

# Three times as fast with double detection

**A flash chromatography system that combines UV detection and Evaporative Light Scattering Detection, detects more and saves a great deal of time. That is the experience of researcher Danny Geerdink of the Department of Bio-organic Chemistry of Groningen University, where this new technology was introduced in the Netherlands.**

Before the arrival of this combined technique, taking separate measurements easily took an hour and a half. That is now done three times as fast. Geerdink: "We were working with standard glass columns filled with silica. After that, we still had to analyze all fractions using thin layer chromatography (TLC) to determine location of compound presence of component(s). Now we can see in *real time* if a compound is present. Because we can skip the TLC step, we usually finish in half an hour. Moreover, the separation is fully automated. You can just work on something else in the meantime."

## Purity

The department where Geerdink is conducting his doctoral research is involved in the synthesis of complex organic molecules. These are often natural substances that must be as pure as possible for the research and development of medicines.

*Researcher Danny Geerdink:*

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Geerdink is working on the synthesis of glyco-lipids that occur in the cell wall of *Mycobacterium tuberculosis*, the pathogen of tuberculosis. These molecules have both a highly hydrophilic part, a disaccharide, and a hydrophobic part with two long fatty acid chains. Geerdink: "The hydrophobic side is on the exterior of the cell wall and forms a strong barrier to antibiotics. That partially explains why these medicines have only a modest effect and tuberculosis patients must undergo relatively long antibiotics treatment. Also, there are indications that the glycolipids have an antigenic effect and therefore stimulate an immune response. For further research, it is essential to obtain them in a pure state. You can start from bacteria cultures, but their extraction and purification is difficult and the yield limited."

### Synthesis steps

Due to their complexity, synthesizing these substances is a fundamental challenge for organic chemistry. "There are many different residues esterified on the disaccharides and the fatty acids have very long functionalized alkyl chains."

## Flash chromatography

'Flash' is a form of column chromatography in which the solvent is forced through the column under medium pressure. This pressure is considerably lower than the 200 bar sometimes used with HPLC. The Reveleris® system has a maximum pressure of 200 psi (13.8 bar). The Groningen chemists actually work at far lower pressures. Depending on the speed and viscosity of the solvent desired, the pressure in their columns is between 20 and 60 psi.

The synthesis therefore includes a large number of steps. "Before you can combine them into an end product, you have to synthesize various building blocks. The combination of both hydrophobic and hydrophilic components makes this synthesis more complicated. Besides that, we do not know where the fatty acids are esterified to the disaccharide."

### Optimization

The Groningen researchers have not had one day of regret since the arrival last year of the Reveleris® Flash chromatography system from Grace Davison Discovery Sciences. They use this apparatus literally in every step of the synthesis, Geerdink notes. "First, we use it to purify the intermediary products as best as possible. But we also want to know which other substances are in the reaction mixture. This is the only way we can track the progress of the reactions accurately and optimize each separate synthesis step." He emphasizes that using UV detection alone is not sufficient in his department. "Both the sugars and the lipids are not UV active, yet some of the intermediary compounds and impurities are. That is why we need both UV and *Evaporative Light Scattering* (ELS) detection."

### Automatic

A convenient touch screen quickly guides the end users. Relevant variables, such as column type, eluent, gradient and pressure are easy to set. The display shows the peaks for each detector separately. "Besides that, it is also useful to be able to collect only the components (peaks) in the tubes. [The instrument] also remembers which peak is collected in which tube."

Changing columns and loading other solvents is very simple. Various pre-loaded columns are available. These are automatically recognized by the instrument, and relevant parameters are customized by the software. Standard tasks such as equilibrating the columns are done automatically.



Left: the Flash cartridge by Grace (silica 40 micrometer) that can withstand a maximum pressure of 200 psi.

It is also possible to slowly change the solvent gradient during a run. This makes it possible to extract components that adsorb to the column and start eluting by mixing a second solvent. "The equipment is rather flexible to use", Geerdink says. It can be used for all applications of column chromatography where more than one detection method is needed, he said. "And particularly when the mixture to be separated contains both UV active and UV inactive components."

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