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# Reducing Acetonitrile Usage Using the LaChromUltra® Liquid Chromatography System for Analysis of Fatty Acids

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Chromatography requires eluents that are common solvents to produce results on a fiscally responsible scale. Due to the high cost and low supply of acetonitrile on a global level recently, alternative eluents have been used, as well as other strategies to reduce the need for acetonitrile. Acetonitrile is a popular eluent due to its polarity and its miscibility with water and other common solvents. Many chromatographic methods use acetonitrile, but due to high demand for the solvent, companies have limited their use of acetonitrile to control costs.

LCGC magazine[1,2] has published articles outlining ways to reduce usage of acetonitrile. The most obvious way to reduce solvent use is to shorten the run time by using columns of smaller inner diameter. Reducing the inner diameter of a column from 4.6mm to 3.0mm (and therefore the flow rate of the instrument) can result in over 50% reduction in overall solvent requirements. This approach maintains selectivity, resolution, and analysis time, but reduces the volume of solvent needed. Another approach is to use a shorter column with smaller particles in the stationary phase. While reducing column length might affect resolution, single component analytes are unaffected. A third alternative is to reduce the column length, internal diameter, and particle size–or basically using fast LC. Hitachi's LaChromUltra LC system decreases overall dead volume in the pump, autosampler, and detector flow cell, while speeding up analysis time and slowing the flow rate simultaneously. This can lead to up to 80% reduction in solvent use. A fourth option is to use a different organic solvent with similar characteristics. Methanol, for example, can be substituted in some cases for acetonitrile. This option must be used with caution because unexpected results could occur including: the use of more methanol by volume (due to methanol's higher polarity), the reduction of selectivity or resolution of analytes, switching of retention times of analytes, and violating company policy of method deviation from standardized SOP's. The last of LCGC's recommendations is to recycle the acetonitrile. This option allows for diversion solvent in isocratic methods, using the detector, similar to fraction collection.

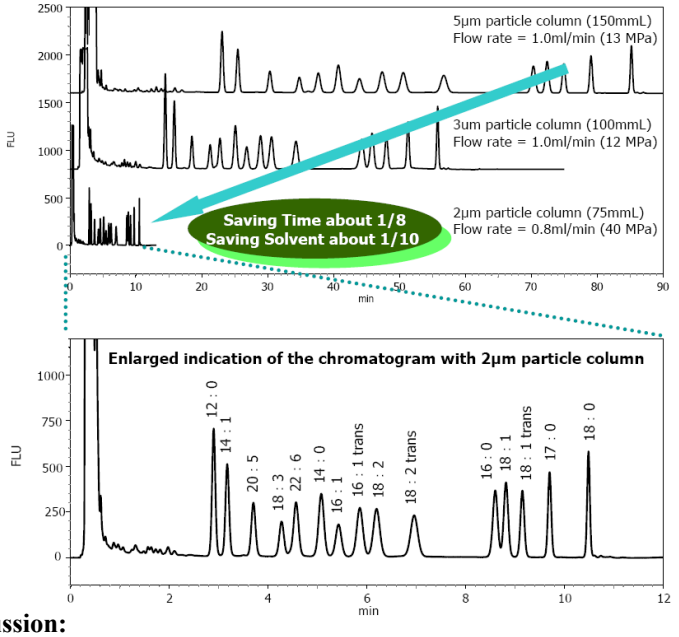
Regardless, acetonitrile is a necessary solvent in the HPLC lab, but one application for fatty acid analysis has overcome the need for massive volumes of acetonitrile. The following is a post-column derivatization for fatty acid analysis that uses 90% less acetonitrile than the standard LaChrom Elite® method, taking advantage of some of the above recommendations[3]. The following fatty acids were derivatized with a post-column reactor, using ADAM (9-Anthryldiazomethane): Dodecanoic Acid (12:0), Myristic Acid (14:0), Myristoleic Acid (14:1), Palmitic Acid (16:0), Plamitoleic Acid (16:1), Palmitelaidic Acid (16:1 trans), Oleic Acid (18:1), Elaidic Acid (18:1 trans), Linoleic Acid (18:2), Linolelaidic Acid (18:2 trans), EPA (20:5), DHA (22:6), Heptadecanoic Acid (17:0), and Stearic Acid (18:0).

Overall, following the above techniques for conservation of acetonitrile (or a combination thereof), laboratory solvent consumable cost can be controlled.

**Chromatographic Conditions:**

| Module | Conditions and Other |
|---|---|
| Pump (Hitachi L-2160U) | A: ACN/MeOH/water (40/50/10)<br>B: ACN/ethyl acetate (90/10); Flow = 0.8 mL/min |
| Column and Oven (Hitachi L-2300) | Hitachi LaChromUltra C-18 75mm x 2.0mm 2µm, maintained at 40°C |
| FL Detector (Hitachi L-2485U) | Excitation: 365 nm; Emission: 412 nm |

**Results:**




**Discussion:**

Hitachi's LaChromUltra Liquid Chromatography System, equipped with a Hitachi C-18 RP 2.0 x 75mm 2-µm particle size column and Sensivate Elite post-column reactor shortens the run time of the analysis, therefore conserving acetonitrile usage. Effective use of fast LC techniques can save both time and acetonitrile usage for methods requiring the use of acetonitrile.

**References:**

1- John Dolan, "LC Troubleshooting", LCGC Magazine. February 2009.
2- Jason Lam (Phenomenex), "Five Ways to Reduce Acetonitrile Consumption", The Peak Magazine, February 2009.
3- Hitachi Application Note, Hitachi-HHT, "LaChromUltra System for Fatty Acid Analysis, Reducing Organic Solvent Usage".

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