Your GPS to avoiding 8 roadblocks in vitamin research

The road to vitamin synthesis is as satisfying as it is challenging. But there are certain roadblocks you could do without. In this short guide, we navigate you through the workflow of vitamin R&D by offering you solutions to common challenges. Ready to go along for the ride?

VITAMIN PURIFICATION

YIELD

I am trying to save money during purification of fat-soluble vitamins

When trying to select the most appropriate mobile and stationary phase for your fat-soluble vitamins, perform either Thin Layer Chromatography (TLC) or analytical HPLC before purifying via flash or prep HPLC. These smaller-scale experiments will save you resources. Fat soluble vitamins can be purified via normal (NP) or reversed phase (RP) conditions:

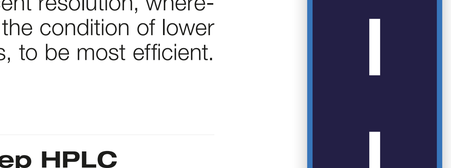
	Normal phase (NP) chromatography	Reversed phase (RP) chromatography
Stationary phase polarity	Polar	Nonpolar
Stationary phase examples	Bare silica	C18 / Amino or Diol
Solvent examples	· Highly non-polar organic solvent, such as hexane · Medium polar solvent, such as ethyl acetate	· Dichloromethane (DCM) · Acetonitrile · Methanol · But no water!

The selection should be based on sample solubility in the solvents and retention on the stationary phase. The compound must be soluble in the starting condition of the run so that it can be easily injected into the system. The compound must also interact with the stationary phase for good retention and a sufficient separation.

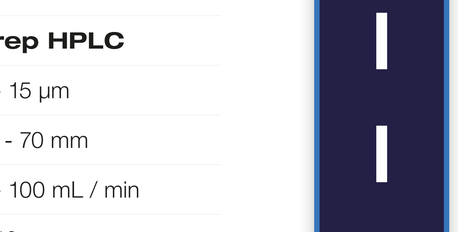
I am getting poor yields when purifying polar vitamins

Since polar vitamins are soluble in water, reversed phase chromatography would be a better choice. However, highly polar compounds typically show no retention and instead run with the solvent front through the cartridge since the stationary phase is too nonpolar for adsorption / retention. In this case, either a less nonpolar stationary phase, such as Diol or Amino, can be used under the same solvent conditions or you could try a different method, namely Hyrophilic Interaction Liquid Chromatography (HILIC). In HILIC, the separation takes place on a polar stationary phase but under reversed phase solvent conditions using water. The mobile phase forms a water-rich layer on the surface of the polar stationary phase vs. the water-deficient mobile phase. In contrast to reversed phase, the gradient elution begins with a low-polarity organic solvent and elutes polar analytes by increasing the polar water content as shown in the figure to the right:

Retention



Elution



VITAMIN CONCENTRATION

5-15

The purity of my vitamin is lower than what I am hoping to achieve

If you are using flash chromatography, try prep HPLC instead. Flash chromatography is mainly used as a pre-purification step to purify large sample quantities at a decent resolution, whereas in prep HPLC the goal is to achieve highest resolution (purity) under the condition of lower loading capacities. A common approach is to combine both techniques, to be most efficient. Differences between the two methods are outlined in the table below:

	Flash chromatography	Prep HPLC
Particle size	15 - 63 µm	5 - 15 µm
Column ID	12 - 115 mm	10 - 70 mm
Flow rate	15 - 250 mL / min	5 - 100 mL / min
Loading Capacity	< 300 g	< 10 g
Maximum pressure	50 bar	300 bar

50

I am trying concentrate fruit juices, but the juice foams when I concentrate it, making this task very tedious

Juices can be concentrated on a Rotavapor®. As this concentration is performed under reduced pressure, the boiling point is lowered, so the heating temperature is also decreased. A heating bath of around 50 °C is sufficient for the concentration. The advantage of the Rotavapor® technology is that it can be upscaled to 20 L and 50 L evaporating flasks. Juices are challenging samples as they tend to foam. A foam sensor on your Rotavapor® will ease the concentration of foaming samples as it will automatically aerate the systeme and break the foam.

My vitamin is degrading during formulation

Vitamins are prone to degradation, hence, it is recommended to remove or avoid the presence oxygen, moisture, and high temperatures to maintain biological properties, such as antioxidant activity. The degradation process is complex and depends on the conditions of vitamin formulation and storage.

My vitamin is light-sensitive and degrades when I try to concentrate it

The standard evaporating flasks are in clear glass which does not protect light sensitive compounds. To prevent this issue, amber evaporating flasks are available to protect the sample from UV light.

VITAMIN FORMULATION

I am losing the desired properties of my vitamin when I use conventional drying

Conventional drying by using heat treatment to remove solvents is popular due to its easy application, low cost and scale-up possibilities. But loss of product and product properties is inevitable. Consider using one of these methods instead:

- **Freeze-drying:** thanks to low process temperatures and an oxygen-free environment during solvent removal, freeze-drying offers the best preservation results. Samples can be dried directly in the final vessel or container and can be sealed with inert gas to avoid oxidative reaction during storage.
- **Spray drying:** this method uses convective drying at elevated temperature. The drying substances samples are further stabilized by addition of stabilizing and protecting agents, such mono- and polysaccharides, polymers. These agents increase stability by infusing the sample in a matrix. The final dry samples are then pre-formulated and gain additional desirable properties such as free flow, defined particle sizes and others.

Freeze drying is expensive. I am afraid I will lose too many resources trying to optimize the process

There are three factors you should consider for optimizing freeze drying right from the get-go. In general, the freezing rate, shelf temperature and pressure are the key factors for a successful drying, where sample property and drying time must be balanced.

- The higher the shelf temperature, the faster the sublimation process and the shorter the drying time. With increasing shelf temperature, the risk of product degradation also rises.
- At low pressure, the oxygen content in the freeze dryer is approximately zero so that the sample integrity is at the maximum. On the downside, the drying process takes more time.

You have now reached your destination: an isolated vitamin whose desired properties are stable and preserved.

www.buchi.com

BÜCHI SWITZERLAND