Waters Lab Highlights

An Internal Communication of Applications and Techniques

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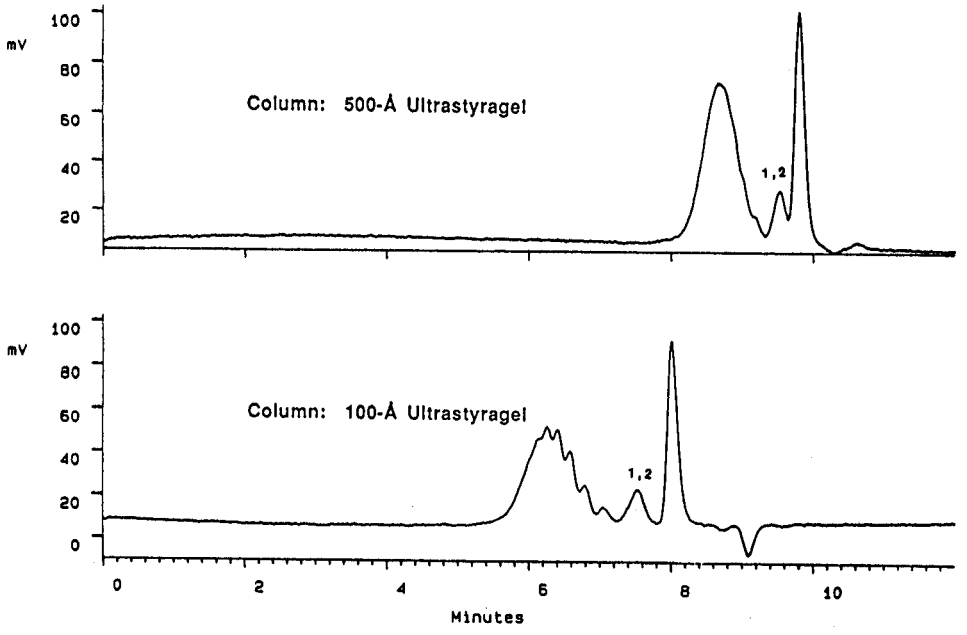
Rapid Analysis of an Antifungal Cream by SMGPC

GPC provides a separation based on the effective size of components in solution. Originally developed as a method for characterizing polymers[1,2], GPC is also useful for separating small molecules from certain complex matrices[3]. For example, many creams and ointments consist of components which represent a wide range of molecular weights. Small molecule GPC (SMGPC) is a method which avoids a time consuming sample cleanup. The only thing to do is dissolve the sample in the eluent, filter the resulting solution and inject. A previous Lab Highlight discussed the analysis of benzocaine from an ointment[4].

A recent application of SMGPC is the analysis of an antifungal cream which contains the active component tolnaftate along with BHT as preservative. The cream also contains monoamylamine and propylene glycol as well as the high molecular weight components (polyethylene glycol and carbomer 934P). The antifungal cream was dissolved in THF and the solution was filtered through a Millex® SR-cartridge and injected.

The columns most useful for SMGPC are the 500Å and 100Å Ultrastyragel® columns. The separations obtained with these columns are shown in Figure 1. The separation was done with THF as eluent, and the chromatograms were monitored with a differential refractometer.

Figure 1: Separation of antifungal cream.
Solvent: THF at 1ml/min
Detector: R401
Column: as noted
Solutes: 1)Tolnaftate, 2)BHT



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The high molecular weight components elute early and are well resolved from components of interest. The separation on the 100Å column offers improved resolution compared to the 500Å column. On the 100Å column, the tolnaftate-containing peak is baseline separated from other components and the high molecular components are even a little resolved. Unfortunately, BHT and tolnaftate still coelute so neither separation is adequate for a quantitative analysis.

In a true GPC mechanism there are not many variables to adjust. However, since GPC separates on the effective size of molecules, a different solvent may enhance the resolution[5] due to eluent-solute interactions such as hydrogen bonding. Therefore, we injected the mixture on a 100Å Ultrastyragel column using methylene chloride as eluent. In Figure 2, this chromatogram is compared to that obtained when THF was used. Note that UV detection at 280nm is used here, unlike Figure 1. Tolnaftate and BHT are well separated when methylene chloride is the eluent.

In summary, SMGPC is a method in which sample preparation is quite simple compared to other methods. Another advantage is that the run time is highly predictable since the analysis is done within one column volume. There are not many variables to adjust, but a solvent effect may alter resolution due to eluent-solute interactions that can change the effective size of the molecule in solution.

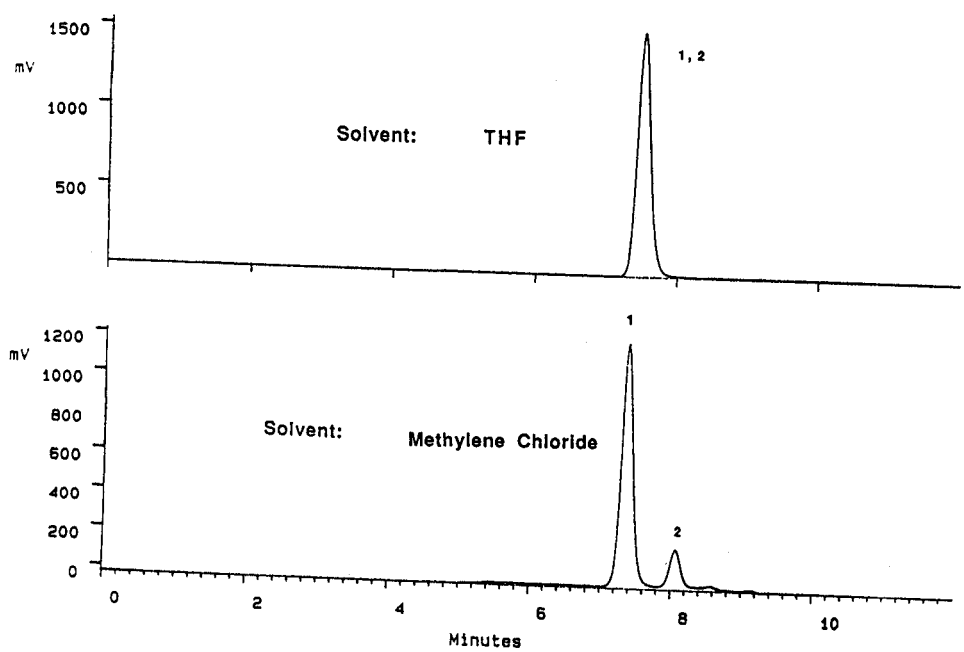

Figure 2: Resolution of Tolnaftate and BHT.
Column: 100Å Ultrastyragel
Detection: UV at 280nm
Solvent: as noted, at 1ml/min

References:

1. LAH 0343 11/87.
2. LAH 0344 11/87.
3. LAH Volume 2, #5
4. LAH Volume 2, #1
5. B.A. Bidlingmeyer and F.V. Warren LC-GC, 6 (1988) 780.

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