 Guest Author – Technical Expert Genevieve Hodson

Liquid Chromatography is reliant upon a accurately made mobile phase. Many chromatographic problems can be attributed to an incorrectly prepared mobile phase. Contamination due to degraded or poor quality mobile phase additives, shifting peaks because of poorly buffered mobile phase and incorrect retention times while trying to replicate Monograph are a few of the problems that can occur. I will discuss the main components and steps to successfully making a mobile phase in this blog post and will close by providing an example buffered mobile phase preparation procedure.

All About Reagents

If you put bad in then you will get bad out. When preparing a mobile phase for LC you need to start with the correct grade of solvent for your system. If using an HPLC this will be HPLC grade solvent like Methanol or Acetonitrile, if using a Mass Spectrometer then you would need to choose an even higher grade. Water will need to come from a bottle with the correct solvent grade or from a MilliQ filtration system. Once a bottle of water is opened, an expiration date of one week should be assigned to ensure enough time does not pass to allow for microbials to form in the open bottle. For MilliQ water sources, ensure that the filters and membranes are being replaced as part of the preventative maintenance as to not allow poor quality of water to enter the LC system.

Any chemicals added should be the correct quality as well. For these it is vital that they not be expired. The degradants from expired additives can be the identity of unknown peaks in the blank chromatogram. Certain additives may go bad quicker than the expiration on the bottle once opened, which should be investigated during troubleshooting unknown peaks. A high percentage of organic can pull out impurities in a lower grade solvent that can look like sample impurities. Running a blank of your samples matrix will allow these to be taken into consideration. Using the correct grade of solvents will minimize these contaminations and extend the longevity of your LC column.

All About pH

Buffers have the ability to keep the mobile phase at a constant pH even if slightly more acid or base is added to the system. Hydrophobicity is a term used to describe if a compound favors water or organic more. The LogP, or partition coefficient, is a measure of the solubility of a compound in two immiscible solvents, typically octanol and water are used. The LogP of a compound is affected by the pH, in other words, how hydrophobic a compound behaves can depend on the pH it is being run at in the method. A lower or higher pH could change the retention of a compound. If a mobile phase is prepared at a different pH, then the retention time can shift for compounds sensitive to pH.

HPLC Application

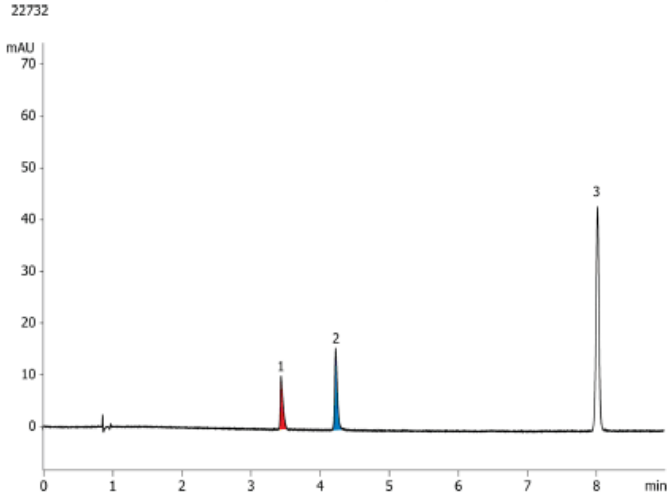
ID No.: **22732**

ABN mix at neutral pH using Kinetex 5u EVO C18 150x4.6mm

Column: Kinetex® 5µm EVO C18 100 Å, LC Column 150 x 4.6 mm, Ea
Dimensions: 150 x 4.6 mm ID
Order No: 00F-4633-E0
Elution Type: Gradient
Eluent A: 20mM Sodium phosphate pH 7
Eluent B: Acetonitrile

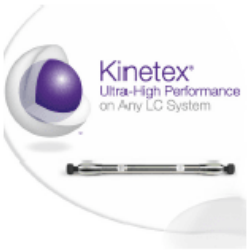
Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	80	20
	2	10	25	75

Flow Rate: 1.5 mL/min
Col. Temp.: 30 °C
Detection: UV-Vis Abs.-Variable Wave.(UV) @ 254 nm (ambient)



ANALYTES:

1. Ibuprofen
   Retention Time: 3.45 min
2. Diphenhydramine
   Retention Time: 4.26 min
3. Ethylbenzene
   Retention Time: 8.12 min



Products used in this application:

KINETEX®

HPLC Application

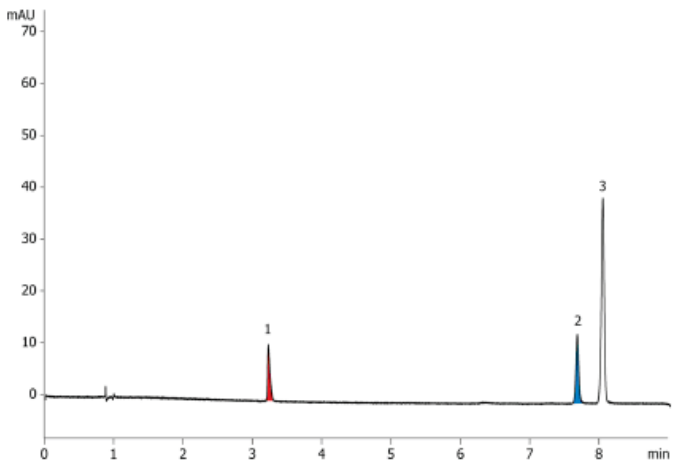
ID No.: **22733**

ABN mix at high pH using Kinetex 5u EVO C18 150x4.6mm

Column:	Kinetex® 5µm EVO C18 100 Å, LC Column 150 x 4.6 mm, Ea			
Dimensions:	150 x 4.6 mm ID			
Order No:	00F-4633-E0			
Elution Type:	Gradient			
Eluent A:	20mM Sodium phosphate pH 10			
Eluent B:	Acetonitrile			
Gradient Profile:	**Step No.**	**Time (min)**	**Pct A**	**Pct B**
	1	0	80	20
	2	10	25	75
Flow Rate:	1.5 mL/min			
Col. Temp.:	30 °C			
Detection:	UV-Vis Abs.-Variable Wave.(UV) @ 254 nm (ambient)			

Products used in this application:

22733



ANALYTES:

1. Ibuprofen
   Retention Time: 3.21 min
2. Diphenhydramine
   Retention Time: 7.62 min
3. Ethylbenzene
   Retention Time: 8.08 min

The pH that the mobile phase is at can also impact the species of the compound. For ionizable compounds this can mean the difference of a nice single peak or a split peak where two different forms are present at the same time.

Author Note: *a helpful tool I have used in the past is www.chemicalize.com for chemical calculations, search, and name-structure conversion.*

Two common mobile phase additives used in LC buffers are phosphate and acetate. Phosphoric acid has three pKa's 2.15, 7.20 and 12.33. The buffering capacity will be ± 1 pH unit from the pKa of the additive, which means the 3 buffering ranges for phosphate will be 1.15-3.15, 6.20-8.20 and 11.33-13.33. If pHed to 5, for example, a phosphate buffer will lose its buffering capacity and no longer be able to maintain a pH for an ionizable compound.

buffer	pH range	LC-MS compatible
phosphate (pK_1)	1.1 – 3.1	X
phosphate (pK_2)	6.2 – 8.2	X
phosphate (pK_3)	11.3 – 13.3	X
acetate[1]	3.8 – 5.8	YES
citrate (pK_1)	2.1 – 4.1	X
citrate (pK_2)	3.7 – 5.7	X
citrate (pK_3)	4.4 – 6.4	X
trifluoroacetic acid (0.1%)	2.0	YES
phosphoric acid (0.1%)	2.0	X
formic acid (0.1%)	2.7	YES
ammonium formate	2.7 – 4.7	YES
ammonium bicarbonate	6.6 – 8.6	YES
borate	8.3 -10.3	YES

When preparing a buffered or pHed mobile phase, mix additive with water first then pH using counter ion. Examples of the proper counter ions to use for potassium phosphate would be phosphoric acid to lower the pH or potassium hydroxide to raise the pH. It is important not add the organic before pHing, this will cause the pH reading to be incorrect and the mobile phase to be incorrectly prepared. Organic should be added after the proper pH has been reached for the aqueous solution.

All About Filtering

Once the mobile phase is well mixed, the final step in preparation is filtering. A glass vacuum filtration system, like the one pictured below, can be used for this step. Mobile phase filters come in different types of membranes and should be chosen based on the solvents that are being filtered. Regenerated Cellulose (RC) membranes are commonly used for Reverse Phase methods as their hydrophilic nature is good for aqueous solvents. A pore size of at least 0.45 um should be used for HPLC systems, while 0.20 um is considered for UHPLC systems. This step removes any left over particulates from the mobile phase preparation to help keep the system and column from clogging.

Have a question about anything discussed above? Reach out to Genevieve or one of our other technical experts through Live Chat any time on any day with fast response times. Start chatting now: www.phenomenex.com/chat

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