GOOD LABORATORY PRACTICE for HPLC

I- Preparation of solvents

Correct solvent preparation is very important. It can save vast amounts of time spent troubleshooting spurious peaks, baseline noise, etc…

I-1. Quality

All reagents and solvents should be of the highest quality. HPLC grade reagents may cost slightly more than lower grade reagents, but the difference in purity is marked. HPLC grade reagents contain no impurities to produce spurious peaks in a chromatogram baseline.

Ensure that any water used in buffer preparation is of highest purity. Deionized water often contains trace levels of organic compounds and so therefore is not recommended for HPLC use. Ultra pure HPLC water (18MΩ resistivity) is generated by passing deionized water through an ion exchange bed. Modern water purification instruments use this mechanism to produce water of suitable quality in high volumes. Alternately, HPLC grade water can be purchased from solvent suppliers.

I-2. Buffers

All buffers should be prepared freshly on the day required. This practice ensures that the buffer pH is unaffected by prolonged storage and that there is no microbial growth present. Changes in pH and microbial growth will affect chromatography.

Buffer reagents can contain a stabilizing agent, for example, sodium metabisulphite. These stabilizing agents often affect the optical and chromatographic behaviour of buffer solutions, so it is often worth buying reagents that contain no stabilizer. Containers of solid reagent are easily contaminated by repeated use. For this reason, we recommend that reagents be purchased in low container weights.

I-3. Filtration

All HPLC solvents should be filtered through a 0.45 µm filter before use. This removes any particulate matter that may cause blockages.

After filtration, the solvents should be stored in a covered reservoir to prevent contamination with dust, etc…

Filtering HPLC solvents will benefit both your chromatography and HPLC system. Pump plungers, seals and check valves will perform better and lifetimes will be maximized.

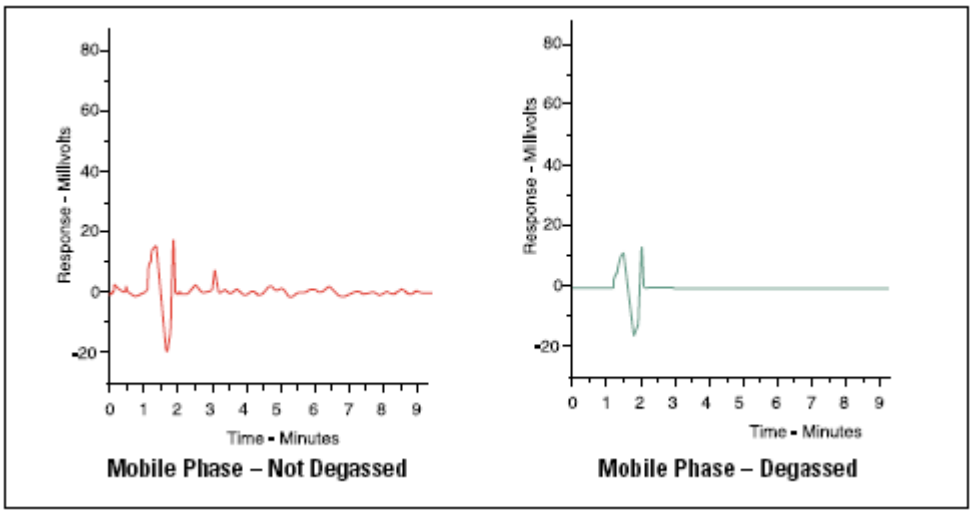
I-4. Degassing

Before the freshly prepare mobile phase is pumped around the HPLC system, it should be thoroughly degassed to remove all dissolved gasses.

Dissolved gas can be removed from solution by

- Bubbling with helium
- Sonication
- Vaccum filtration

If the mobile phase is not degassed, air bubbles can form in the high-pressure system resulting in problems with system instability, spurious baseline peaks, etc.



The most efficient form of degassing is bubbling with helium or another low solubility gas. If this method is available, we recommend that the mobile phase is continually degassed at very low levels throughout the analysis. This will inhibit the re-adsorption of gases over the analysis time.

II- Solvent use

Each solvent line should be fitted with an inlet filter. This is the first line of system defence against particulate contamination from solvents. The filters should be kept clean to prevent cross contamination. When they are not being used, it is recommended that they stored in a solution of 50% acetonitrile / 50% water. This will inhibit microbial growth and stop dust and dirt from embedding in the filter pores.

The solvent lines should be clean, growth-free and should have no sharp bends or creases in them. Solvent reservoirs should be placed as high as possible on or in the instrument – always higher than the pump inlet manifold.

Mobile phase properties:

Do not use highly acidic or basic solvents unless your HPLC system and column have been engineered to accommodate them. Seals, plungers, etc… can be damaged by extreme pH conditions.

The use of highly aqueous mobile phase is becoming more popular as safety guidelines demand less exposure to organic solvents. Care should again be taken that the HPLC column has been engineered to accommodate highly aqueous solvents – traditional alkyl chain media can be prone to phase collapse in low organic composition solvents mixes, for example at less than 5% organic solvent.

Highly aqueous mobile phases are ideal breeding grounds for microbes. Ensure that an organic solvent is flushed through the HPLC system and column at least once every 48 hours to kill unwanted microbial growth. Alternatively add a small amount of sodium azide to the aqueous solvent to inhibit growth.

Note: *Never allow a HPLC column or system to stand with water or buffer in it for an extended period of time. Always flush with a solvent mix that contains a minimum of 20% organic in water.*

III- Changing solvents

III-1. Buffered phase to wash or storage phase

Ensure that the buffer is soluble in the proposed wash or storage phase. Is it is not, first flush the system with a solvent mix that is highly aqueous to remove the buffer from the system and column, then change to the proposed wash or storage solvent mix.

III-2. Normal to reversed phase and Vice versa

Few columns like Hypercarb™ can be used in both normal and reversed phase, then with both solvent types.

To convert a normal phase system/column to a reversed phase system/column, flush with a solvent that is miscible with both the current normal phase solvents and ideally, the proposed reversed phase solvents. If the final reversed phase solvents include a buffer, then it is advisable to move from the 100% methanol flush to a 50% aqueous methanol flush.

For example:

Normal phase	Hexane/Ethyl acetate
Flush	IPA (isopropenyl acetate) then Methanol finally (50:50) Methanol/Water
Reversed phase	Buffered aqueous methanol

To convert a reversed phase system/column to a normal phase system/column, follow a similar path to the one listed previously, but in reverse…

For example:

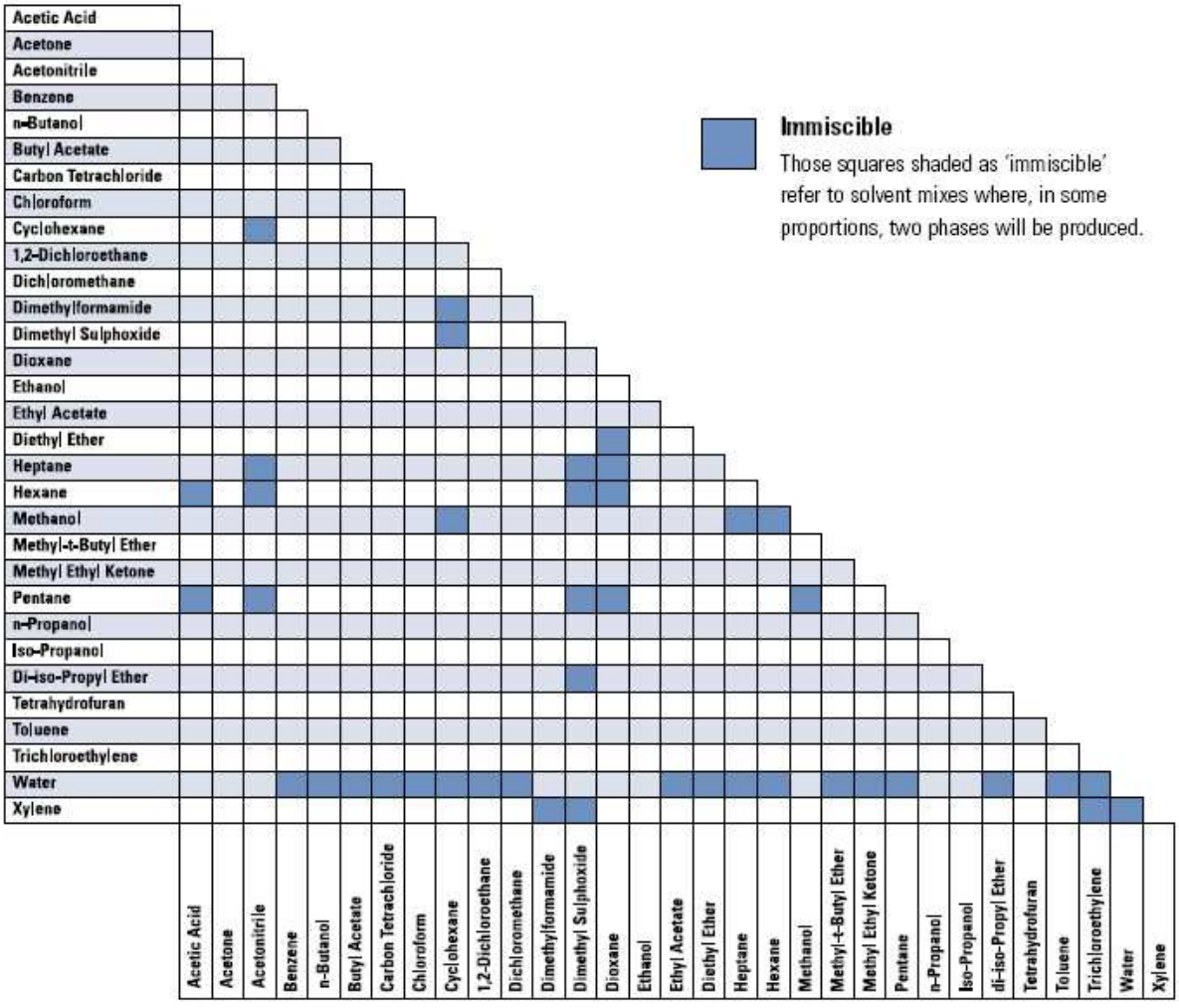
Reversed phase	Buffered aqueous methanol
Flush	(50:50) Methanol/Water
	Methanol then IPA
Normal phase	Hexane/Ethyl acetate

III-3. General

Before attempting any solvent change, ensure that the solvent already in the system and column is compatible with the new solvent.

If the miscibility or physical properties of the two solvents are unknown, then it is better to mix the solvents in a beaker to see the reaction than to go ahead and pump the second solvent into the first on the HPLC instrument mixing problems are easier to rectify before HPLC.

III-4. Solvent miscibility chart



IV- Solvent properties

The following table list a series of commonly used HPLC solvents and their most pertinent physical properties, including viscosity and miscibility number.

The miscibility numbers can be used to predict the miscibility of solvents.

If the smaller miscibility number is subtracted from the larger and the difference is 15 units or less, then the 2 liquids are soluble in all proportions at 15°C.

If the smaller miscibility number is subtracted from the larger and the difference is 16 units, then the 2 liquids have a critical solution temperature between 25 and 75°C with 50°C as the optimum temperature.

If the smaller miscibility number is subtracted from the larger and the difference is 17 or greater, then the 2 liquids are immiscible, or their critical temperature is greater than 75°C.

Solvent	Polarity Index	Viscosity (cp) at 20°C	Boiling Point (°C) at 1 atm	Miscibility Number	Refractive Index	UV Cut Off (nm)
Acetic Acid	6.2	1.26	117.9	14	1.372	230*
Acetone	5.4	0.32	56.3	15, 17	1.359	330
Acetonitrile	6.2	0.37	81.6	11, 17	1.344	190
Benzene	3.0	0.65	80.1	21	1.501	280
Chloroform	3.4	0.57	61.2	19	1.443	245
Cyclohexane	0.0	0.98	80.7	28	1.427	200
Dimethyl Sulphoxide	6.5	2.24	189.0	9	1.477	268
p-Dioxane	4.8	1.54	101.3	17	1.422	215
Ethanol	5.2	1.20	78.3	14	1.361	210
Ethyl Acetate	4.3	0.47	77.1	19	1.370	256
Formamide	7.3	3.76	210.5	3	1.446	≈260
Hexane	0.0	0.31	68.7	29	1.372	195
Methanol	6.6	0.60	64.7	12	1.329	205
Methyl Ethyl Ketone	4.5	0.43	80.0	17	1.381	330
1-Propanol	4.3	2.30	97.2	15	1.380	210
2-Propanol	4.3	2.35	117.7	15	1.380	205
i-Propyl Ether	2.2	0.33	68.3	26	1.368	220
Tetrahydrofuran	4.2	0.55	66.0	17	1.408	230
Toluene	2.3	0.59	101.6	23	1.496	285
Water	9.0	1.00	100.0	–	1.330	190
p-Xylene	2.4	0.70	138.0	24	≈1.50	290

** Value refers to a 1% solution in water*

Solvents have a double miscibility number are immiscible with other solvents at extremes of the lipophilicity scale. The lower of the 2 numbers relates to solvents with high lipophilicity and the second to solvents with low lipophilicity.

Solvents with double miscibility numbers can, in some circumstances, be immiscible with each other.

V- Buffer properties

The following table list a series of commonly used HPLC buffers, their alternative name, where applicable, and their pKa values at 20℃.

Buffer transparency is a variable that should be measured prior to buffer use, as it will vary with salt concentration.

Buffer choice will be very dependent on the analyte and the instrumentation used. Ideally, LC/MS applications should use a volatile buffer as this will not form a containing deposit on the cone and source. Inorganic acids, involatile buffers and ion-pair reagents should all be avoided.

Typical LC/MS buffers include:

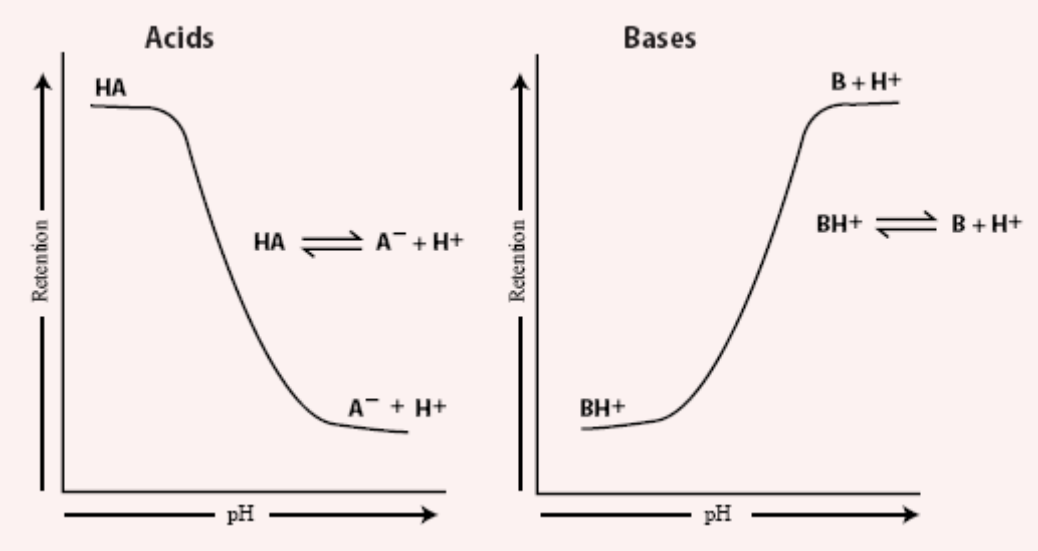
- ammonium acetate/formate/hydrogen carbonate (< 50 nM)
- formic/acetic acid (0.01 – 1% v/v)
- trifluoroacetic acid (< 0.1% v/v)
- trialkylamine and aqueous ammonia type bases
- TRIS (tri-hydroxyméthyl-amino méthane)
- BIS-TRIS propane

Electrolyte additives are often added to LC/MS buffers to improve peak shape. These additives should also be volatile. Care should be taken when choosing a buffer and additive mixture to ensure that a solution of the two does not produce a solid salt which could cause system contamination.

Buffers should always be flushed from the analytical column and instrument after use to avoid salts being deposited on delicate frits, etc…

Buffer	Buffer Range	pK_a at 200C
ACES/N-(2-Acetamido)-2-aminoethanesulfonic Acid	6.4 – 7.4	6.9
Acetamidoglycine/N-(2-Acetamido) Glycine		7.72
Acetic Acid/Ammonium (K- & Na-) Acetate	3.8 – 5.8	4.8
ADA/N-(2-Acetamido)-iminodiacetic Acid	6.4 – 7.4	6.6
Mono- & Di- Ammonium (K- & Na-) Carbonate	5.4 – 7.4 9.3 – 11.3	6.4 10.3
Ammonium Hydroxide (Chloride)/Ammonia	8.2 – 10.2	9.2
BES /N,N-Bis(2-hydroxyethyl)-2-aminoethane-sulfonic Acid	6.6 – 7.6	7.15
Bicine/N,N-Bis(hydroxyethyl) glycine	7.8 – 8.8	8.35
BIS-TRIS Propane/1,3-Bis[tris (hydroxymethyl)methylamino]propane	5.8 – 7.8	6.8
Borate	8.2 – 10.2	9.24
CAPS	9.7 – 11.1	10.40
CHES	9.0 – 10.1	9.55
Cholamine Chloride/(2-Aminoethyl) trimethylammonium Chloride Hydrochloride	–	7.1
Citric Acid/Tri potassium citrate	2.1 – 6.4	3.1 4.7 5.4
Diethylamine Hydrochloride/Diethylamine	9.5 – 11.5	10.5
Formic Acid/Ammonium (K- & Na-) Formate	2.8 – 4.8	3.8
Glycinamide/Glycinamide Hydrochloride	–	8.2
Glycine Hydrochloride/Glycine	8.8 – 10.8	9.8
Glycylglycine	–	8.4
HEPES/N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic Acid	7.0 – 8.0	7.55
HEPPS	7.6 – 8.6	8.00
MES/2-(N-Morpholino)-ethanesulfonic Acid	5.8 – 6.5	6.15
1-Methylpiperidine Hydrochloride/1-Methylpiperidine	9.1 – 11.1	10.1
MOPS	6.5 – 7.9	7.20
Phosphoric Acid; Mono & Di potassium phosphate	<3.1 6.2 – 8.2 11.3 – 13.3	2.1 7.2 12.3
PIPES/Piperazine-N,N'-Bis (2-ethanesulphonic Acid)	6.4 – 7.2	6.8
TAPS/N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic Acid	7.7 – 9.1	8.4
TES/N-Tris(hydroxymethyl)methyl-2-amino-ethanesulfonic Acid	7.0 – 8.0	7.5
Trifluoroacetic Acid	1.5 – 2.5	>>2
Tricine/N-Tris(hydroxymethyl)methylglycine	7.6 – 8.8	8.15
Triethylamine Hydrochloride/Triethylamine	10.0 – 12.0	11.0
TRIS/Tris(hydroxymethyl)aminomethane	7.3 – 9.3	8.3

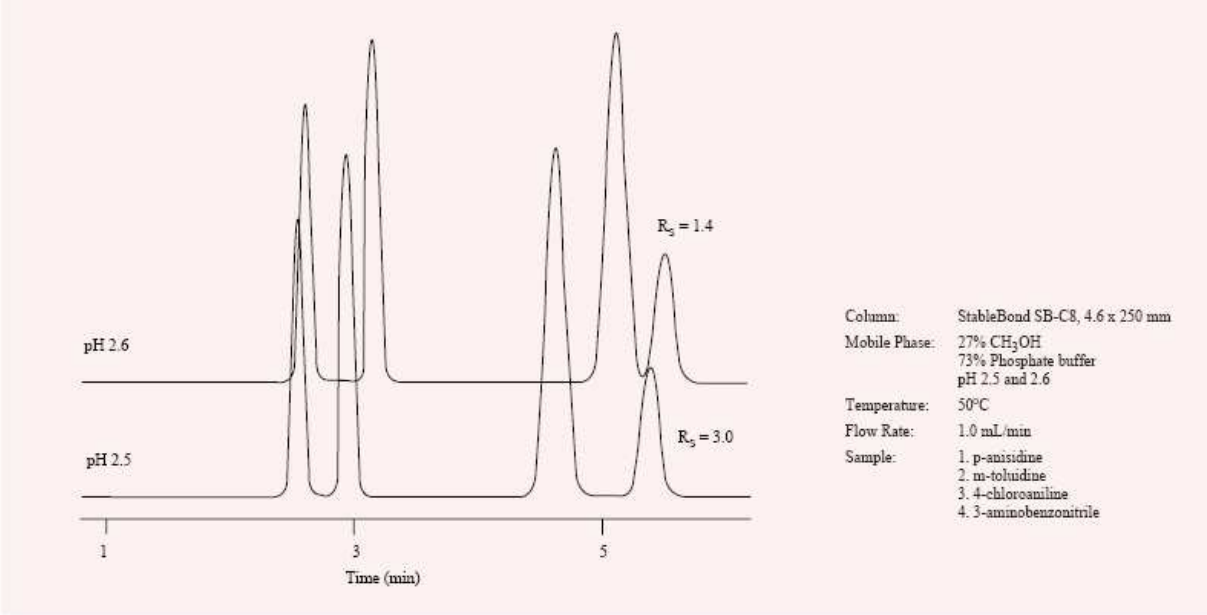
In reversed phase HPLC, the retention of analytes is related to their hydrophobicity. The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic therefore its decreases.



As acids lose a proton and become ionized (with increasing pH), their retention decreases.
As bases gain a proton and become ionized (with decreasing pH), their retention decreases.

When separating mixtures containing acids and/or bases by reversed phase HPLC, it is necessary to control the pH of the mobile phase using an appropriate buffer in order to achieve reproducible results.

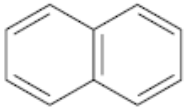
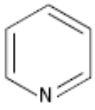
Example



Chromatographic separation where resolution goes from an unacceptable 1.4 to an acceptable 3.0 when the mobile phase pH is decreased by only 0.1 units.

VI- Chromophore detection wavelengths

Chromophores are light absorbing groups. Their behaviour is used to allow the detection of analytes. They have one or more detection wavelengths, each of which has a molar adsorbtivity associated with it. The information contained in the following table is intended as a guide to common chromophores. It is not an exhaustive list.

Chromophore		λmax (nm)	εmax (L/m/cm)
Acetylide	-C≡C-	175 – 180	6000
Aldehyde	-CHO	210	Strong
		280 – 300	11 – 18
Amine	$-NH_2$	195	2800
Azidin	>C=N-	190	5000
Azo	-N=N-	285 – 400	3 – 25
Benzene		184	46,700
		202	6,900
		255	170
Carboxyl	-COOH	200 – 210	50 – 70
Ester	-COOR	205	50
Ether	-O-	185	1000
Ethylene	-C=C-	190	8000
Ketone	>C=O	195	1000
		270 – 285	18 – 30
Napthalene		220	112,000
		275	175
		312	5,600
Nitrate	$-ONO_2$	270	12
	$-(C=C)_2$ acyclic	210 – 230	21,000
	$-(C=C)_3$	260	35,000
	C=C-C=C	219	6,500
	C=C-C=N	220	23,000
	C=C-C=O	210 – 250	10,000 – 20,000
	$C=C-NO_2$	300 – 350	Weak
Nitrile	-C≡N	160	
	-ONO	220 – 230	1000 – 2000
		300 – 400	10
Nitro	$-NO_2$	210	Strong
Nitroso	-N=O	302	100
Oxime	-NOH	190	5000
Pyridine		174	80,000
		195	6,000
		251	1,700
Sulfone	$-SO_2-$	180	
Sulfoxide	>S-O	210	1500
Thioether	-S-	194	4600
		215	1600
Thiol	-SH	195	1400

VII- Column cleaning and regeneration

In all instances, the volume of solvent used is 40-60 column volumes unless otherwise stated.

The column efficiency, capacity factor etc…should be measured at the start and end of the clean-up procedure to ensure that it has been performed successfully and has improved the column performance.

Ensure that no buffers/samples are present on the column and that the solvent used prior to the clean-up is miscible with the first wash solvent.

After the clean-up, ensure that the test mobile phase is compatible with the last solvent in the column.

VII-1. Normal phase media

1. flush with tetrahydrofuran
2. flush with methanol
3. flush with tetrahydrofuran
4. flush with methylene chloride
5. flush with benzene-free *n*-hexane

VII-2. Reversed phase media

1. flush with HPLC grade water; inject 4 aliquots of 200 µL DMSO during this flush
2. flush with methanol
3. flush with chloroform
4. flush with methanol

VII-3. Anion exchange media

1. flush with HPLC grade water
2. flush with methanol
3. flush with chloroform

VII-4. Cation exchange media

1. flush with HPLC grade water; inject 4 aliquots of 200 µL DMSO during this flush
2. flush with tetrahydrofuran

VII-5. Protein size exclusion media

There are two wash/regeneration procedures associated with the removal of contaminants from protein size exclusion media.

Weakly retained proteins

Flush with 30 mL, 0.1M, pH = 3 phosphate buffer

Strongly retained proteins

Flush for 60 minutes using 100% water to 100% acetonitrile gradient.

VII-6. Porous graphitic carbon

There are four wash or regeneration procedures associated with porous graphitic carbon. The one(s) used will depend on the analytes and solvents that have been used with the column.

Acid/Base regeneration
Suitable for ionized species analyzed in strongly aqueous mobile phases.

1. invert the column
2. flush at 1 mL/min with 50 mL tetrahydrofuran/water (1:1) containing 0.1% trifluoroacetic acid
3. flush at 1 mL/min with 50 mL tetrahydrofuran/water (1:1) containing 0.1% triethylamine or sodium hydroxide
4. flush at 1 mL/min with 50 mL tetrahydrofuran/water (1:1) containing 0.1% trifluoroacetic acid
5. flush with methanol/water (95:5) to re-equilibrate
6. re-invert the column

Strong Organic regeneration
Suitable for applications involving polar and/or ionized species analyzed in aqueous mobile phases.

1. flush at 1 mL/min with 50 mL acetone
2. flush at 1 mL/min with 120 mL dibutylether
3. flush at 1 mL/min with 50 mL acetone
4. flush with aqueous mobile phase until equilibrated

Normal Phase regeneration
Suitable for applications running predominantly in normal phase mobile phases.

1. flush at 1 mL/min with 50 mL dichloromethane
2. flush at 1 mL/min with 50 mL methanol
3. flush at 1 mL/min with 50 mL water
4. flush at 1 mL/min with 50 mL 0.1 M hydrochloric acid
5. flush at 1 mL/min with 50 mL water
6. flush at 1 mL/min with 50 mL methanol
7. flush at 1 mL/min with 50 mL dichloromethane
8. flush with mobile phase until equilibrated

Removal of Trifluoroacetic acid
Suitable for applications running mobile phases containing trifluoroacetic acid.

Flush the column with acetonitrile that has been heated to 75℃. the column should also be maintained at this temperature.

VIII- System plumbing and fittings

The purpose of a well-plumbed HPLC system is to minimize dead volume between it’s components and to eliminate leaks.

System tubing errors show themselves in many ways, for example as band broadening, baseline noise, etc… detection of incorrect diameter tubing is often very difficult once it is in-situ.
The internal diameter of tubing used in a HPLC system varies with the position in the instrument, refer to your system maintenance manuals.

The type of tubing used is determined by the application that is being performed. The 2 most common types of tubing are steel and PEEK™, although others are also available. When changing tubing, make sure that the replacement is manufactured from a material that is compatible with any solvents that may be flushed through it.

The first table contains information on the compatibility of a wide range of solvents at 20°C with PEEK™, polyethylene, polypropylene, PVDF, Teflon® and Tefzel®.

The second table contains information on the compatibility of PEEK with solvents at elevated temperature.

Solvent Compatibility with Polymeric Tubing

Solvent	PEEK	Polyethylene	Polypropylene	PVDF	Teflon	Tefzel
Acetaldehyde	1					
Acetic Acid, 20%	1	1	1	1	1	1
Acetic Acid, 80%	1	1	1	2	1	
Acetic Acid, glacial	1	1	1	1	1	1
Acetone	1	2	1	3	1	1
Acetonitrile	1				1	1
Ammonia, 10%	1	2	1	1	1	1
Ammonia, liquid	2					
Ammonium Hydroxide	1	1	1	1	1	1
Aromatic Hydrocarbons	1	2	3			
Benzene	1	2	3	1	1	1
Butanol	1	1	1	1	1	1
Chloroform	1	2	2	1	1	1
Cyclohexane	1	2	3	1	1	1
Cyclohexanone	1	3	3	3	1	1
Diethylamine	1	3	1	3	1	1
Diethylether	1					
Dioxane	1					1
Ethanol	1	2	1		1	1
Ethylacetate	1	2	1	3	1	1
Hexane/Heptane	1	2	2	1	1	1
Hydrochloric Acid, 100%	1		2	1	1	1
Hydrochloric Acid, 20%	1	1	2	1	1	1
Isopropanol	1	1	1		1	
Isopropyl Ether		1	2	3	1	
Ketones, general	1	2	2	2	2	
Methanol	1	1	1	1	1	1
Methyl Dichloride				3	3	
Nitric Acid, 100%	3	2	3	3	1	1
Nitric Acid, 20%	1	2	1	1	1	1
Perchloric Acid	1	2	2	1	1	
Phosphoric Acid, 100%	1	2	1	2	1	1
Phosphoric Acid, 20%	1	1	1	2	1	1
Sodium Hydroxide, 80%	1	2	1	3	1	
Sodium Hydroxide, 20%	1	1	1	1	1	1
Sulphuric Acid, 100%	3	2	2	1	1	1
Sulphuric Acid, 40%	1	1	1	1	1	1
THF	1	2	2	2	1	1
Toluene	1	2	2	1	1	1
Triethylamine			3	1	1	1

Solvent Compatibility with PEEK Tubing at Elevated Temperature

Solvent	Temperature (°C)	PEEK Compatibility
Acetic Acid	200	2
Ammonia, Liquid	200	1
Hydrogen Sulphide, gas	200	1
Methane, gas	200	1
Methylethylketone	200	3
Phosphoric Acid, 50%	200	1
Sodium Hydroxide, 20%	200	1
Sulfuric Acid, 50%	200	2
Sulfur Dioxide, gas	200	1

1 = Compatible, no adverse effect; 2 = Application dependant;3 = Not compatible/recommended.

IX- Band spreading or Band broadening

Broad peaks, often accompanied by a change in retention time, indicate band spreading. It can occur within the HPLC column, but is more often due to incorrect system plumbing. The following procedure describes a method for measuring band spreading due to the HPLC system. Column effects can be measured using efficiency calculations.

1. Remove the HPLC column from the system and replace with a zero dead volume union.
2. Configure the HPLC system with the following parameters:
   Flow rate: 1 mL/min
   Detector sensitivity: 0.5 to 1.0 AUFS
   Detector time constant: 0.2 or less
   Chart speed (if required): 20 cm/min
3. Perform a ten-fold dilution of the column efficiency test solution. Inject 5 µL of this diluted mix.
4. Adjust the detector sensitivity until the peak height approximately 75% of the full-scale readout.
5. Measure the peak width at 4.4% peak height (5-sigma column efficiency method).
6. convert the peak width to mL using the following conversion:
   Band spread (µL) = peak width x (1/20) x 1 x 1000

Where peak width is measured at 4.4% peak height and expressed in cm; (1/20) represents the chart speed, min/cm; 1 represents the flow rate, mL/min and 1000 represents the volume correction factor, µL/min.

Important: 100 mL ± 30 µL is a typical system band spreading value. Larger values may indicate a problem in the detector, injector, tubing or fittings.

Ensure that your HPLC system does not have built-in extra dead volume, as this will also increase the band spreading value.

7. For high values of band spreading, troubleshoot your HPLC system then repeat the determination of band spreading. If the value decreases to acceptable levels, then the problem is resolved. A partial decrease will require further investigation.

If the problem persists, contact your instrument supplier for technical advice.

The table below shows details of the volume of solvent per unit length contained in tubing of varying volumes. For ease of use, both metric and imperial measurements are shown.

Tubing Diameter (mm)	Tubing Volume (µL/cm)	Tubing Diameter (inch)	Tubing Volume (µL/inch)
0.12	0.127	0.005	0.323
0.17	0.249	0.007	0.632
0.25	0.507	0.010	1.288
0.51	2.026	0.020	5.146
1.02	8.103	0.040	20.581

X- Sample preparation

Sample preparation is about more than just the dissolution of s solid in a liquid. Samples may require other techniques such as filtration, extraction or derivatization as well as accurate weighing and /or dissolution.

Samples require **filtration** if they contain suspended solids. This can be performed on-line using a pre-column filter or as the sample is introduced to the vial.

Samples that require **extraction** usually contain the analyte of interest at low levels. The most common forms of extraction are liquid-liquid, solid-liquid and solid phase extraction. The latter of these three is perhaps the quickest and easiest to perform. The question most often asked is when do I perform an extraction? The simple answer is,

- Whenever the matrix, that is the substance that your analyte is contained in, is liable to contaminate your HPLC system or block it with particulate matter or
- Whenever the analyte is at such low levels that pre-concentration is required. Solid phase extraction is not difficult to perform and requires very little specialist equipment.

Sample **derivatization** can occur before or after the column. It can also be performed manually or automatically. It is generally required for detection purposes, for example, the UV detection of analytes without chromophores. There are many different derivatization techniques and care should be taken to choose one that is suitable for your application and that does not produce side products that will cause chromatographic problems later in analysis.

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ADDING LC TO MS

Good Mass Spectrometry will not compensate for Poor Liquid Chromatography.
Don't forget the LC in LC/MS!!!

1. Select appropriate chromatography grade HPLC solvents.

2. Avoid exotic solvent mixes

MeOH, acetonitrile, water, 0.1% formic acid work best for most of LC/MS applications.

3. Dissolve sample in start mobile phase solvent (weakest solvent possible).

4. Other solvents

Isopropanol, can be used for normal and reversed phases

Dichloromethane, chloroform, hexane for normal phase with APLCI as ionisation.

5. Volatile buffers for LC/MS

Buffer	pKa	pH range
Formate	3.8	2.8 – 4.8
Acetate	4.8	3.8 – 5.8
Carbonate	6.4 10.3	5.4 – 7.4 9.3 – 11.3
Ammonia	9.2	8.2 – 10.2
Diethylamine	10.5	9.5 – 11.5
Triethylamine	11	10 -12

- Ammonium salts if possible
- Concentrations: 10mM (up to 50mM for APCI)
  Formic, acetic acids 0.01 – 1% v/v
  Trifluoroacetic acid < 0.1% v/v
  Alkylamine type bases < 0.1% v/v
- Carbonates are unstable

6. Summary

√ Volatile buffers
√ Low buffer concentration
√ High organic-aqueous ration
√ Formic, acetic acids

× Involatile buffers
× Inorganic acids
× High buffer concentration
× High aqueous content
× > 0.1% TFA