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應用手冊

HPLC to UPLC Method Migration Using Acrylate Analysis as a Model

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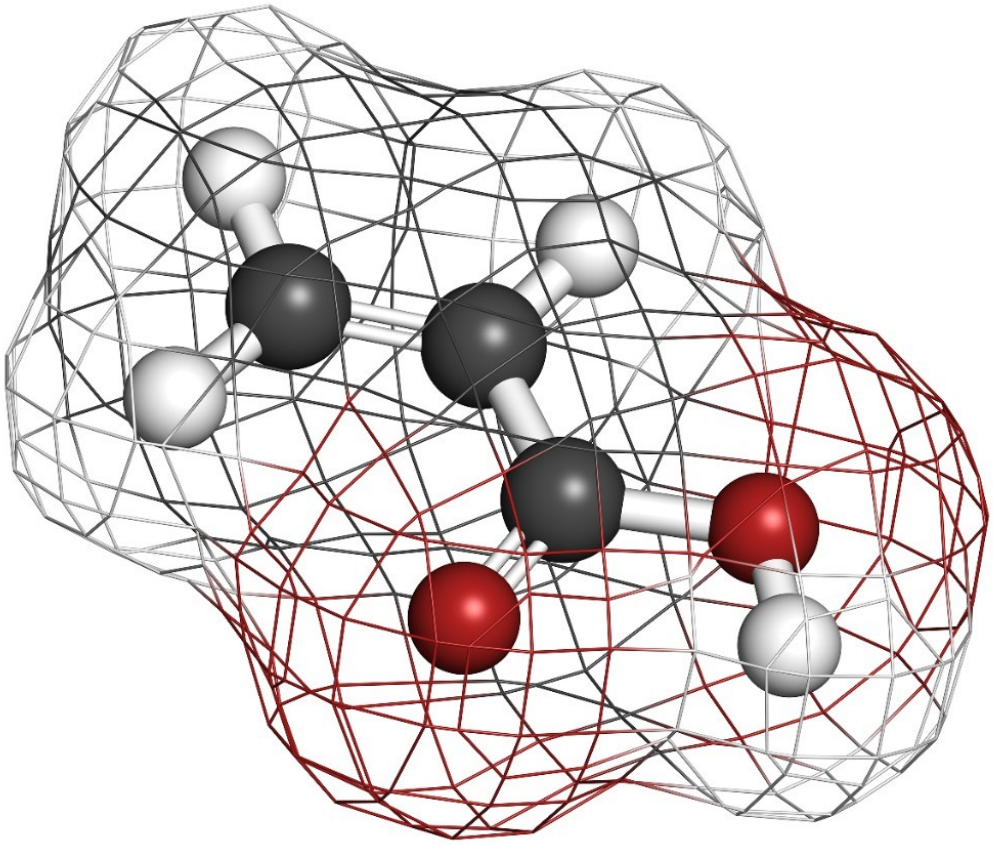


This is an Application Brief and does not contain a detailed Experimental section.

Abstract

For a laboratory running HPLC methods to utilize the power of UPLC, existing methods need to be migrated. This application brief demonstrates the tools that Waters has developed to make HPLC to UPLC method migration a simple and efficient process, saving both time and money for the laboratory.

Benefits

Reduced consumable usage and higher sample throughput, increasing the return on investment for the business.

Introduction

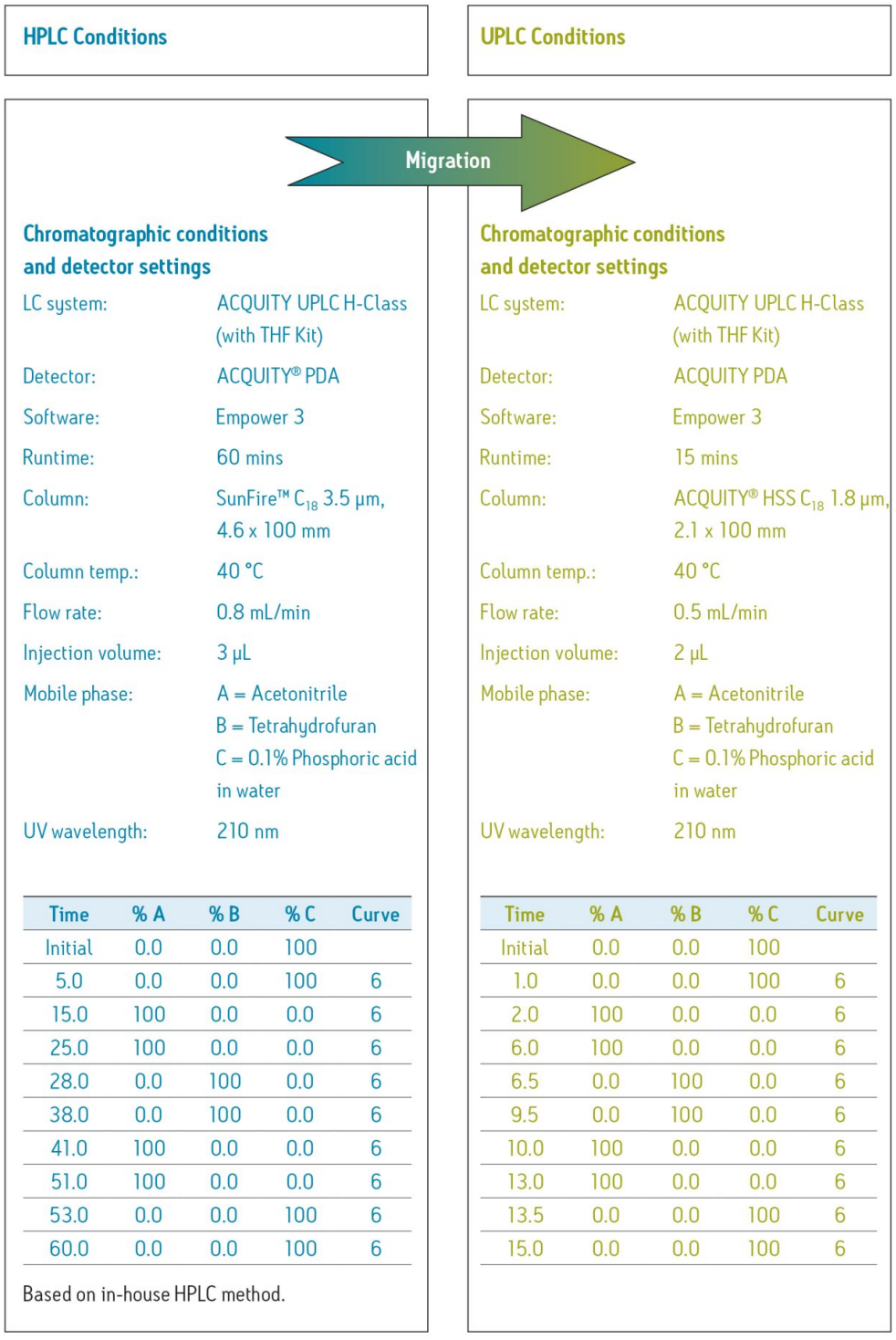
Recent advances in instrumentation and column chemistries have led to the development of UltraPerformance Liquid Chromatography (UPLC). The sub-2-µm particle size and higher pressure capabilities have increased peak capacity, and as a consequence allow shorter run times to be achieved.

Waters ACQUITY UPLC H-Class System has a quaternary pump and flow-through needle, making it an ideal LC system for traditional HPLC users wanting to access the increased productivity and resolution of UPLC. The system has been specifically designed for accelerated method development activities and seamless method transfer.

HPLC analysis of free acrylates has been widely reported. This high level of interest in acrylates is partly due to their diverse use across many industries, including adhesives, coatings, paints, plastics, and textiles.[1]

This application brief uses acrylate analysis as a model to demonstrate the tools Waters has developed to make the method migration as quick and simple as possible. An HPLC method to measure acrylates, developed in-house, has been used to demonstrate an approach to method migration. The benefits of UPLC to a profit and environmentally conscious laboratory have also been highlighted.

Experimental

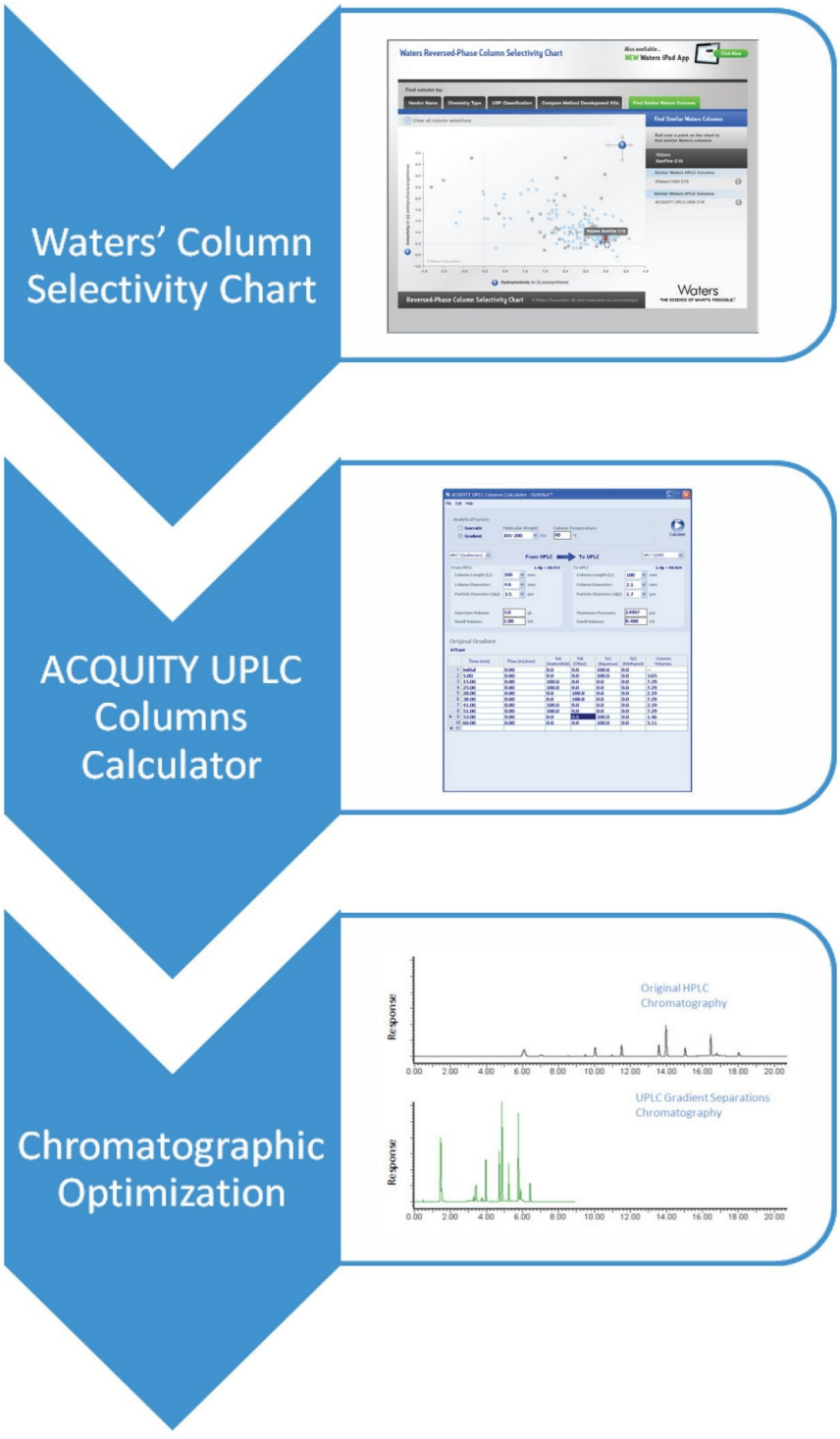


Results and Discussion

Selecting the ACQUITY UPLC column chemistry

The first step in method transfer is selecting the most appropriate UPLC Column for the analysis. ACQUITY UPLC Columns offer a wide range of chemistries with smaller particle size and have higher pressure tolerance, enabling increased resolution and shorter run times to be achieved. Waters' Column Selectivity Chart aids quick and simple column choice, plotting Hydrophobicity versus Selectivity to identify the column required. The program can be found on Waters website at:

http://www.waters.com/waters/promotionDetail.htm?id=10048475



The Column Selectivity Chart can be used for many tasks. Figure 1 shows the steps that are required to find the most appropriate UPLC column when migrating a method.

Step 1 - Select the column vendor from the original HPLC method.

Step 2 - Click on the original column name to highlight the corresponding data point on the chart in red.

Step 3 - Select the tab, Find Similar Waters Columns. The original HPLC column is still highlighted and by placing the mouse over this data point, a list of similar UPLC and HPLC columns are listed on the right side of the screen.

Figure 1. Waters Column Selectivity Chart Software.

Selecting the UPLC conditions

Once the ACQUITY UPLC column has been chosen the next step is to migrate the LC conditions. ACQUITY UPLC Columns Calculator is available with Waters' software and has been developed to aid migrating methods between different systems.

Existing HPLC conditions and some UPLC parameters are entered into the first screen, as shown in Figure 2. Clicking Calculate takes the user to the second window where the UPLC column dimensions and flow rate can be chosen. UPLC conditions, for the selections made, are displayed at the bottom of the page.

The ACQUITY UPLC Columns Calculator provides extra information, such as peak capacity, to help analysts make the best decisions for their analysis. Many different column dimensions and conditions can be quickly and easily compared within the second window.

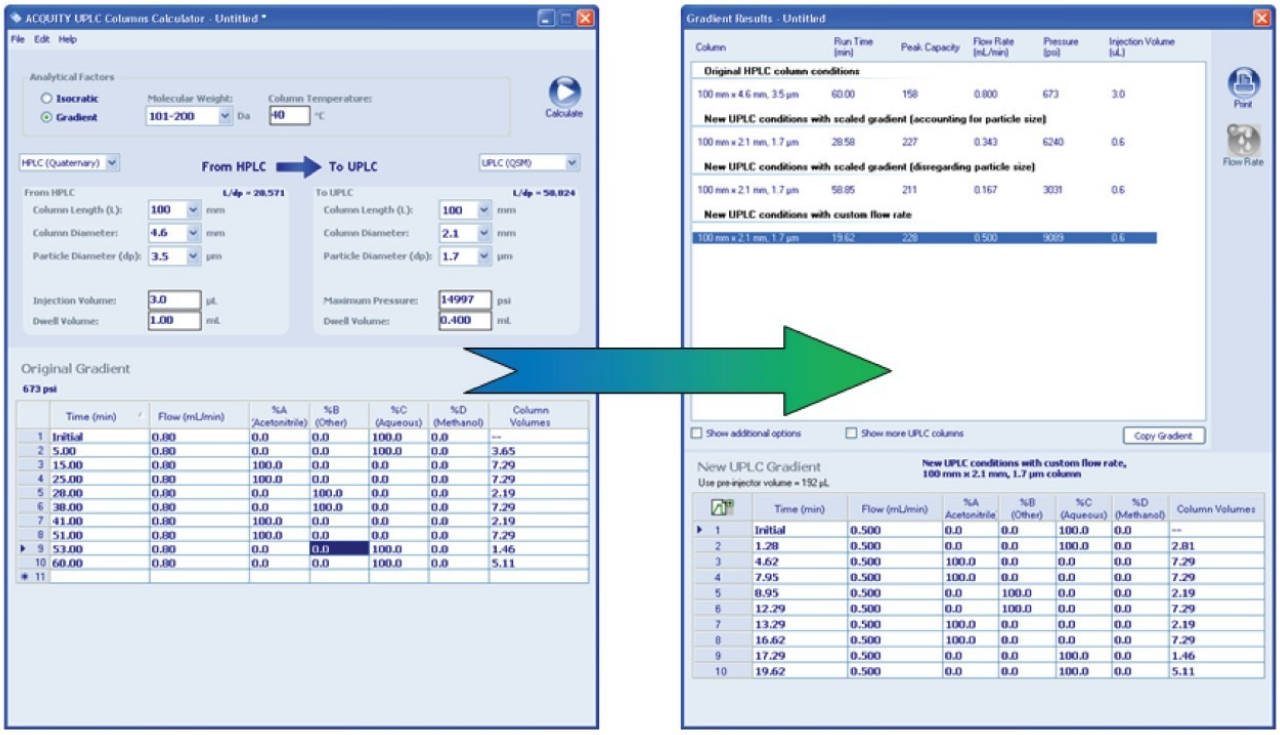


Figure 2. Gradient Separations workflow.

Waters' UPLC systems are compatible with HPLC columns and flow rates. By running the original HPLC and new UPLC conditions on the same system, with the same detector, effects of system dead volume and injection modes are eliminated. Hence a direct comparison is possible.

The Gradient Separations software states that the conditions calculated are a good estimate and some optimization may be required. Figure 3 shows three chromatograms of the same 100 ppm mixed acrylate standard, using the original HPLC conditions, and UPLC conditions, with and without optimization. The elution

order of the nine compounds was consistent in all conditions. The final section of the gradient was 100% THF and is not shown in Figure 3, as it was used to remove the polyacrylates from the column following free acrylate separation and quantification. A significant increase in response was observed.

Optimization was carried out to shorten the total run time without compromising chromatographic separation. A final run time of 15 minutes was achieved, 45 minutes less than the original HPLC method. This reduces the run time to approximately 25% of the HPLC method.

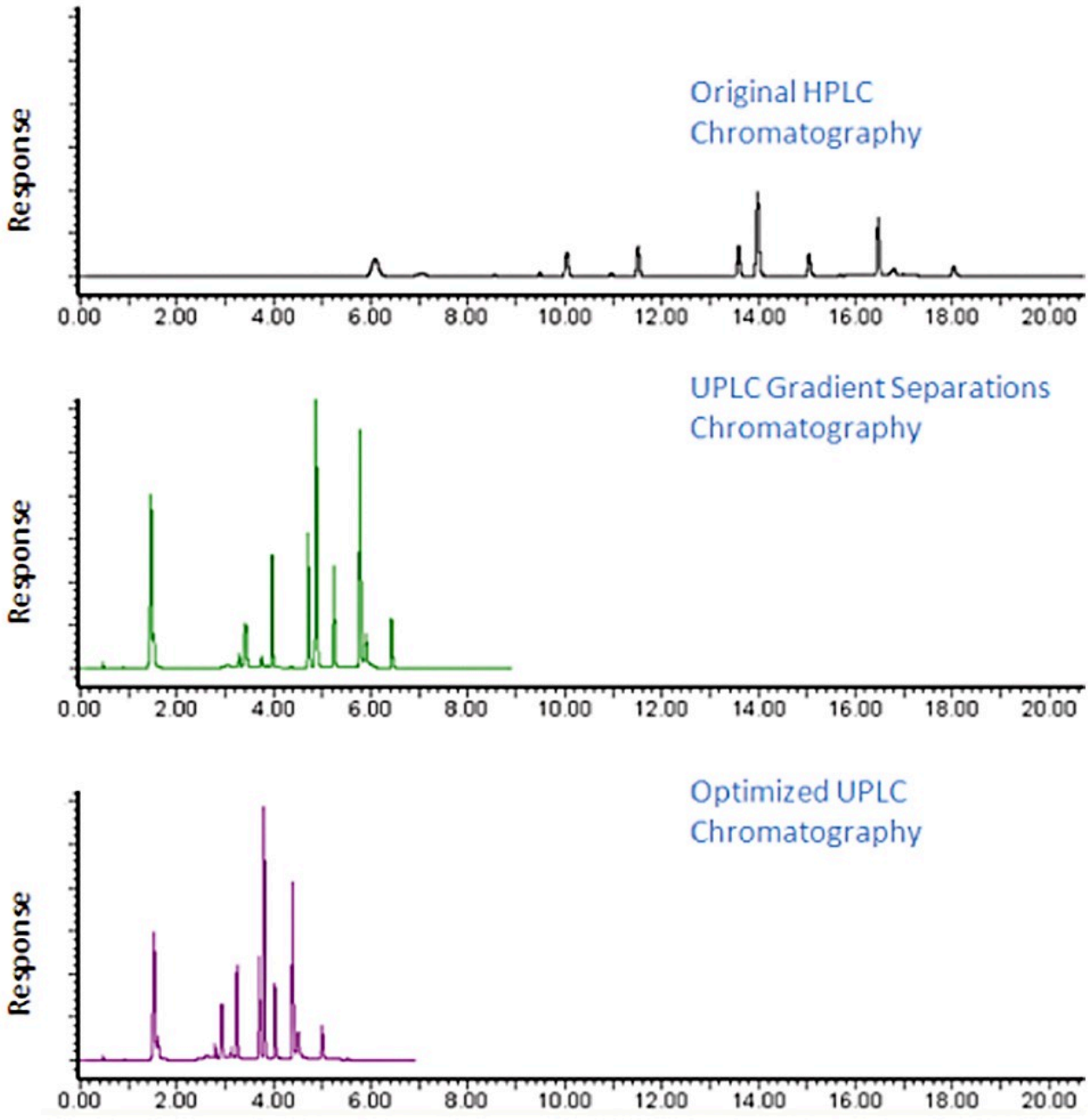


Figure 3. Original HPLC chromatography and two UPLC chromatograms, before and after optimization. 100 ppm mixed standard containing acrylic acid, methyl acrylate, ethyl acrylate, butyl acrylate, toluene, hexyl acrylate, BHT, isodecyl acrylate, and lauryl acrylate.

Significant solvent and time savings have been achieved by migrating the measurement of free acrylates from HPLC to UPLC. These savings can be seen in Table 1.

	HPLC	UPLC	Savings
Flow rate (mL/min)	1.0	0.5	
Run time / sample (mins)	60	15	45
Approximate mobile phase / sample (mL)	60	7.5	52.5

Table 1. Mobile phase usage and run times for UPLC and HPLC.

Figure 4 shows UPLC chromatographic results for a 100 ppm mixed acrylate standard and two sample polyacrylates. The polyacrylate samples were dissolved in THF at a concentration of approximately 5 mg/mL. Toluene and BHT were included in the standard because they were present at low levels in the raw materials used. This allowed identification of the peaks and confirmed there was no co-elution with the peaks on interest.

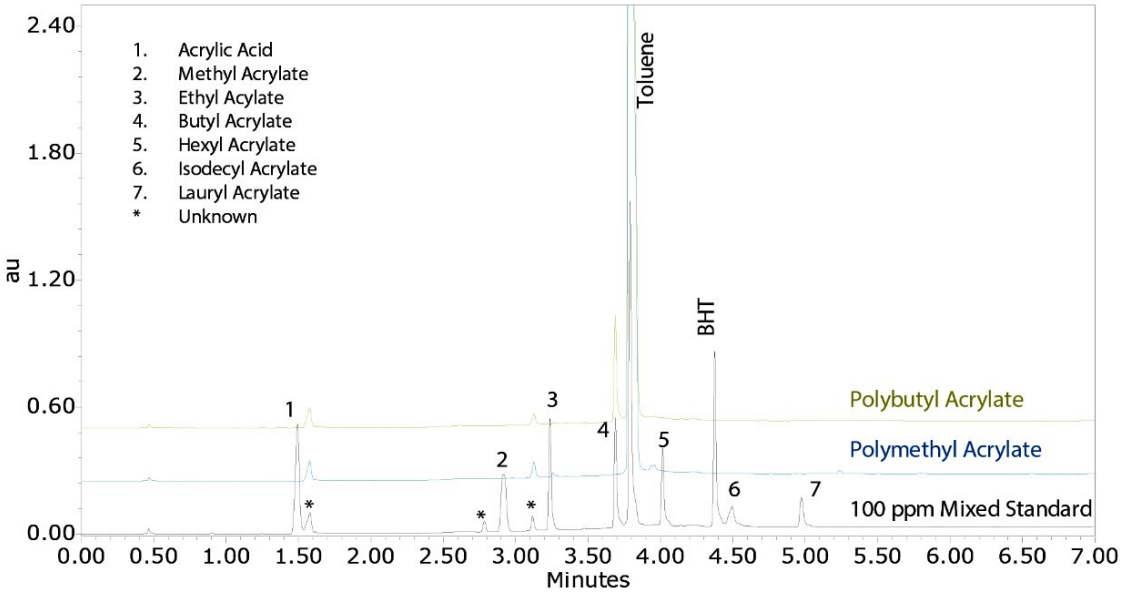


Figure 4. Polyacrylate samples (Polymethyl acrylate and Polybutyl acrylate) and 100 ppm mixed monomer standard.

Conclusion

Waters' UPLC solutions offer many benefits to laboratories carrying out liquid chromatography. The increased peak capacity and higher pressure tolerance allow shorter run times to be achieved, compared with HPLC. This translates into reduced consumable usage and higher sample throughput, increasing the return on investment for the business.

The ACQUITY UPLC H-Class System is an ideal LC system for traditional HPLC users, due to its familiar configuration with quaternary pump and flow-through needle. The system has been specifically designed for accelerated method development activities and seamless method transfer.

For a laboratory running HPLC methods to utilize the power of UPLC, existing methods need to be migrated. This technical note demonstrates the tools that Waters has developed to make HPLC to UPLC method migration a simple and efficient process, saving both time and money for the laboratory.

References

1. http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0487/0901b80380487c6c.pdf?filepath=productsafety/pdfs/n
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