

# Non-Aqueous Reverse Phase

with RediSep Rf Gold<sup>®</sup> C18

**Chromatography Application Note**  
**AN58**

## Abstract

Non-aqueous reverse-phase is a useful method to purify non-polar compounds allowing resolution of materials that are difficult to purify on silica gel. The method is suitable for long-chain, non-polar compounds such as lycopenes or acyl glycerides. Some symmetric molecules such as crown ethers may also be purified with this method. The purification of acyl glycerides is described.

## Experimental

### RediSep Rf Gold C18

Acyl glycerates, 0.1 mL, from fish oils were run on a 15.5 g RediSep Rf Gold C18 column (part number 69-2203-334). Solvent A was methanol; solvent B was acetone. Acetone was chosen as the B solvent because there is a “hole” in its absorbance allowing detection of the sample components at 205 nm. The standard C18 method was modified as described in the results and discussion.

### RediSep Rf Gold silica, diol, cyano, and amine

A 0.1 mL sample of acyl glycerates was injected on a 12 g RediSep Rf Gold column (20–40  $\mu$ ) column with a standard method with a hexane/acetone gradient. The detection wavelength was 205 nm.

## Results and Discussion

The non-aqueous reverse phase method was able to purify the mixture into three main groups of peaks. Silica gel and the other media failed to purify the mixture. Method development on silica gel for these compounds is difficult because a small amount of strong “B” solvent causes the mixture to move together on a TLC plate.

Non-aqueous reverse-phase can be used with a variety of solvents. Methanol/methylene chloride can be used as can methanol/ethyl acetate. Acetonitrile is a substitute for methanol. The C18 is chemically bonded to the silica so using strong organic solvents will not harm the column and it can be used for 20 to 30 runs. The column should be stored in methanol, acetonitrile, or isopropanol.

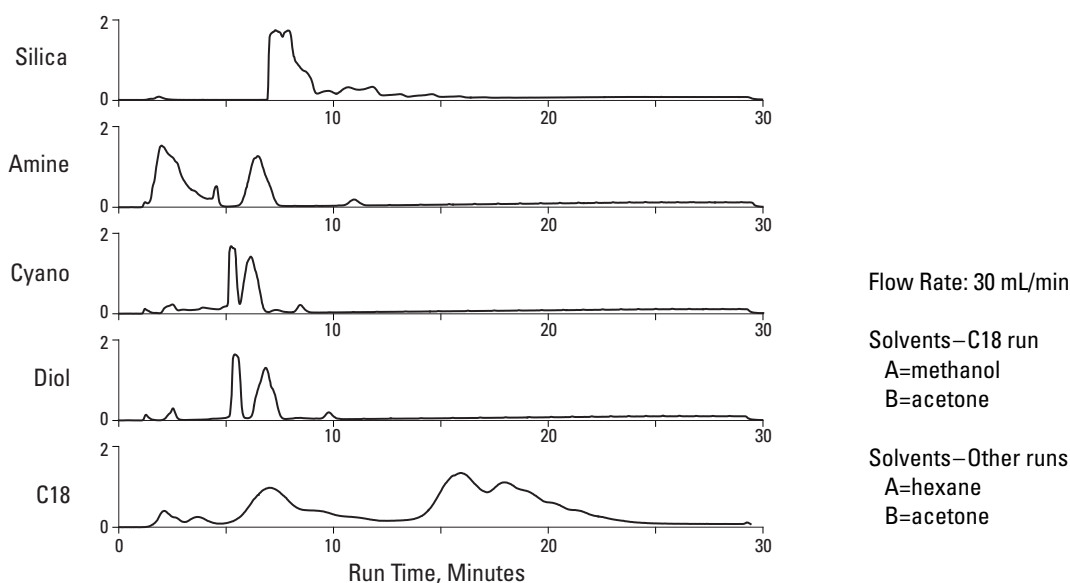
### Changes to the C18 method

The standard C18 method was modified as follows for non-aqueous reverse phase:

- The initial segment was changed to 0% B.
- The gradient was ramped to 100%
- The final segment was changed to 0% B. By returning to 0% B, the column is ready for the next run.

#### Note

The column should be stored in alcohol or acetonitrile which may be used as the starting solvent.



**Figure 1: Acyl glycerate mixture purified** using non-aqueous RediSep Rf Gold C18, diol, cyano, and amine columns

## Use of Solid Load Cartridges

Although these experiments used liquid loading, solid load cartridges filled with C18 media could also be used. When running non-aqueous reverse-phase, the cartridge does not require pre-wetting or “activation.” The compound is loaded in an organic solvent and allowed to dry.

Alternatively, Celite<sup>®</sup> solid load cartridges may be used in the same fashion. The sample is loaded and dried on the cartridge.

## Conclusion

Non aqueous reverse phase is a useful technique when other media work poorly. Non-aqueous reverse phase can be used if the compound elutes slowly (or fails to elute) from a C18 column, even with 100% B solvent.

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