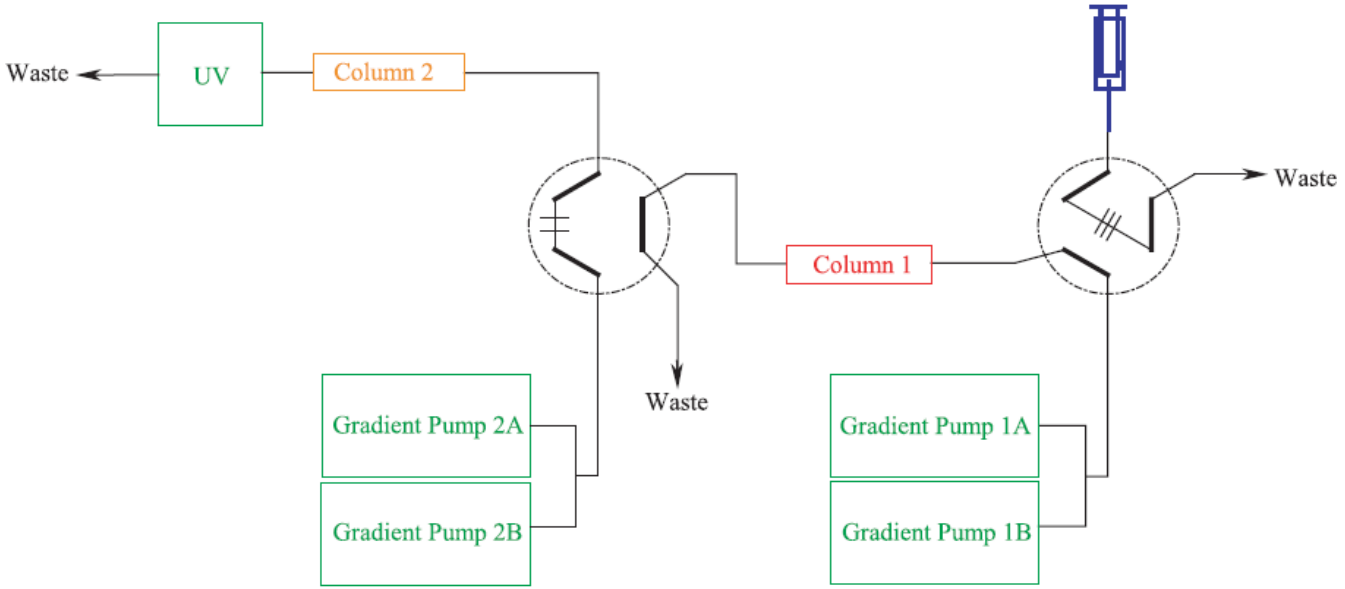
(3) ***High efficiency***: RPLC can generate high column efficiency [10]. Especially with the new generation of sub- 2 μm packing, the separation was sped up (e.g., by a factor of nine and often without a loss in peak capacity or change in selectivity).

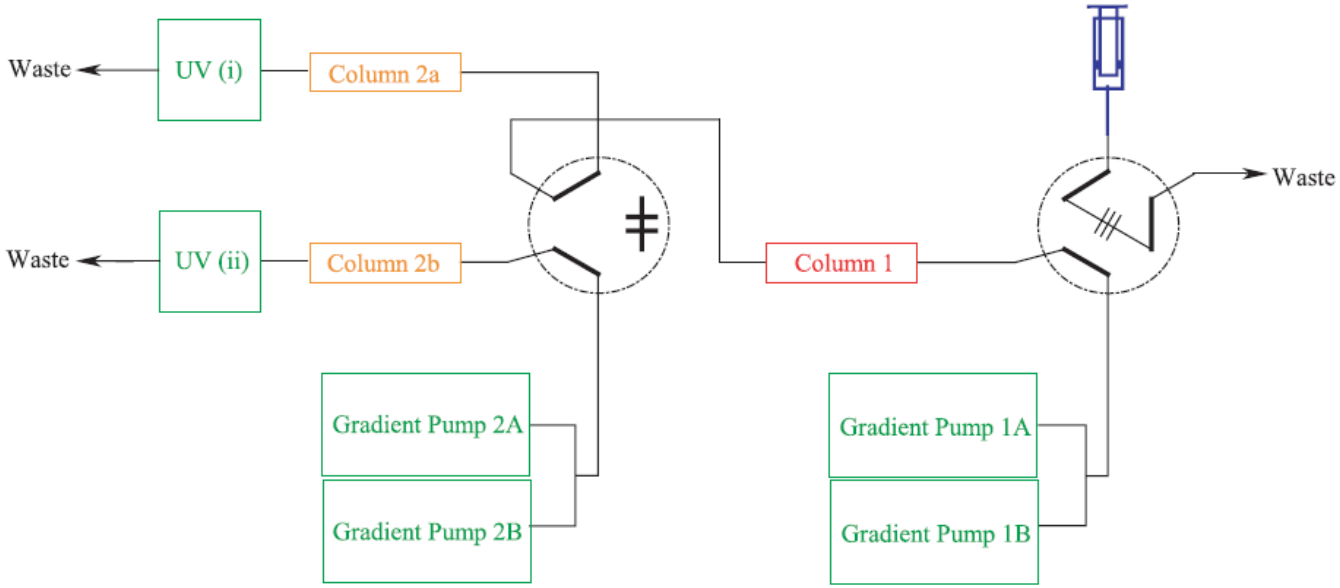
(4) ***Difference in selectivity***: A great number of various stationary phases featured significant differences in selectivity. The stationary phases show differences in the matrix (silica, polymer, inorganic oxide) and, e.g., linked functional groups (C18, Cyano, Phenyl etc.) and various chemical modifications to C18 (like endapping and polar embedded groups etc.).

(5) ***Compatibility with MS***: As has been pointed out by Guiochon et al. [12], “However, we must keep in mind that any serious advance in chromatographic resolution should be made through approaches that permit coupling to mass spectrometry” the compatibility with MS

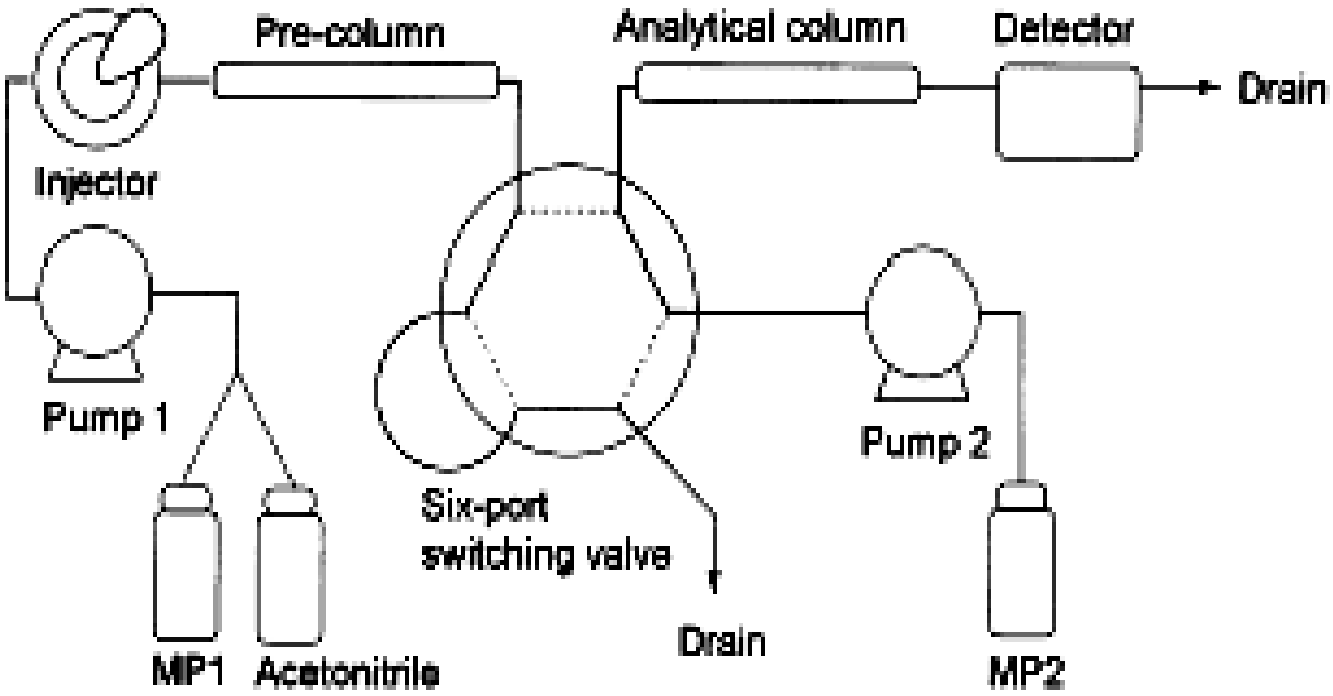


Column combinations in LCxLC systems (up to May 2014)


Waste
UV
Column 2
Waste
Column 1
Waste
Gradient Pump 2A
Gradient Pump 2B
Gradient Pump 1A
Gradient Pump 1B

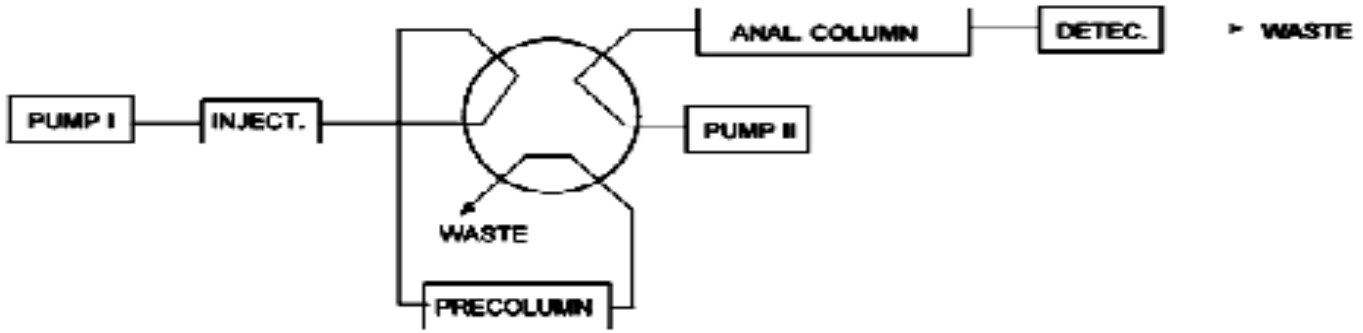

Waste
UV (i)
Column 2a
Waste
UV (ii)
Column 2b
Column 1
Waste
Gradient Pump 2A
Gradient Pump 2B
Gradient Pump 1A
Gradient Pump 1B

Fore Flush System

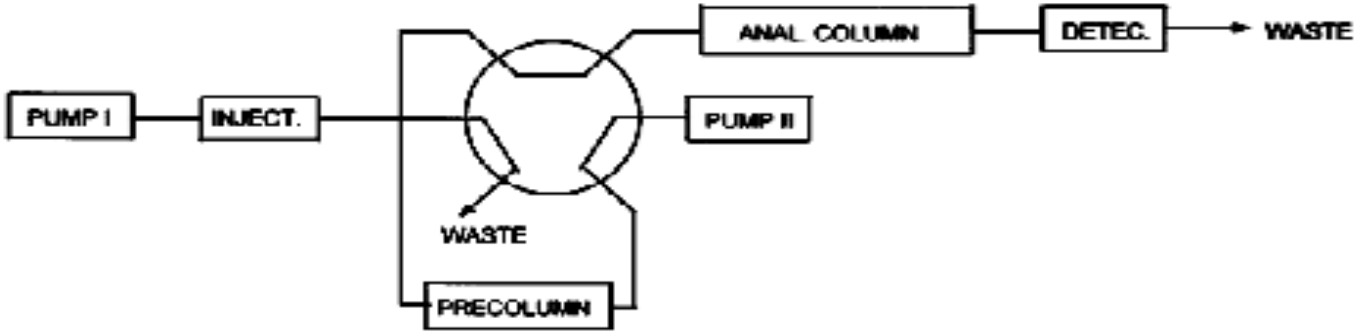


Back Flush System

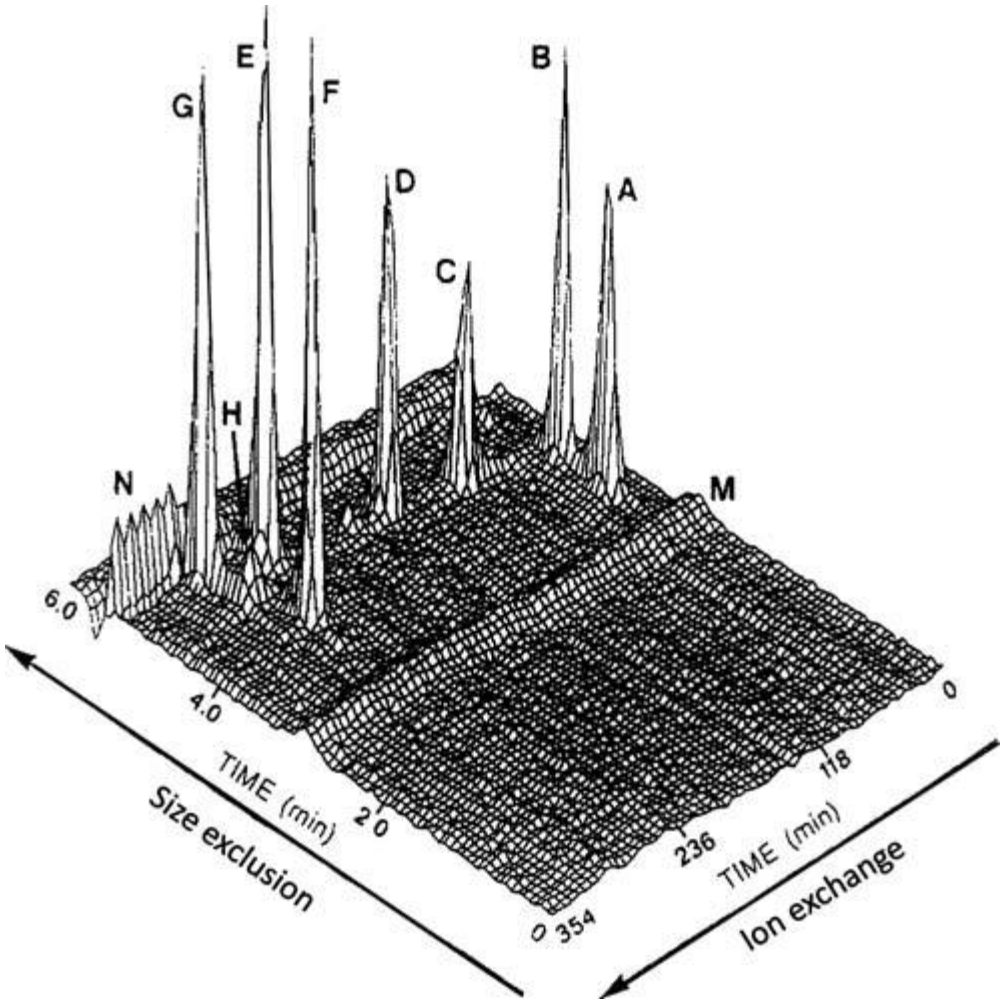
LOAD POSITION



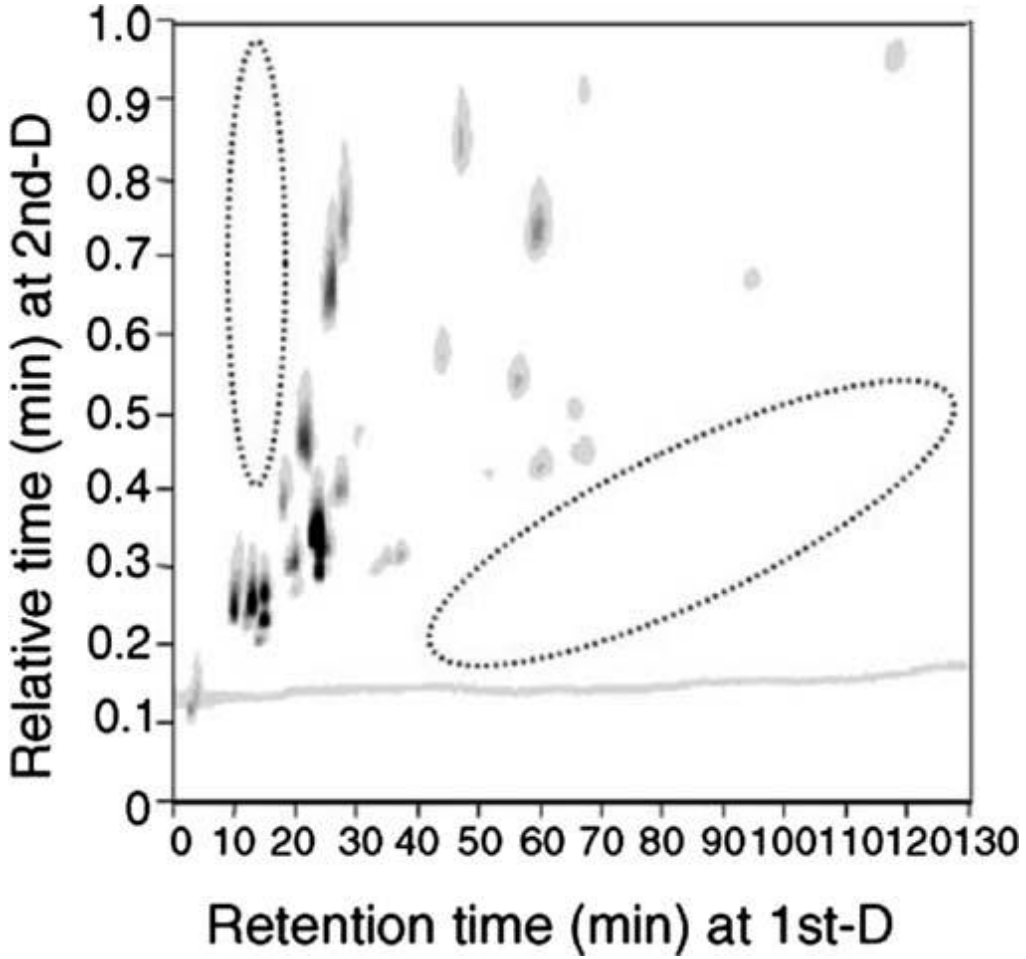
INJECT POSITION



3D plot of protein sample. An ion-exchange column (250 mm×1.0 mm) and a size-exclusion column (250 mm×9.4 mm) were used in the first and second dimensions, respectively



Two-dimensional separation space arising from the LC×LC separation of a test mixture using a 0.65 mL min−1 flow of 80:20 H2O/THF in the first dimension and a 42% methanol second dimension mobile phase (9.5 mL min−1). The column dimensions were 100 mm × 4.6-mm ID and 50 mm × 4.6-mm ID for the first and second dimensions, respectively. The effective peak capacity is about half of the maximum possible peak capacity due to the partial correlation of the two dimensions.



The interest in comprehensive two-dimensional separations is based on the peak capacity product rule

$$n_{2D} = {}^{1}n \times {}^{2}n$$

with n_{2D} as the theoretical peak capacity of an LCxLC analysis, ^{1}n and ^{2}n as the peak capacity of the first and second dimension, respectively.

But this equation is only correct, if the separations in the two dimensions are completely independent from each other and if there is no loss of separation as a result of under-sampling from the first to the second dimension. This is rarely possible. Losses of resolving power are encountered in any implementation of two-dimensional chromatography as a result of back-mixing of the fractions in the sample loops.

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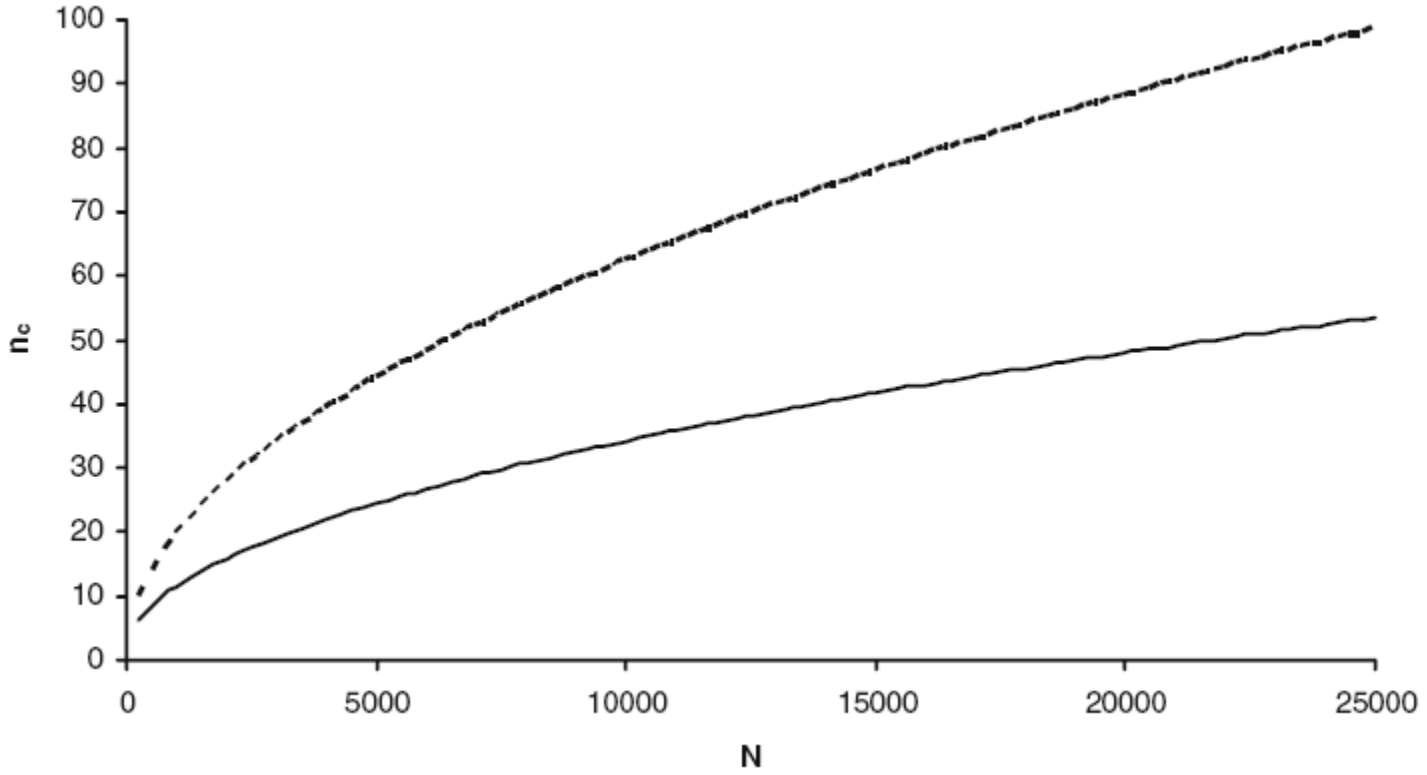
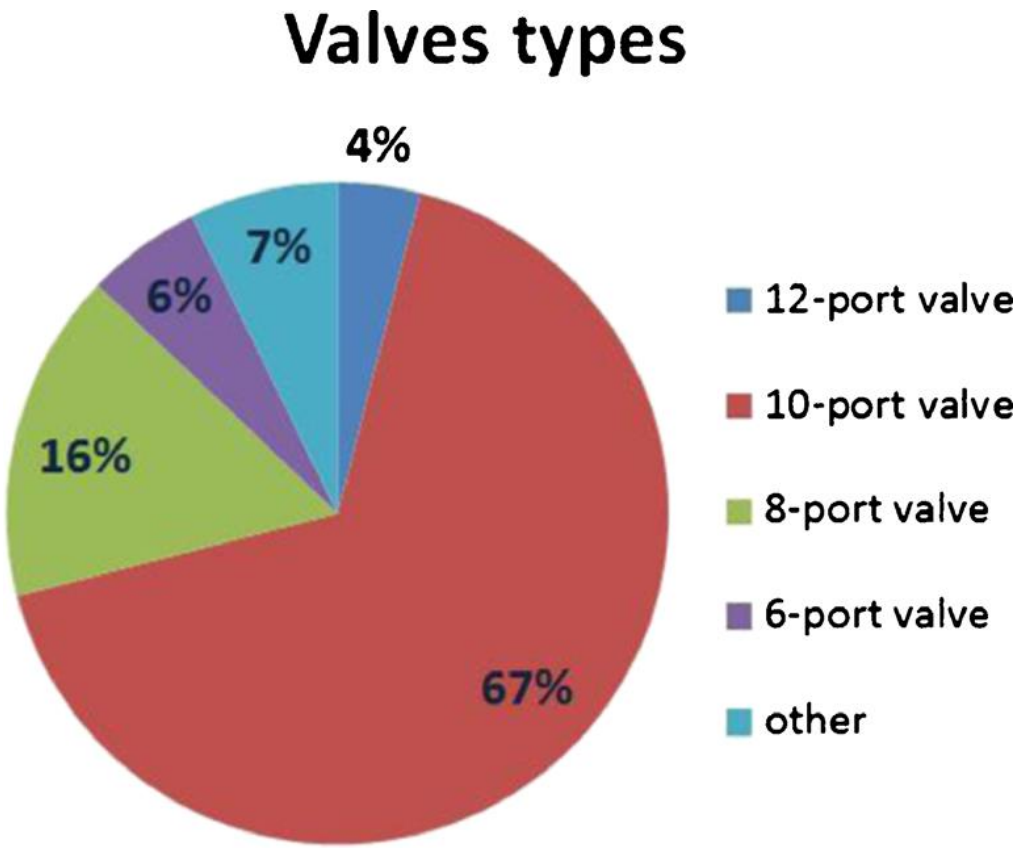
Peak capacity as a function of plate number N. The solid line represents the isocratic case and the broken line is for a gradient separation.

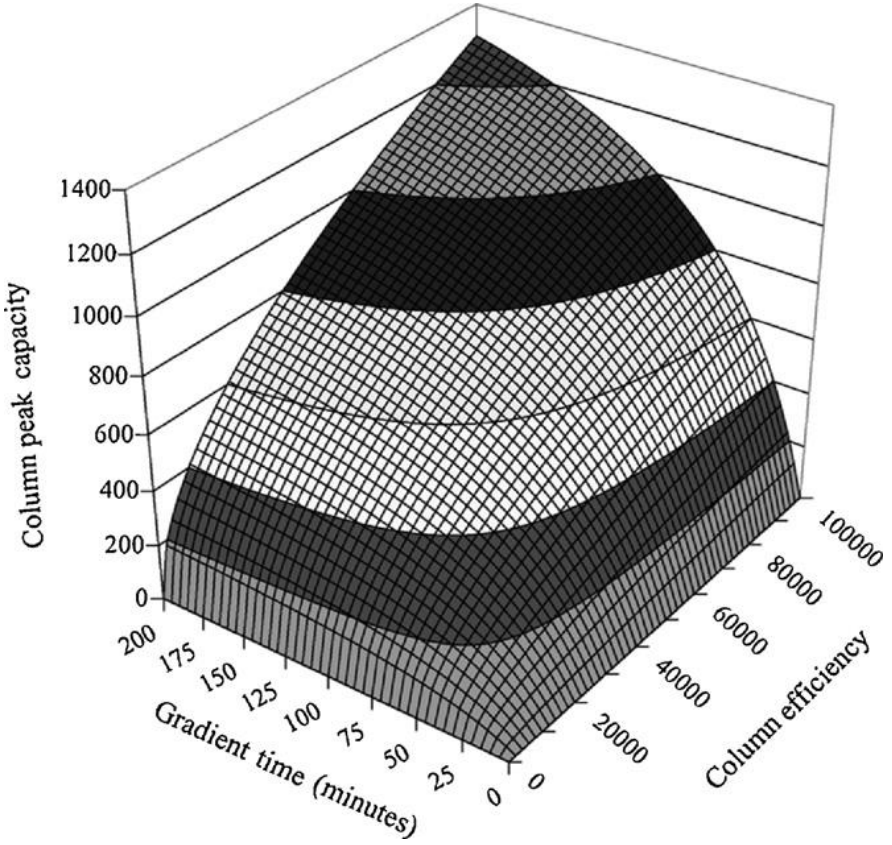




Valve types adopted in LCxLC (up to May 2014)

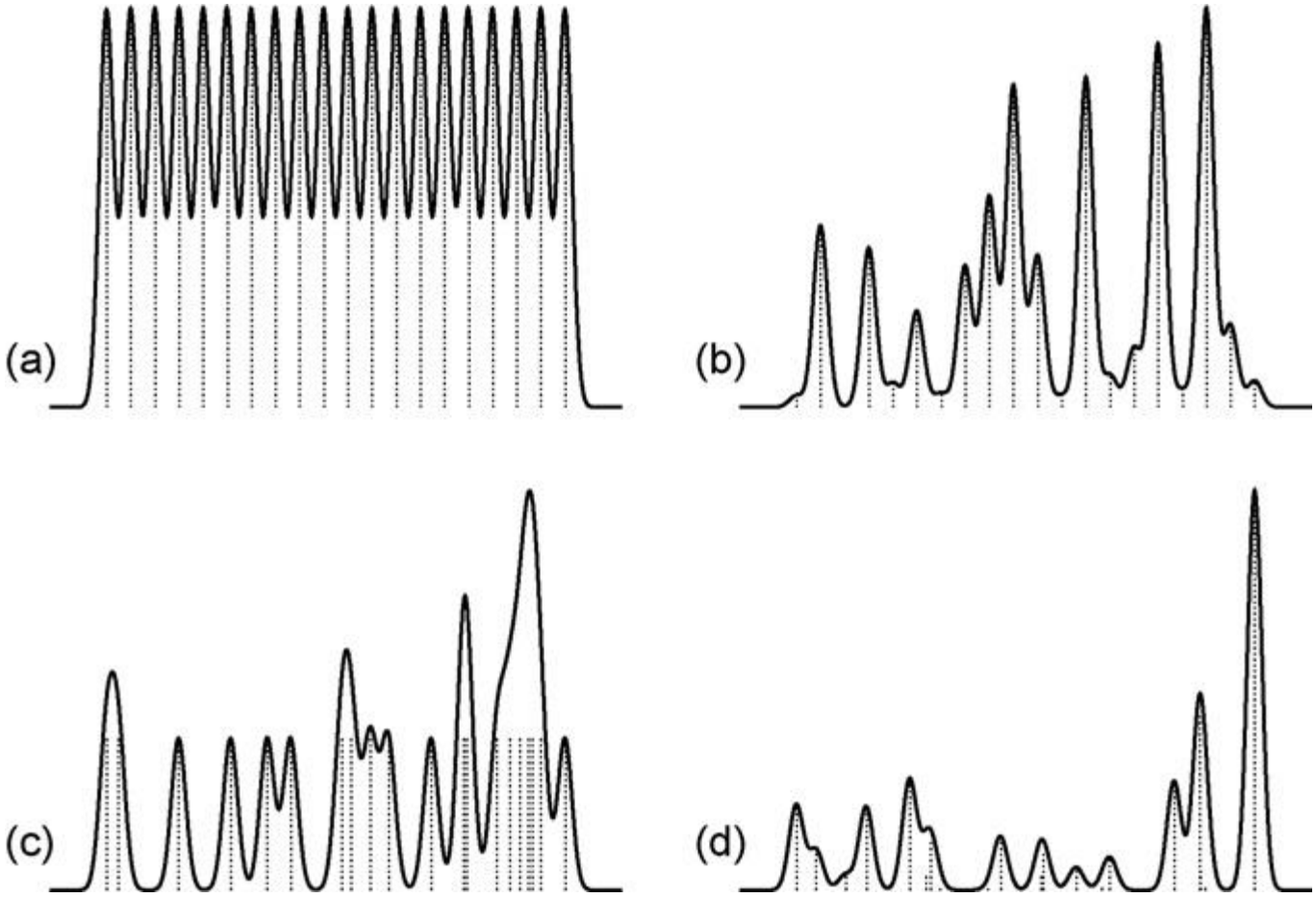
$$n_c = 1 + \frac{\sqrt{N}}{4}\frac{S\,\Delta\phi}{1 + S\,\Delta\phi(t_0/t_G)} = 1 + \frac{\sqrt{N}}{4}\frac{S\,\Delta\phi}{1 + Ss}$$

The important parameters are the gradient time, t_G, *the relationship* between the retention factor, *k, of a compound under* isocratic conditions and the mobile phase composition, and the excursion of this composition, φ. with t_0 *hold-up time and* $s = \varphi t_0/t_G$ *the normalized gradient* slope.



Peak capacity and resolution:

The resolution of 1 is necessary to provide peaks that are easily identified on a chromatogram and 2 is even more may be required.



- Two-dimensional chromatography resolution has been reported as follow, where R_{s2D} *is the resolution between a pair of peaks* in a two-dimensional system, and R_{sD1} *or* R_{sD2} *is the* resolution between a pair of peaks in each of the dimensions.

$$R_{\mathrm{s2D}} = \sqrt{R_{\mathrm{sD1}}^2 + R_{\mathrm{sD2}}^2}$$

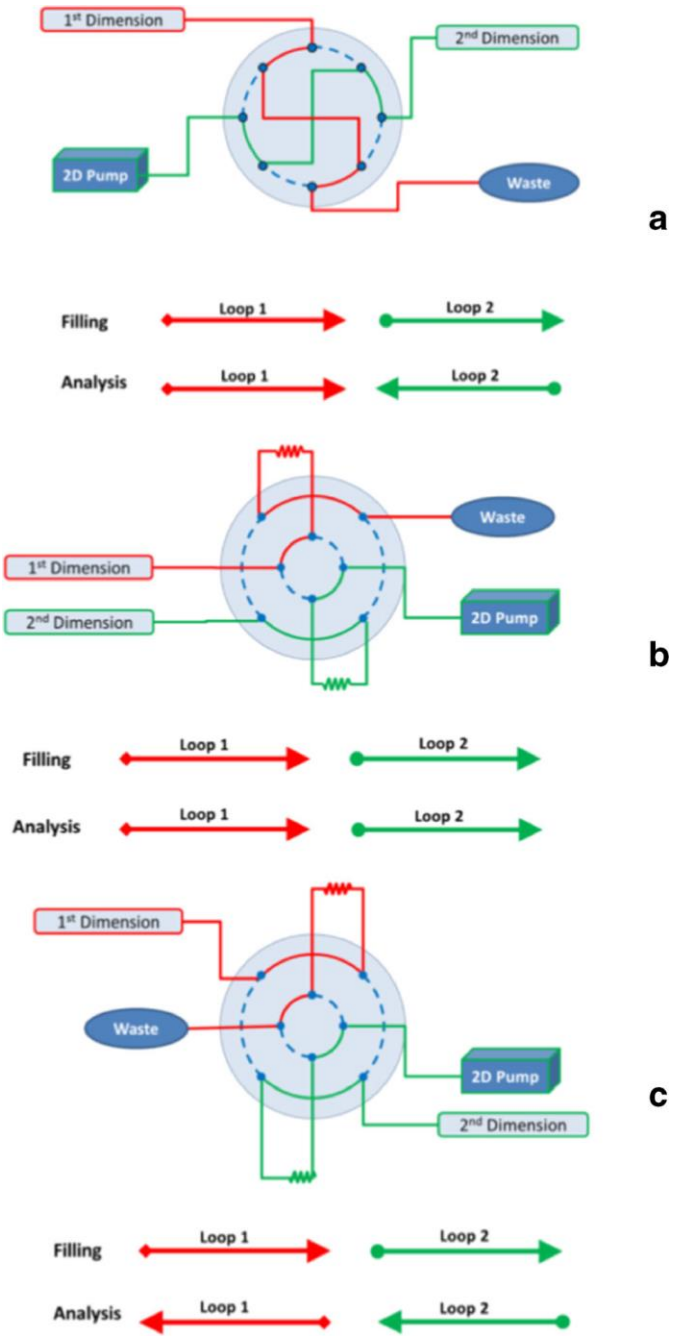
Sampling rate

- The rate of sampling from one dimension to the next is an important consideration in any multi-dimensional system.

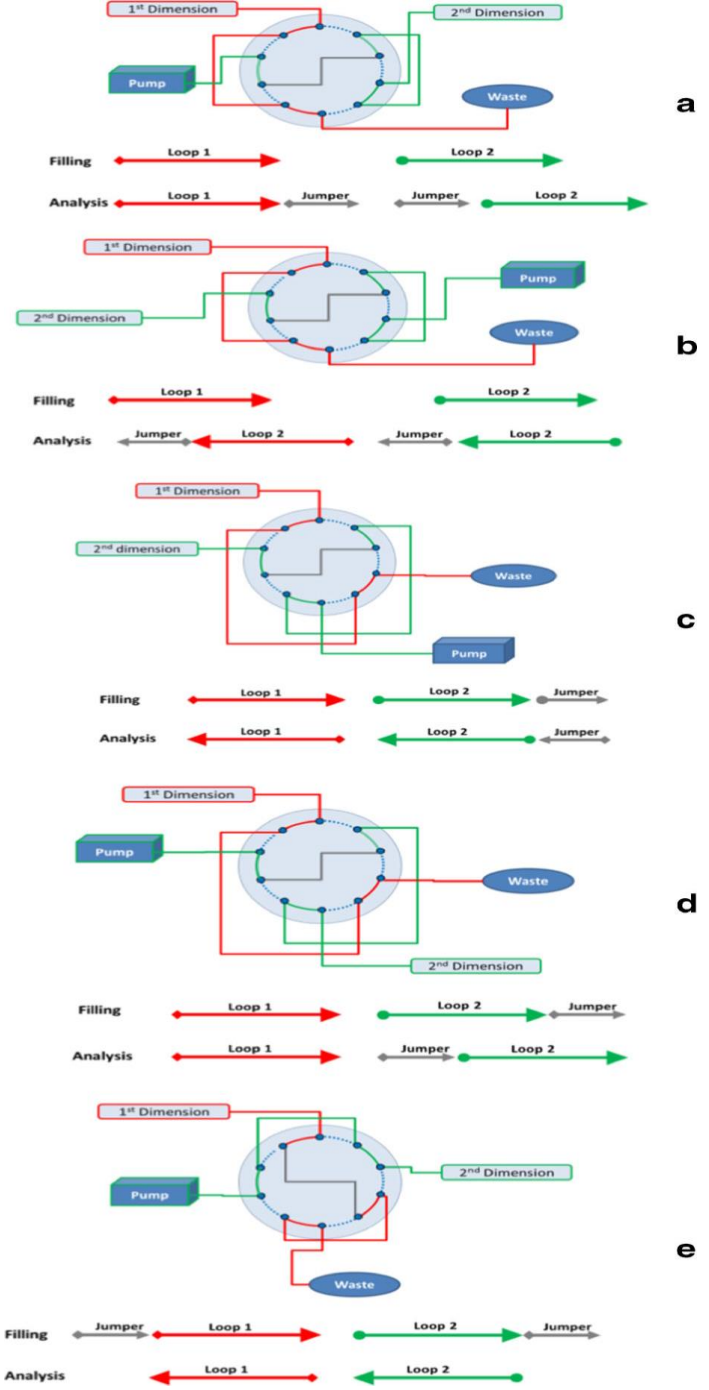
- The sampling period should be <1.5 standard deviations of the peak width to maintain precision. For larger sampling periods the time of the sampling event relative to the peak becomes more critical, the retention time (t_R) *is less accurately estimated and peak width* increases due to the dilution of the solute within the fraction volume.

Dilution factors

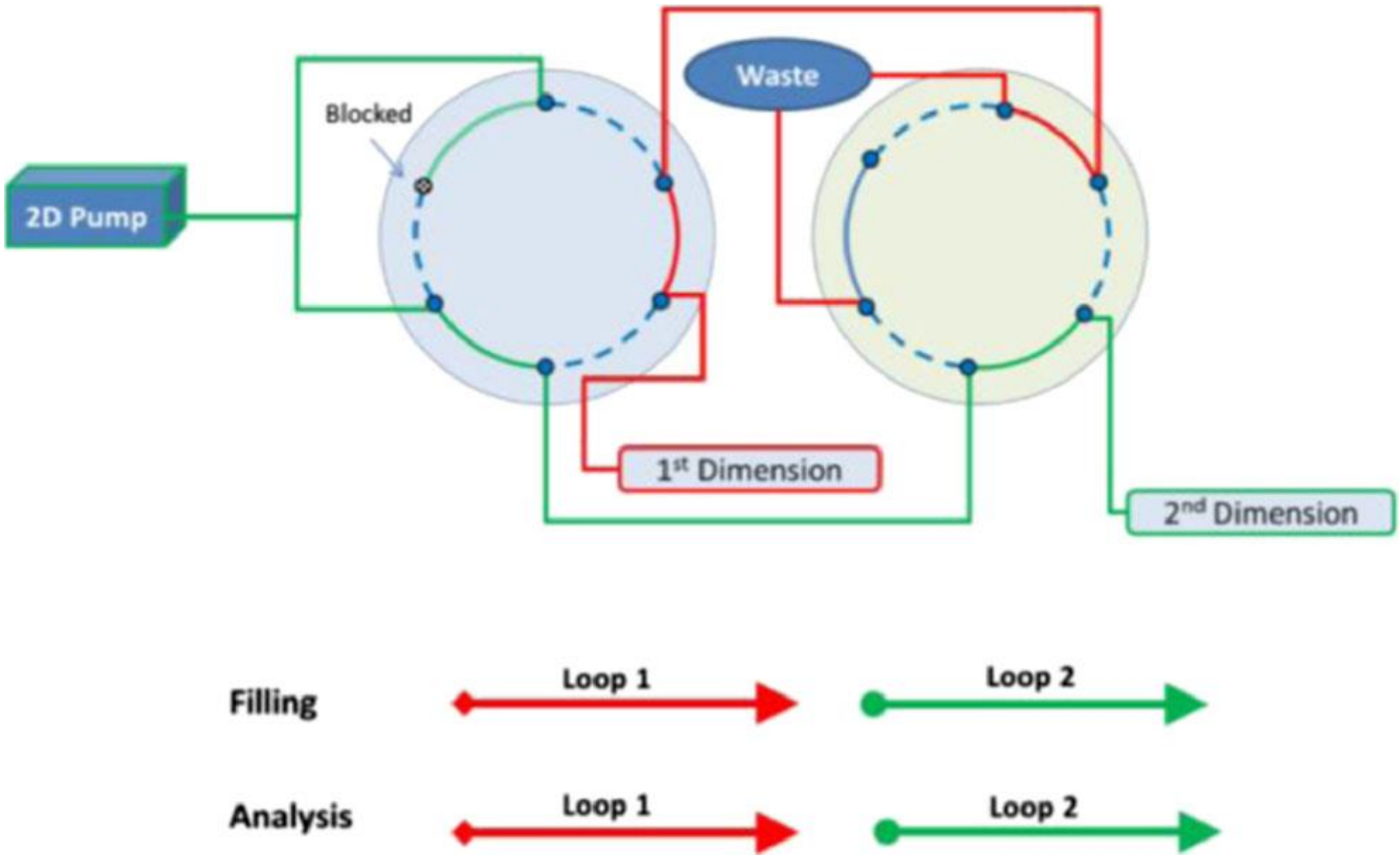
- The final dilution of the sample can be quite large depending on the techniques being coupled. If a system is designed carefully then on-column focussing (e.g. by use of a more polar eluent in RP-HPLC) can re-concentrate the sample in the next dimension, high-efficiency separation can concentrate compounds in narrow bands, and, of course, narrow-bore plumbing can reduce system dead volume.



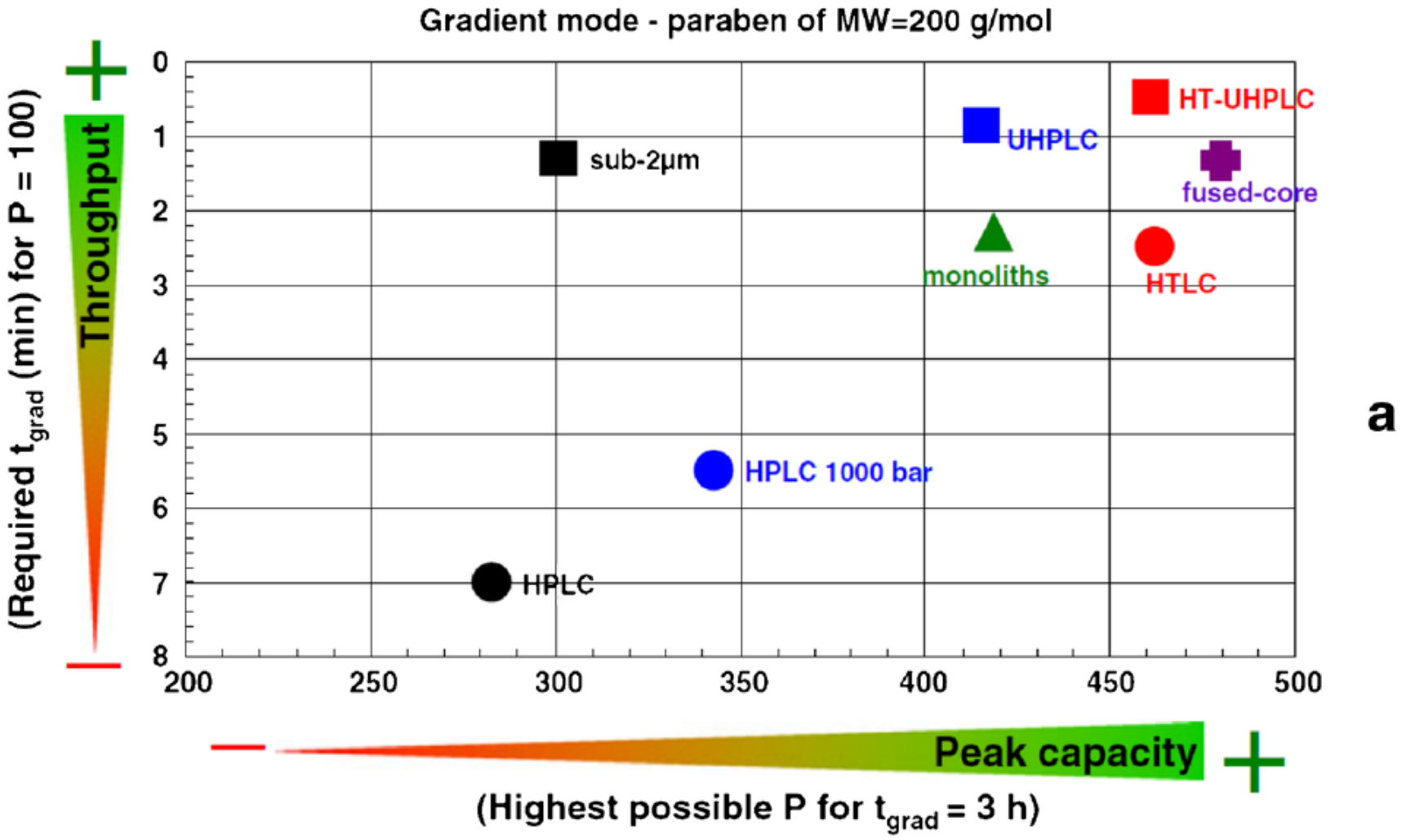
Setups of different eight-port valves in LCxLC



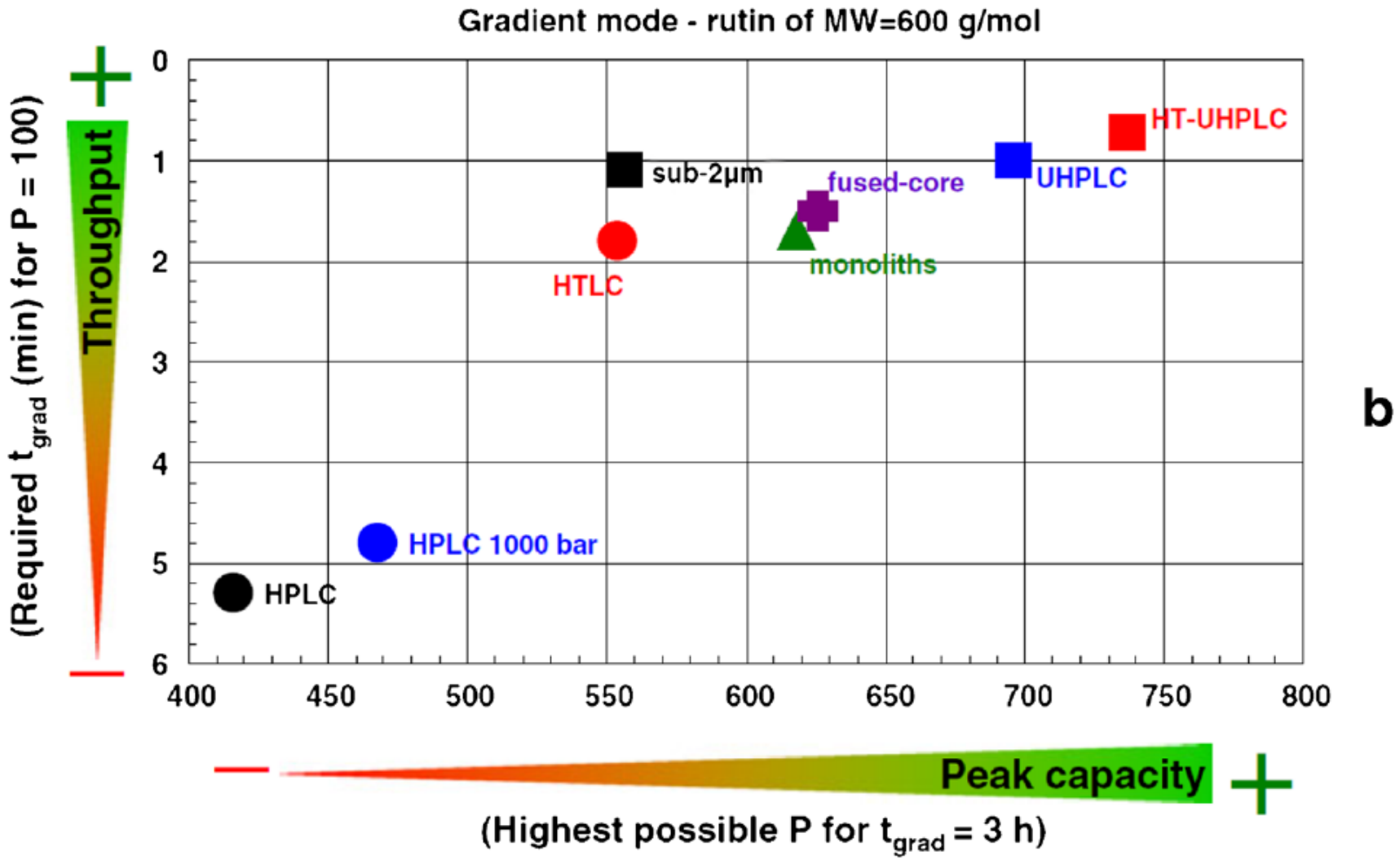
Ten-port valve setups in LCxLC

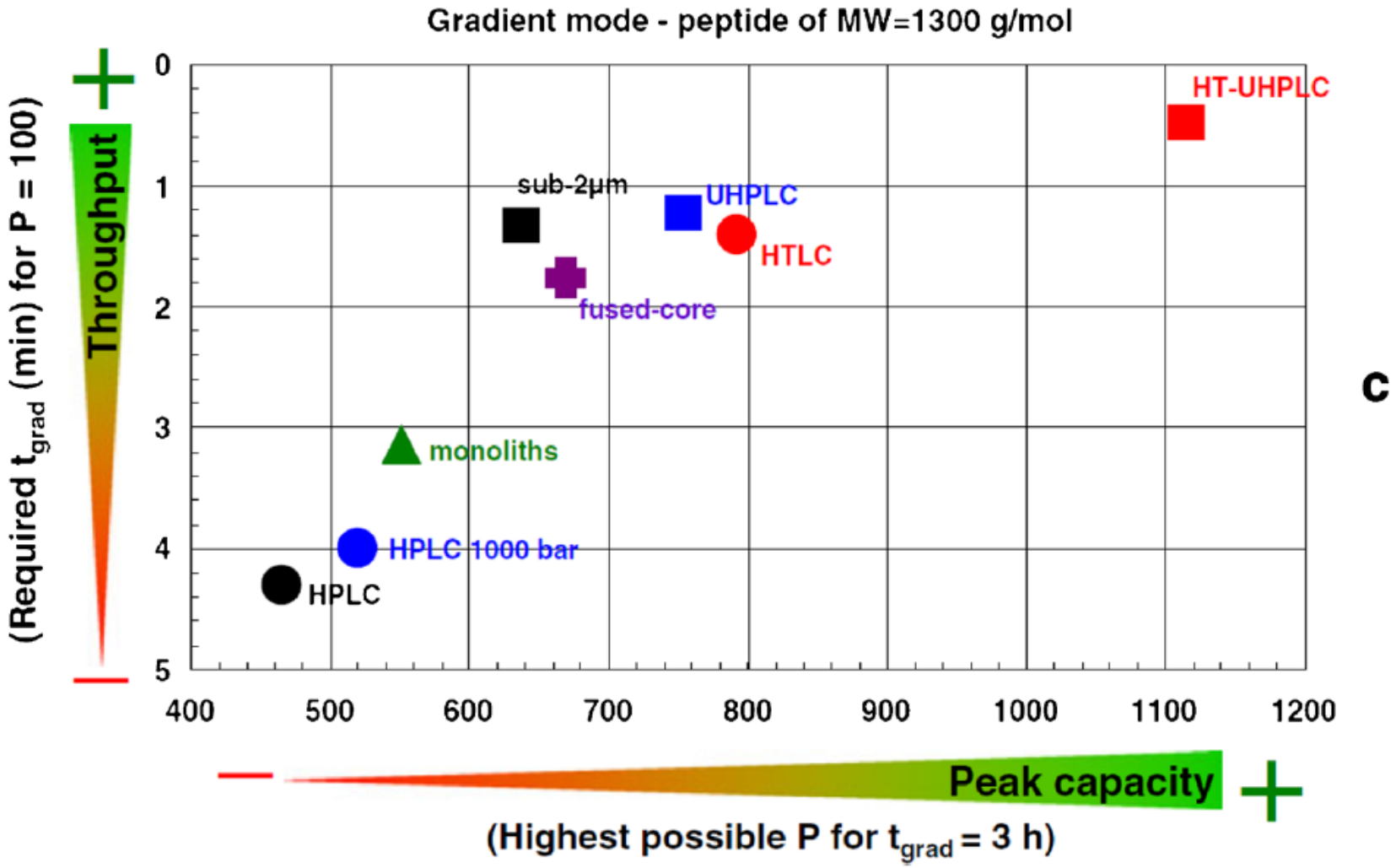


Setup of the two-six-port valves system


Gradient mode - paraben of MW=200 g/mol
(Required t_{grad} (min) for P = 100)
Throughput
0
1
2
3
4
5
6
7
8
200
250
300
350
400
450
500
sub-2µm
UHPLC
HT-UHPLC
fused-core
monoliths
HTLC
HPLC 1000 bar
HPLC
Peak capacity
(Highest possible P for t_{grad} = 3 h)

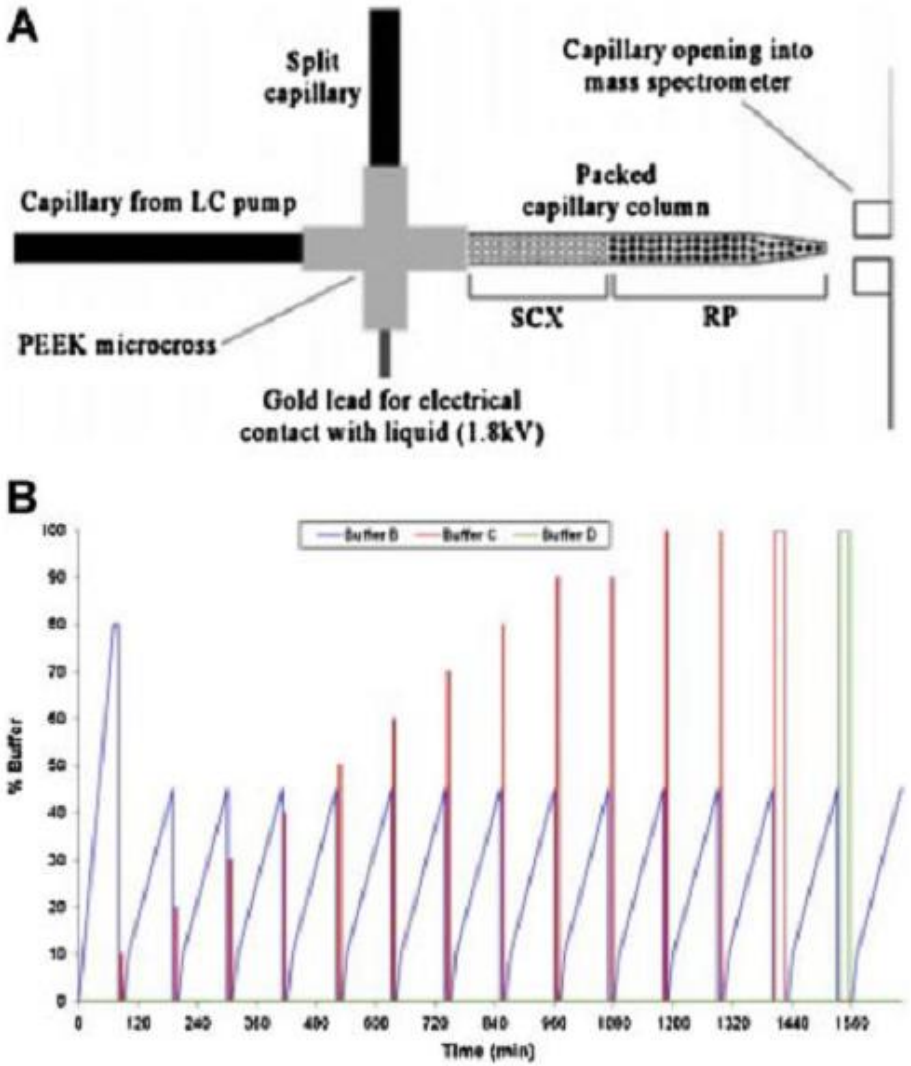
a


Gradient mode - rutin of MW=600 g/mol
(Required t_{grad} (min) for P = 100)
Throughput
+
−
0
1
2
3
4
5
6
400
450
500
550
600
650
700
750
800
HT-UHPLC
sub-2µm
fused-core
UHPLC
HTLC
monoliths
HPLC 1000 bar
HPLC
Peak capacity
(Highest possible P for t_{grad} = 3 h)
b


Gradient mode - peptide of MW=1300 g/mol
(Required t_{grad} (min) for P = 100)
Throughput
0
1
2
3
4
5
400
500
600
700
800
900
1000
1100
1200
HT-UHPLC
sub-2µm
UHPLC
HTLC
fused-core
monoliths
HPLC 1000 bar
HPLC
Peak capacity
(Highest possible P for t_{grad} = 3 h)

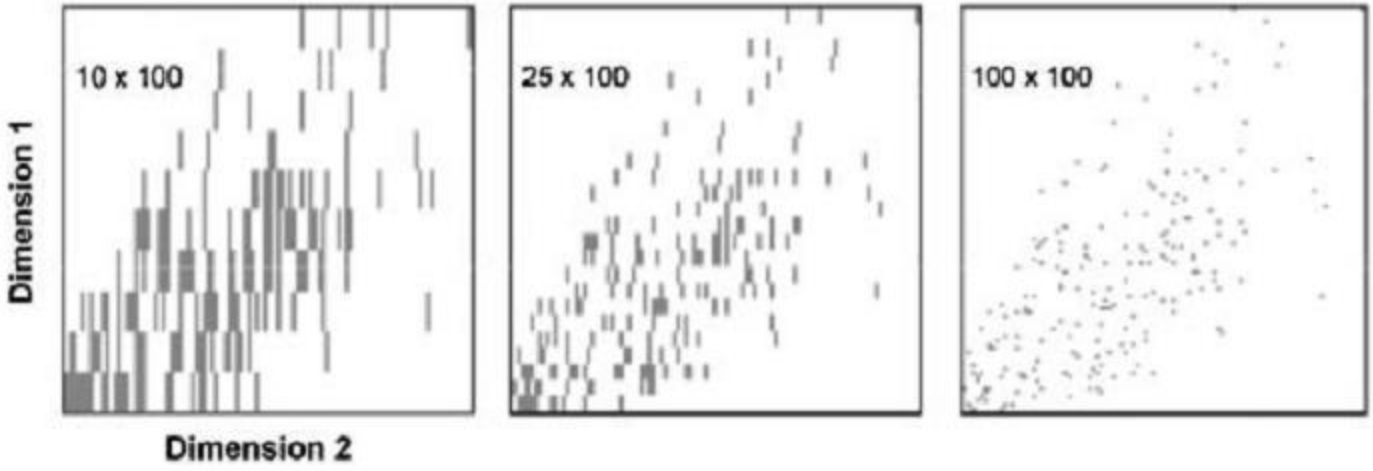
c

Mass Spec Rev 31:523–559, 2012



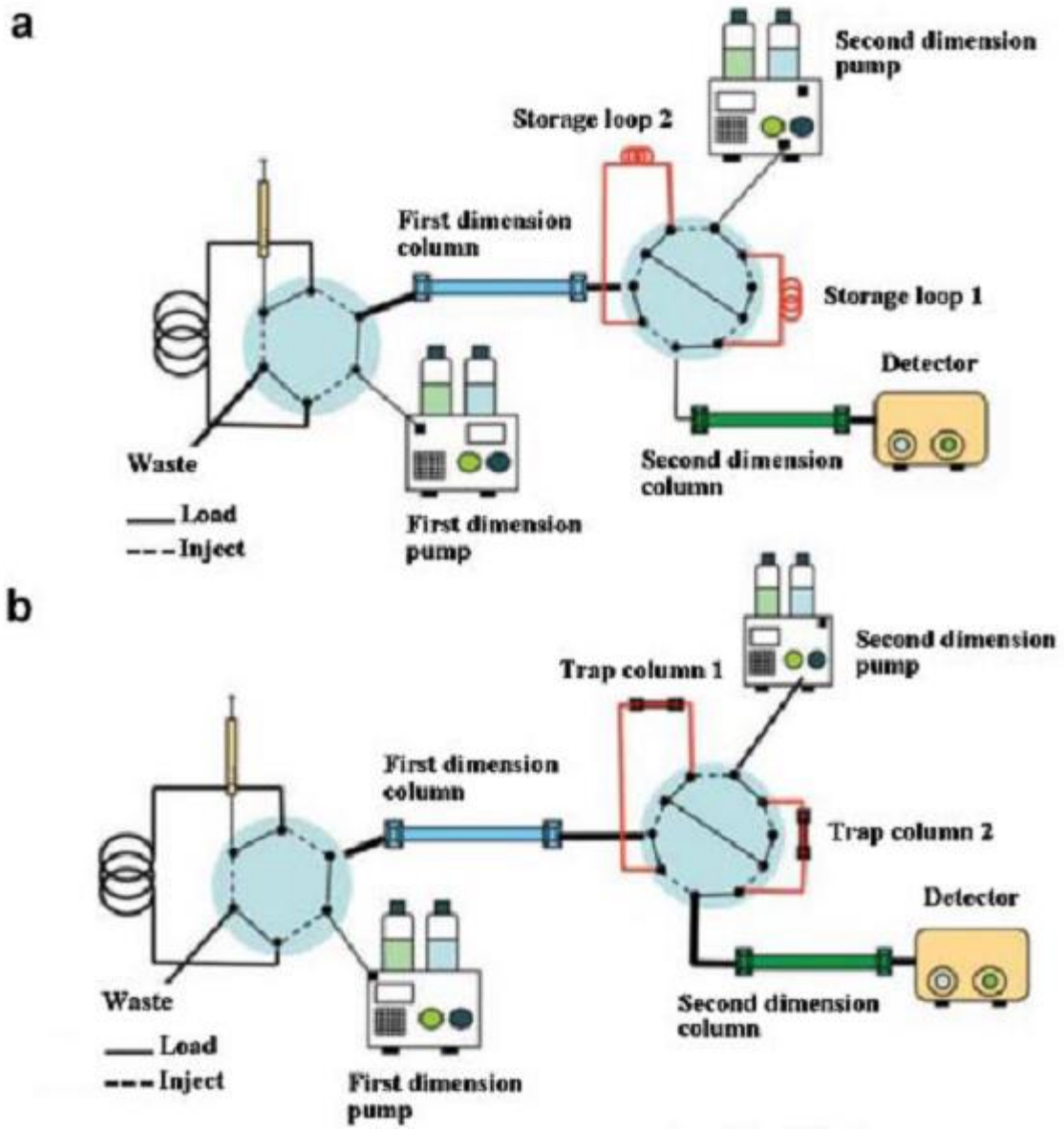
(A) Schematic overview of a biphasic MudPIT system having SCX in the first and RP in the second dimension loaded in the electro-spray needle. (B) Applied gradient elution profile used in the original MudPIT methodology (buffer A: 5% acetonitrile, 0.5% acetic acid; buffer B: 80% acetonitrile, 0.5% acetic acid; buffer C: 5% acetonitrile, 0.5% acetic acid and 250 mM KCl; buffer D: 5% acetonitrile, 0.5% acetic acid, 1 M KCl)

Mass Spec Rev 31:523–559, 2012

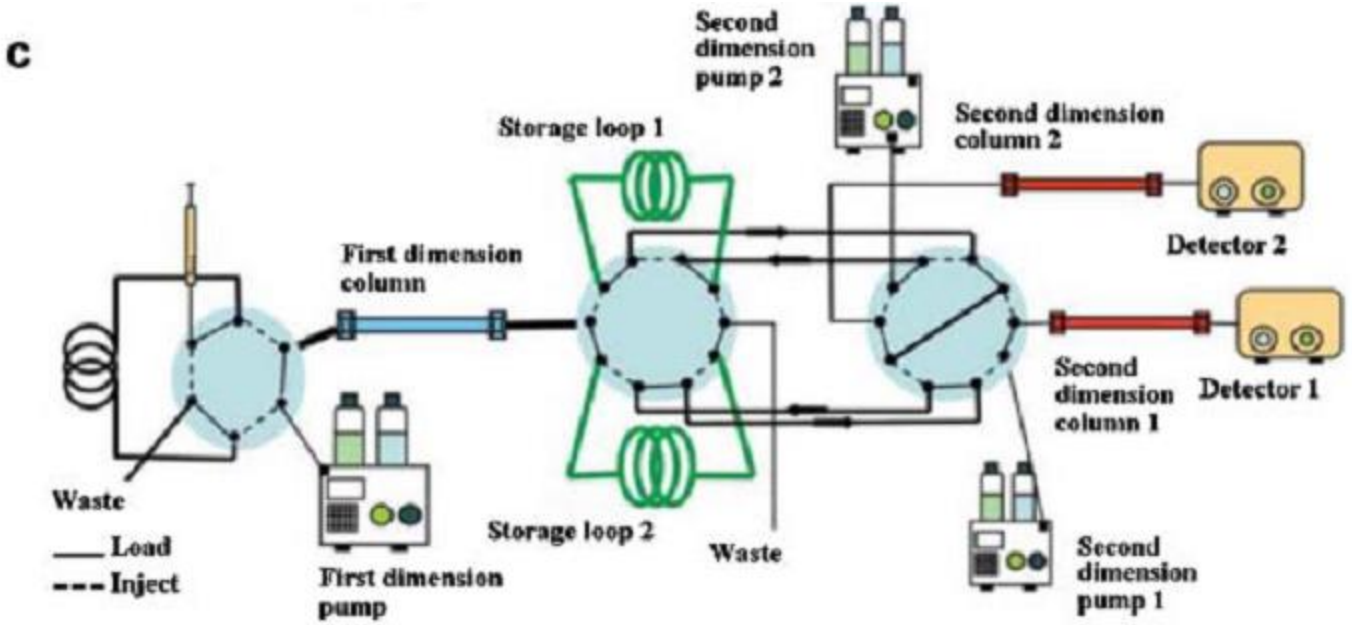


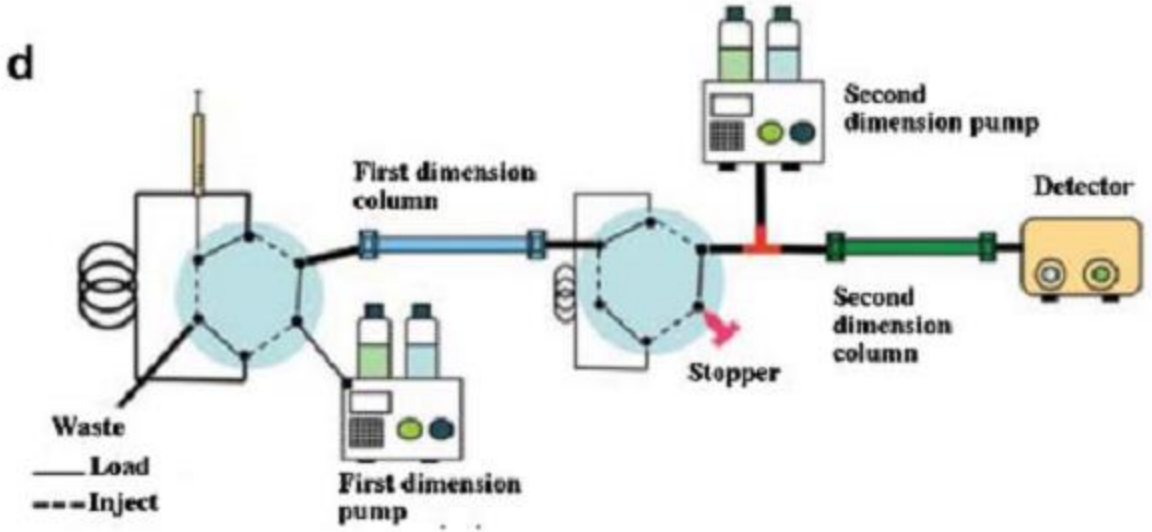
Effect of sampling rate on chromatographic resolution. From left to right, the first dimension is sampled 10, 25, and 100 times. Second dimension nc is 100. The power of a multidimensional system is visible in all cases, but the highest gain in performance is achieved at the highest sampling rate. The plots can also be interpreted as the gain in overall peak capacity upon switching to a more efficient first-dimension separation

Mass Spec Rev 31:523–559, 2012



Column-switching LC×LC instrumentation using (a) storage loops (b) trap columns (c) paralle analytical columns in the second dimension or (d) the stop-flow approach for on-line collection of the first-dimension fractions





Column-switching LC×LC instrumentation using (a) storage loops (b) trap columns (c) paralle analytical columns in the second dimension or (d) the stop-flow approach for on-line collection of the first-dimension fractions

Ref.	Type of interface	Column Packing and Dimensions (Length x i.d., mm, d_p, µm); Flow rate; Elution Mode*		Detection	MS and MS/MS parameters	Application
		First dimension, D1	Second dimension, D2			
[1]	Two-position 8-port valve	IEC 125 x 0.75, 5; 10 µL/min; GP	RP (R2/H) 100 x 0.5; 50 µL/min; GP	UV, ESI-MS	1,000-2,000 *m/z*, 0.1 amu steps, 0.2 ms dwell, 0.5 Hz	Mixture of standard proteins and *Escherichia coli* cell lysate
[2]	2 Two-position 4-port valves equipped with alternated D2 columns	Six SEC 300 x 7.8; 1 mL/min, 0-40 min run; 100 µL/min, 40-140 min run; IP	Two parallel C_{18} 33 x 4.6, non-porous; 1 mL/min; GP	UV, ESI-MS	600-2,600 *m/z*, 0.125-amu steps, 0.190 ms dwell, 0.33 Hz	Ovoalbumin and serum albumin tryptic digests
[3]	Two-position 4-port valve equipped with alternated D2 columns	SEC 300 x 7.8; a) 8 columns, 5 µm; 250 µL/min; GP b) 12 columns, 8 µm; 150 µL/min; GP	Two RP (R2/H) a) 33 x 2.1 b) 100 x 2.1; 1.5 mL/min; GP	UV, ESI-MS, MALDI-TOF-MS	600-2,600 *m/z*, 0.125-amu steps, 0.190 ms dwell, 0.33 Hz	Native and non-native proteins in *Escherichia coli* cell lysate
[4]	Two-position 10-port valve equipped with alternated D2 columns	IEX 35 x 4.6; 1 mL/min; GP	C18 NPR 14 x 4.6; 2.5 mL/min; GP	UV, MS	-	Protein and peptide mapping
[5]	No interface; directly coupled-columns	Biphasic 140 x 0.1, 5; SCX: 40 mm capillary inlet, C_{18}: 100 mm distal segment; 0.15-0.25 µL/min; GP		ESI-MS/MS	400-1,400 *m/z*, CID 35% (30 ms activation time), repeat count and duration: 2 and 0.5 min	Peptides and proteins in *Saccaromyces cerevisiae* lysate
[6]	Two-position 10-port valve equipped with alternated D2 columns	IEC 35 x 4.6, 5 non-porous; 1 mL/min; GP	Four C_{18} 14 x 4.6, 5 non-porous; 2.5 mL/min; GP	UV, MALDI-TOF-MS	Reflectror and linear mode: 20 kV voltage, 150 ns delay time	Human emofiltrate and human fetal fibroblast cell lysate
[7]	Two-position 10-port valve equipped with alternated D2 columns	IEC 35 x 4.6; 400 µL/min; GP	a) Two C_{18} 33 x 4.6, 1.5; b) C_4 50 x 2.1, 3.5; 0.5 mL/min; GP	UV, ESI-TOF- MS	3,000-46,000 *m/z*, 65 µs flight time, MCP at 2700 V	Mixture of standard proteins and yeast ribosomial fraction

[8]	Two-position 10-port valve	IEC 50 x 2.1, 5; 50 µL/min; GP	C_{18} monolithic a) 25 x 4.6; 5 mL/min (to UV) b) 100 x 0.1; 3µL/min (to MS); GP	UV, ESI-TOF-MS	400-2,000 *m/z*, 2 Hz	Bovine serum albumin tryptic digest
[9]	3 Two-position 10-port valves equipped with alternated D2 columns	IEC 35 x 4.6, 5 non-porous; 0.5 mL/min; GP	Four parallel C_{18} 14 x 4.6, 1.5 non-porous; 2 mL/min; GP	UV, MALDI-TOF-MS	1,300-4,100 *m/z*, ion source voltage: 25 kV	Human emofiltrate tryptic digest
[10]	2 Two-position 10-port valves	IEC 150 x 0.3; 15 µL/min; IP	C_{18} 1500 x 0.075; 200 nL/min; GP	ESI-TOF-MS/MS	90-10,0000 *m/z*, CID energy: 51.2 V	Human hepatocellular carcinoma proteins and peptides
[11]	2 Two-position 6-port valves equipped with alternated D2 columns	SEC (Macrosphere GPC) 250 x 4.6, 7; GP	C_{18} 7.5 x 4.6, 5; 1.0 mL/min; GP	UV, ESI-IT-MS	-	Protein mapping
[12]	Two-position 10-port valve equipped with two C18 trapping columns (5 x 0.3)	SAX 250 x 0.32; 5 µL/min; GP	PSDVB 100 X 0.3 20 µL/min; GP	UV ESI-TOF- MS	200-2,500 *m/z*	Protein mapping
[13]	Two-position 10-port valve equipped with two C18 trapping columns (5 x 1.0)	SAX 150 x 1.0; 50 µL/min; GP	PSDVB 150 x 0.3; 15 µL/min; GP	UV, MALDI-TOF-MS, ESI-TOF- MS, ESI-QTOF- MS	200-2,000 *m/z*, CID energy: 20-60 eV	Protein mapping
[14]	Two 10-port column selectors system with SPE columns	ZIC-HILIC 150 x 0.3, 5; 70 µL/min; IP	PLRP-S C_{18} 150 x 0.3, 3; 5 µL/min; GP	UV, ESI-TOF-MS	200-1,300 *m/z*	Arg-bradykinin and bradykinin in rat muscle tissue
[15]	A multi-channel interface equipped with three-way microsplitter valves used as stop and flow matrix	SCX 70 x 0.32; 5 µL/min; GP	C_8 10 (250 x 0.25); 8 µL/min; GP	UV MALDI-TOF-TOF-MS	700-3,500 *m/z*	Protein and peptide mapping
[16]	Two-position 6-port valve	IEC 50 x 0.5, 5; 12 µL/min; GP	SB- C_{18} 150 x 0.5, 3.5; 20 µL/min; GP	ESI-TOF-MS	350-1,250 *m/z*, scan time: 0.88 s, interscan delay: 0.1 s, MCP at 2700 V	Recombinant proteins tryptic digests

[17]	Two-position 6-port valve	SCX 200 x 2.1, 5; 0.20 mL/min; GP	PSDVB monolithic 60 x 0.10; 0.70 μL/min; GP	UV MALDI-TOF-MS	800-3,000 *m/z*, 200 Hz	Protein mapping
[18]	Switching valve equipped with an RP trap column	SCX 10 x 0.32, 5; 5 μL/min; GP	C_{18} 15 x 0.075, 5; 200 nL/min; GP	ESI-TOF-MS	400-2,000 *m/z*, 1.0 scan/s	Protein mapping
[19]	Two-position 10-port valve	Four serially coupled C_{18} 60 x 2.1, 2.7; 0.1 mL/min; GP	C_{18} 3 x 4.6, 2.7; 4 mL/min; GP	ESI-IT-TOF-MS	200-2,000 *m/z*	α-casein and dephosphorylated α-casein tryptic digests

* IP, isocratic program; GP, gradient program.

[1] Opiteck et al. (1997a); [2] Opiteck, Jorgenson, and Anderegg (1997b); [3] Opiteck et al. (1998); [4] Unger et al. (2000); [5] Wolters, Washburn, and Yates, (2001); [6] Wagner et al. (2002); [7] Liu et al. (2002); [8] Kimura et al. (2004); [9] Machtejevas et al. (2004); [10] Wang et al. (2005); [11] Winther et al. (2005); [12] Pepaj et al. (2006a); [13] Pepaj et al. (2006b); [14] Wilson et al. (2007); [15] Liu and Zhang (2007); [16] Kajdan et al. (2008); [17] Melchior et al. (2010); [18] Zhang et al. (2010); [19] Donato et al. (2011).

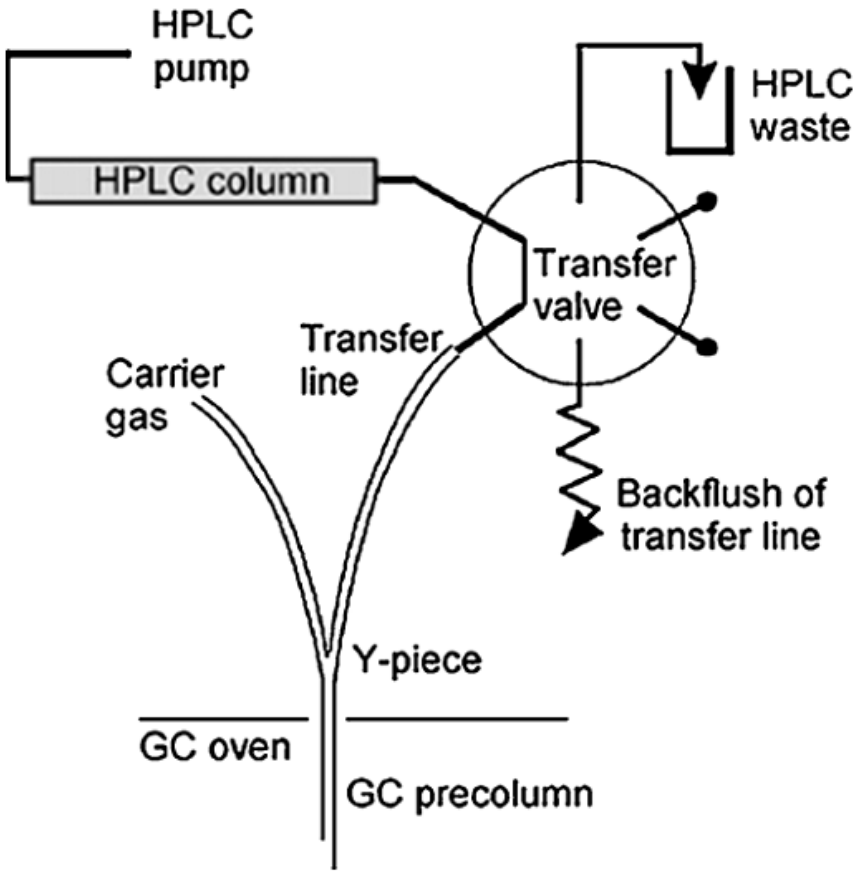
LC-GC

However, if an adequate separation process is carried out before MS detection, data interpretation is much easier and more reliable.

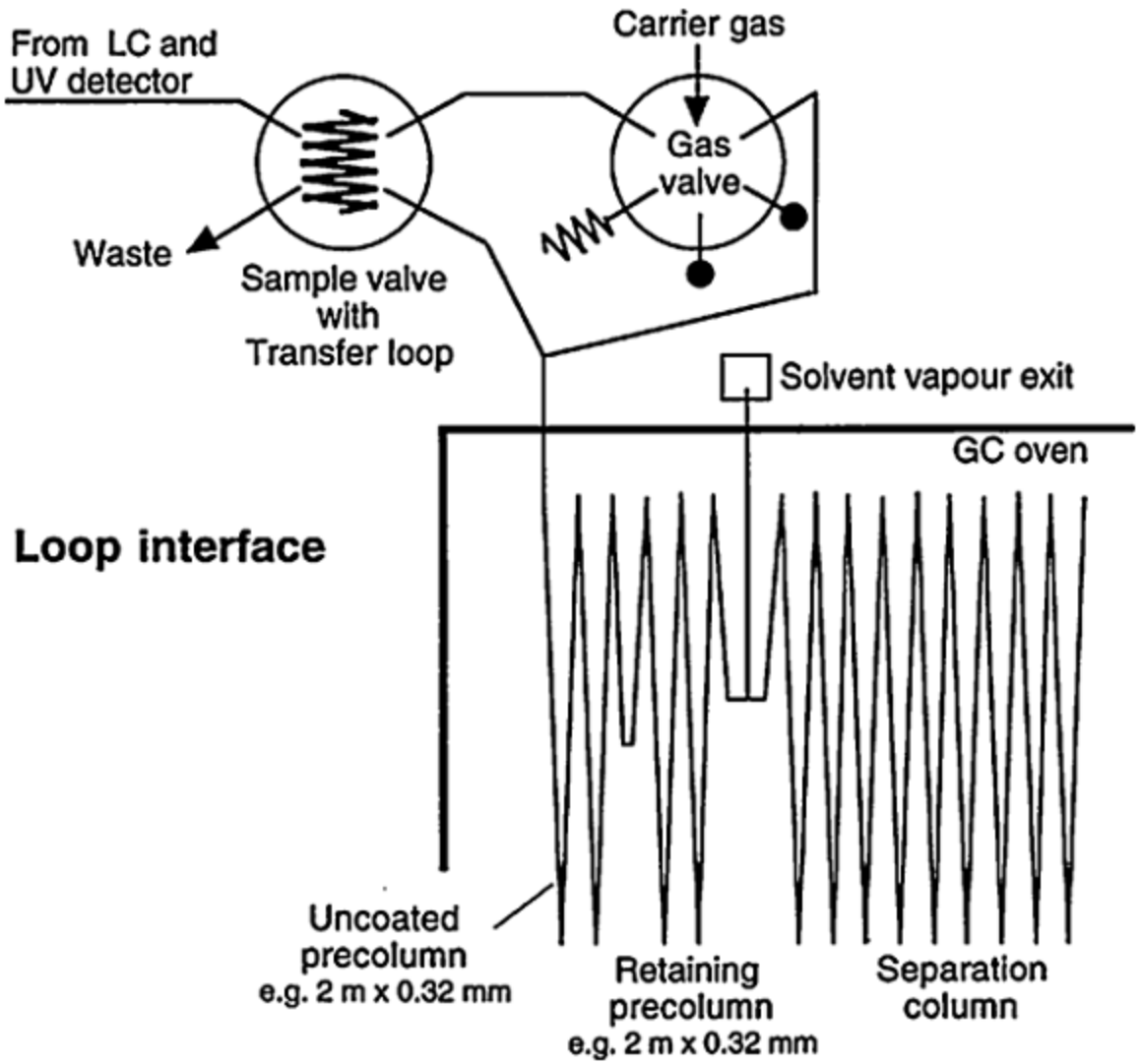
Two approach were perform:

1. The combination of the same form of chromatography in a heart-cutting (LC–LC and GC–GC) or a comprehensive mode (LC × LC and GC × GC), has been extensively studied over the last decades.
2. Using of strength data base of MS and specific data procedure such AMDIS.

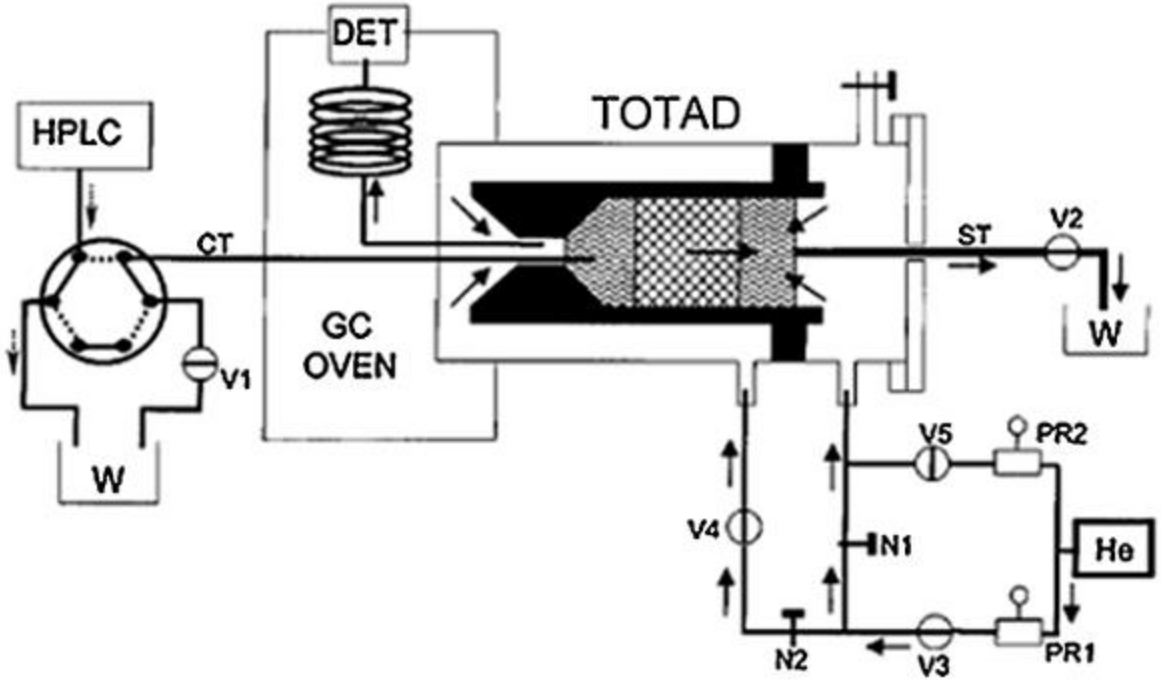
Scheme of the Y-interfac



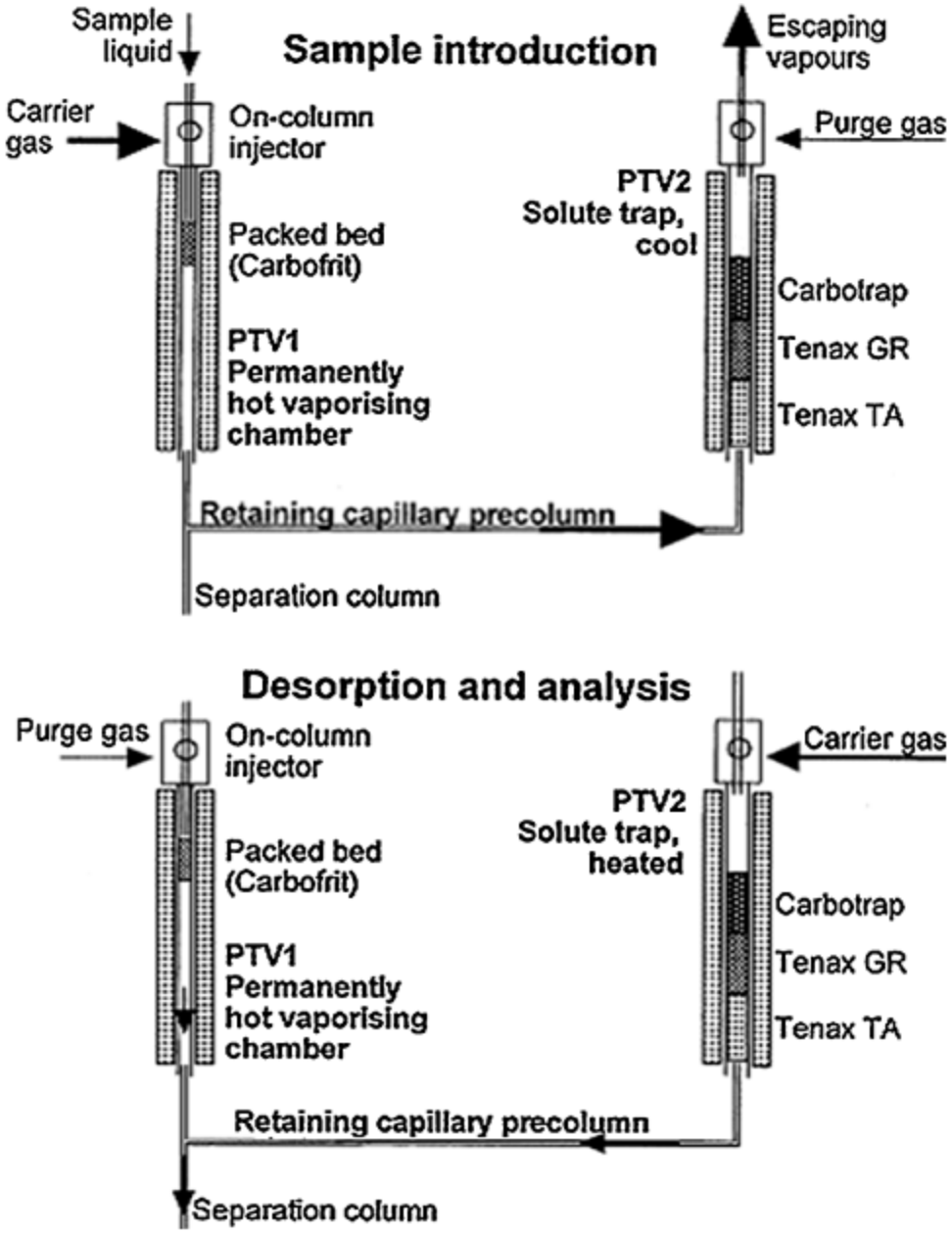
They consist of a switching valve, which directs the LC eluent to the waste or, during the transfer, to a retention gap (sometimes following by a coated pre-column) connected through a Y-union to the GC column and a solvent vapour exit (SVE). The SVE is used To accelerate solvent elimination and to prevent large amounts of solvent vapours from reaching the detector.



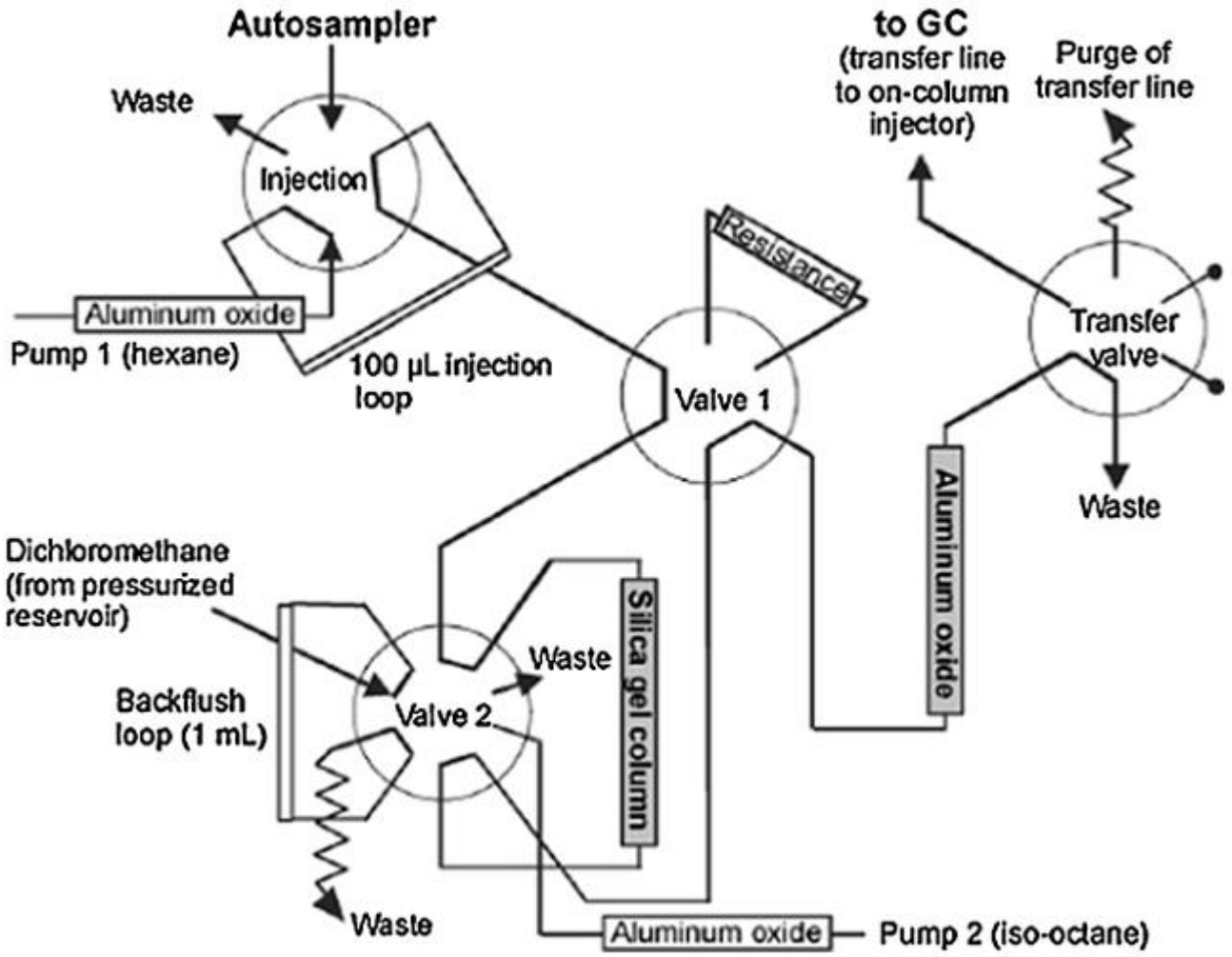
Scheme of the loop-interface



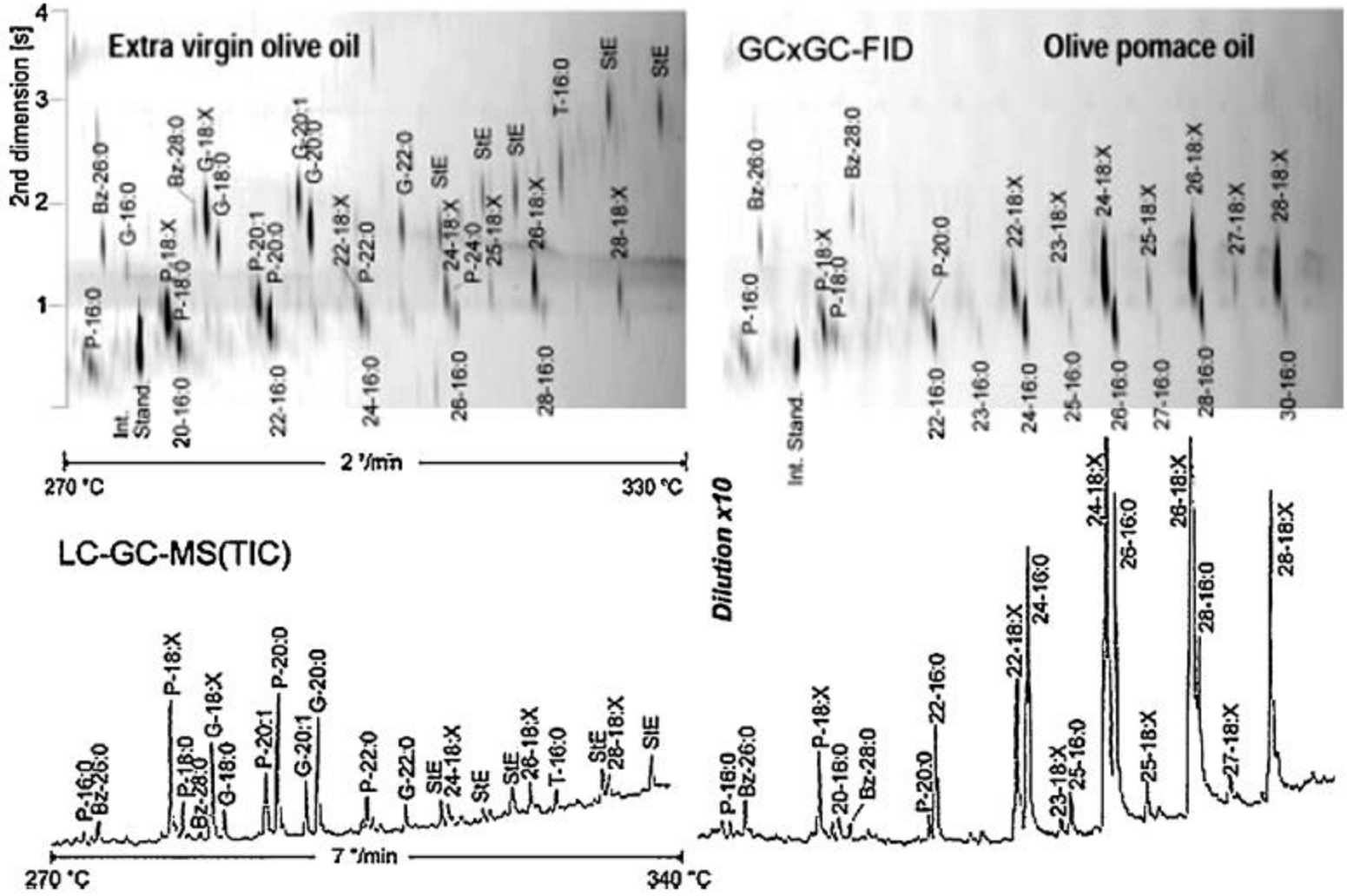
. Scheme of the Through Oven Transfer Adsorption Desorption (TOTAD) inter-



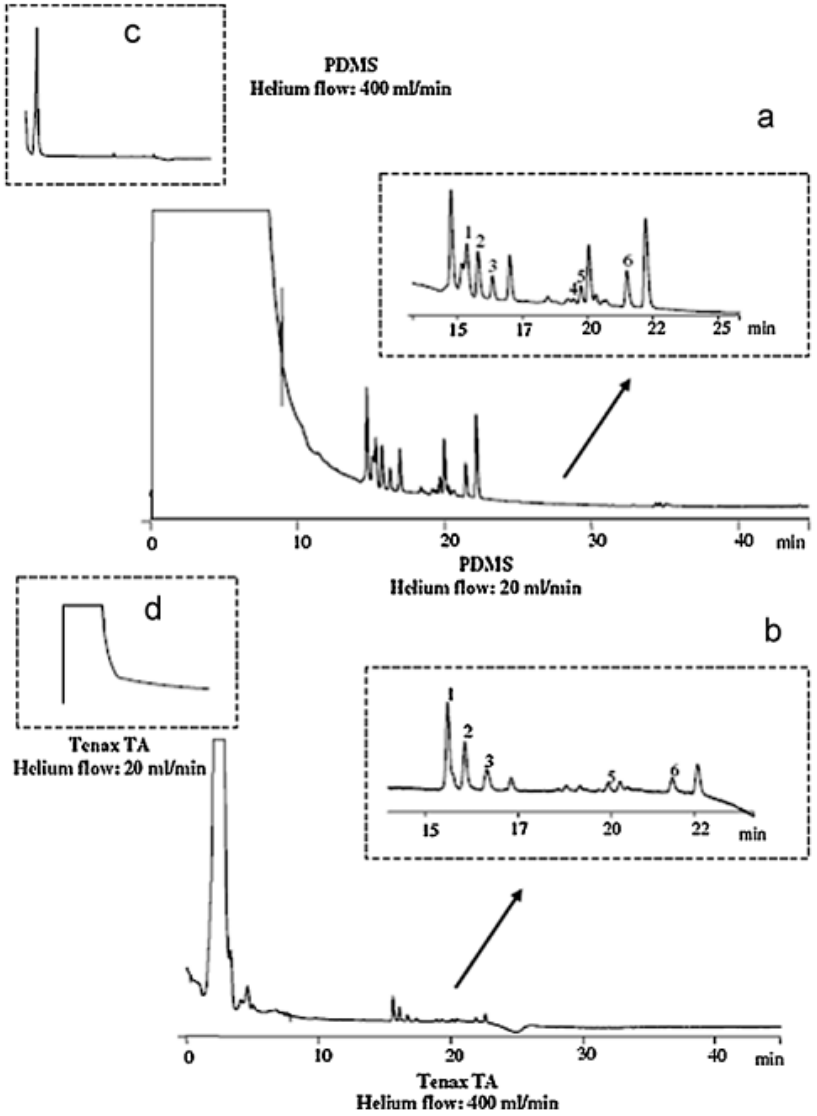
Scheme of the swing system interface in the injection and in the desorption mode.



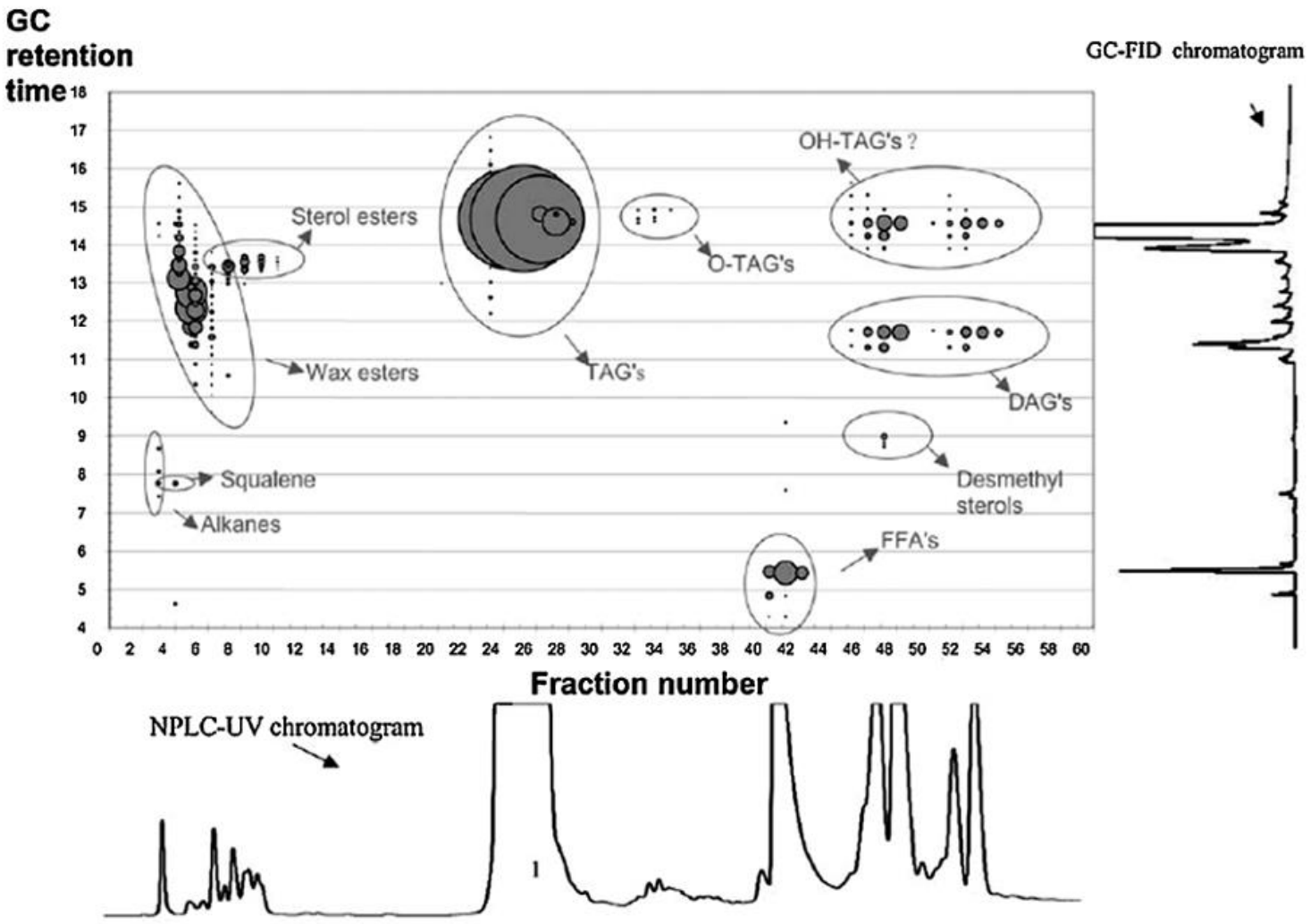
LC system for pre-purification in mineral oil analysis.



. LC–GC–MS chromatogram on the bottom and GC × GC-FID plots on the top of an extravergin olive oil and of an olive pomace oil. Peak identification, G, geranylgeraniol; P, phytol; T, -tocopherol; Bz, benzylalcohol; StE, unidentified esters probably of sterols; fatty acids by number of carbon atoms and double bonds; Int.Stand., internal standard.



Comparison between the chromatograms resulting from the RPLC–GC analysis of orange juice using different PTV-liner packaging material and conditions: (a) PDMS and 20 mL/min helium flow, (b) Tenax TA and 400 mL/min helium flow. Chromatograms in box c and d represent the analysis performed by inverting the helium flow: 400 mL/min with PDMS and 20 mL/min with Tenax, respectively.



Fingerprint contour plot of an olive oil sample obtained by NPLC–GC. On the bottom and on the right side are reported the monodimensional chromatogram obtained by the LC and GC analysis, respectively.