



# Care and Use Notes for Lux AMP Chiral Columns

This guide will help your Lux AMP column live a long and happy life. Please read this before using your Lux AMP column as chiral columns require extra care.

## General Information

### Shipping Solvent

85/15 Acetonitrile/Water (Reversed Phase)

### Equilibration

Please equilibrate Lux AMP column with a minimum of 10 column volumes of mobile phase. A: 5 mM Ammonium Bicarbonate, adjusted to pH 11 with ammonium hydroxide, B: Methanol (40:60).

### Test Certificate

Each column is individually tested before shipment. A test certificate showing the separation parameters for trans-stilbene oxide is enclosed with each column. A test certificate can also be found on our website using column part number and serial number.

### Operating Backpressure

The mobile phase flow rate should be set such that the column backpressure stays below 310 bar (4500 psi).

### Operating Temperatures

With standard co-solvent/modifier (such as alcohol and acetonitrile) the column can be used in the temperature range 0-50°C.

## Column Care, Maintenance, and Storage

### Extending Lifetime and Removal of Contaminants

Ideally, samples must be completely dissolved in the mobile phase modifier and filtered through a syringe filter of approximately 0.45 µm porosity. To remove potential contaminants after extended use of your Lux column, we recommend flushing the column with 100% methanol or with ethanol for 2-3 hours at the appropriate flow rate. Back flush can also be used to clean the column.

### Column Storage

Columns used in high pH conditions should be flushed with 10 column volumes of 85/15 Acetonitrile/Water prior to long term storage. The column can be stored in 85/15 Acetonitrile/Water.

### Trademark

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## Mobile Phase

### Lux 3 µm AMP High pH Mobile Phase FAQ's

1. How much Ammonium Hydroxide do I use to adjust the 5 mM Ammonium Bicarbonate solution to pH 11?

Make up a 1 Liter solution of 5 mM Ammonium Bicarbonate and mix well. Transfer to a larger flask or beaker and follow the procedure below. The solution will easily get to pH 9 with a small amount of concentrated Ammonium Hydroxide. In order to get to pH 11 you should expect to add an amount of concentrated Ammonium Hydroxide around 5-10% of the total volume of your solution. For example if you were to make a 1 Liter solution of 5 mM Ammonium Bicarbonate then expect to add as much as 50-100 mL of concentrated Ammonium Hydroxide.

2. How often should I make new mobile phase? Do I need to make a completely new mobile phase or can I keep adding new mobile phase to the old mobile phase?

Since Ammonium Bicarbonate is a volatile buffer it is best to make new mobile phase every day but we have found that this buffer is stable for as long as 2 days.

It is best practice to make completely new mobile phase every time and not to keep adding the old to the new mobile phase.

3. Will the high amount of ammonium salts (ammonium bicarbonate and ammonium hydroxide) cause issues with my mass spec detector?

No. Ammonium Bicarbonate is a volatile buffer which is appropriate for mass spec detectors. A preliminary lifetime study shows no salt build up on the ion source of the mass spec.

4. Is the pH of the mobile phase stable?

Yes, if this volatile buffer is in a capped HPLC solvent reservoir, the pH will remain stable.

The mobile phase is a buffered solution, a solution that resists changes in pH when acid or alkali is added to it. Buffers typically involve a weak acid or alkali together with one of its salts. There will be changes in pH when the composition of the mobile phase changes; e.g., Ammonium Bicarbonate will volatilize (reducing the amount in solution) and in turn causing the pH to decrease if the reservoir containing the buffer solution is not capped. For this reason fresh buffer should be made daily.

5. More tips and tricks.

Do not filter the mobile phase to degas the solution. Allow an inline degasser to do all the work.

### Mobile Phase Restrictions

Lux AMP is a pH (1-11.5) stable chiral column. Like other Lux columns, any solvent dissolving the polysaccharide derivative (such as tetrahydrofuran, acetone, chlorinated hydrocarbons, ethylacetate, dimethylsulfoxide, dimethylformamide, N-methylformamide, toluene, methylethyl ketone and methyl tert-butyl ether, etc) must be avoided even in trace amounts (e.g. sample solvent).

### Mobile Phase Additives

For the separation of substituted amphetamines we recommend using Ammonium Hydroxide (NH<sub>4</sub>OH) to adjust pH.

### Strategies for Successful Separation

<http://www.phenomenex.com/application/detail/23631>