

ULTRA PERFORMANCE LC[®] FOR ONLINE MONITORING OF A CONTINUOUS PROCESS

Waters
THE SCIENCE OF WHAT'S POSSIBLE.[®]

Aaron D. Phoebe¹, Robert J. Tinder² and Charles H. Phoebe, Jr.¹

¹ Waters Corporation, 34 Maple Street, Milford, MA 01757 and ² Proteaf, 904 Silver Spur Road, Rolling Hills Estates, CA 90274

INTRODUCTION

In 2004 Waters introduced Ultra Performance LC (UPLC) technology into the market place. It was quickly realized that this technology provided real-time analytical speed that also provided enhanced resolution, selectivity, specificity, and dynamic range. These abilities are the foundation for the use of liquid chromatography as a sensor in Process Analytical Technology (PAT) replacing the slower HPLC technology. However, the recognized speed of UPLC analysis was now limited by the ability to prep and introduce samples into the chromatographic system. In order to overcome the sample introduction limitation, Waters introduced a family of UPLC Process Analysis Systems that included the new Process Sample Manager (PSM) with unique attributes for process development, scale-up, and manufacturing. These attributes include:

1. Online sample introduction from a process stream
2. Programmable dilution of standards and samples
3. Atline sample introduction with a barcode directed operation
4. Ability to interface with Distributed Control System (DCS)

Using the first three attributes described above, we have successfully used UPLC technology to investigate a design of experiments (DoE) for the two-step synthesis of 5-amino-benzyl-1H-1,2,3-triazole-4-carboxamide (referred to as the 1,2,3-triazole) by a continuous process and the monitoring of low level potential genotoxic impurities (i.e benzyl bromide and benzyl azide).

METHODS

PATROL™ UPLC[®] Pilot Process Analysis System

- ACQUITY UPLC Quaternary Solvent Manager
- ACQUITY UPLC Process Sample Manager
- ACQUITY UPLC Column Manager (CM-A)
- ACQUITY UPLC PDA eλ
- Empower 3 Chromatographic Data Software (CDS)

Reactor Instrumentation

- Chemtrix KiloFlow[®]
- Waters 515 HPLC pumps (4)
- VWR circulating water bath

UPLC Conditions

- Column: ACQUITY UPLC[®] BEH C18 1.7μm, 2.1x50mm
- Column Temperature: 40°C
- Eluents: A water, B acetonitrile, D 2% formic acid in water
- Flow Rate: 1 mL/min
- Gradient: (A/B/D) 92.5/2.5/5.0 initial to 0/95/5 over 2.51 minutes, return to initial conditions at 2.51 minutes

Triazole Synthesis Chemistry

- Sodium Azide: 0.5 M dissolved in Water/1-methyl-2-pyrrolidinone (NMP) (20/80)
- Benzyl Bromide: 0.5 M dissolved in NMP
- Sodium Hydroxide: 1.0, 0.75, and 0.5 M dissolved in Water
- Cyanoacetamide: 1.0, 0.75, and 0.5 M dissolved in NMP
- Flow rates of all four reagents at 1 mL/min
- Reaction Temperatures: 70, 80, and 90°C

DISCUSSION

The difference in the time of analysis between UPLC and HPLC is illustrated in Figure 1. The synthetic pathway is shown in Figure 2 and the instrumentation used in this study is shown in Figure 3 and 4. The synthesis started by the formation of benzyl azide from the reaction of benzyl bromide with sodium azide. The benzyl azide was then immediately reacted with a combination of sodium hydroxide and cyanoacetamide to form the 1,2,3-triazole. To fully understand the reaction, two different sampling lines were plumbed into the reactor (Figure 5). This allowed for the monitoring of each step of the reaction as well as the tracking of the low levels of the potential genotoxic impurities. (Figures 6 and 8.)

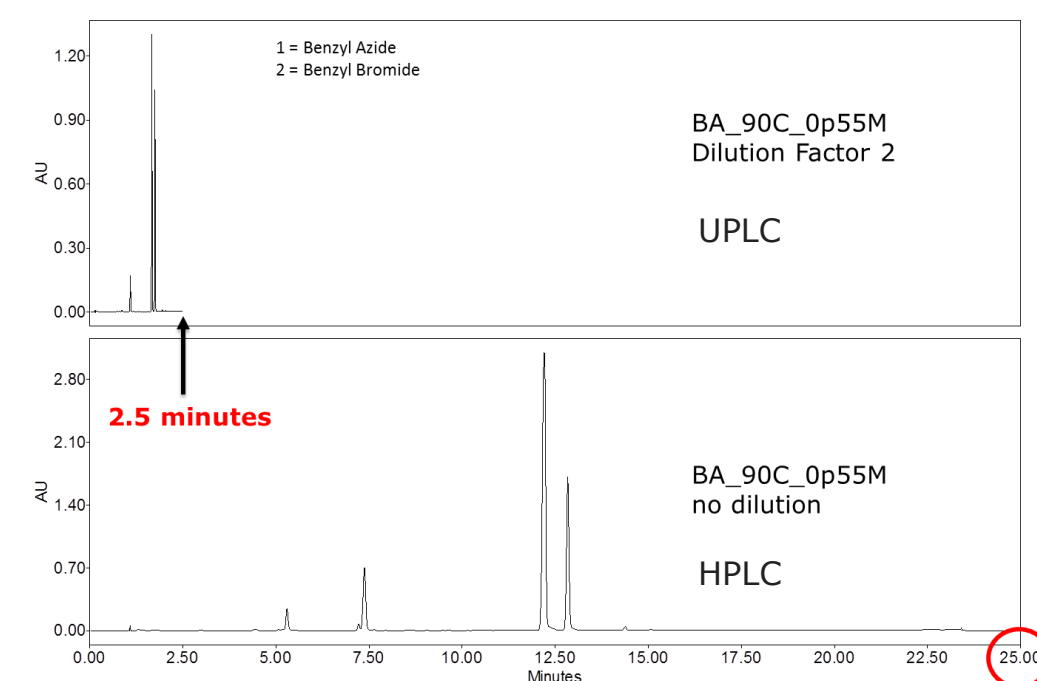


Figure 1. Comparison of the speed of analysis using UPLC versus standard HPLC

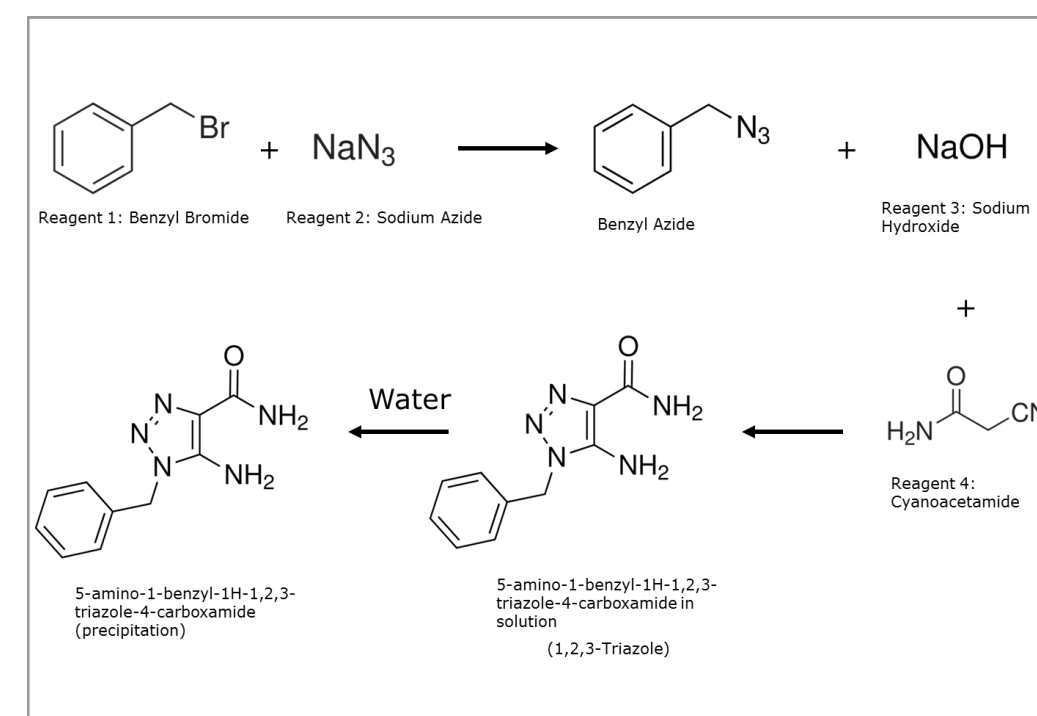


Figure 2. Reaction sequence used for the Design of Experiments (DoE) for the synthesis of the 1,2,3-triazole



UPLC Detector

UPLC Column Manager

UPLC Process Sample Manager

UPLC Pump

Figure 3. System components of a PATROL UPLC Pilot Process Analysis System on the PATROL Pilot cart

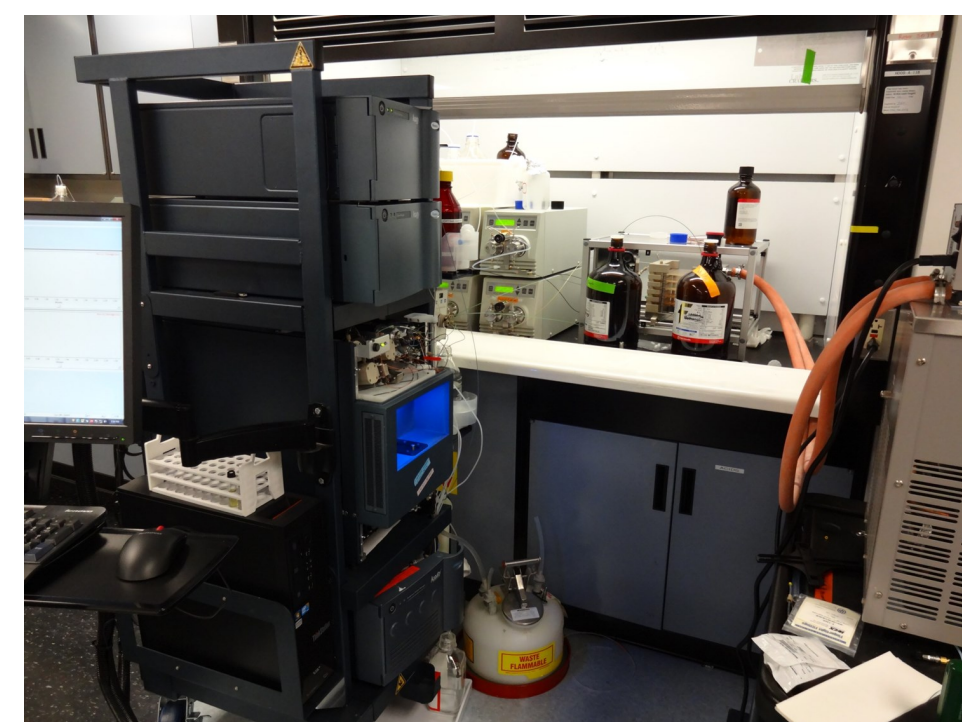


Figure 4. System components used for the UPLC analysis and the Design of Experiments for the synthesis of the 1,2,3-triazole

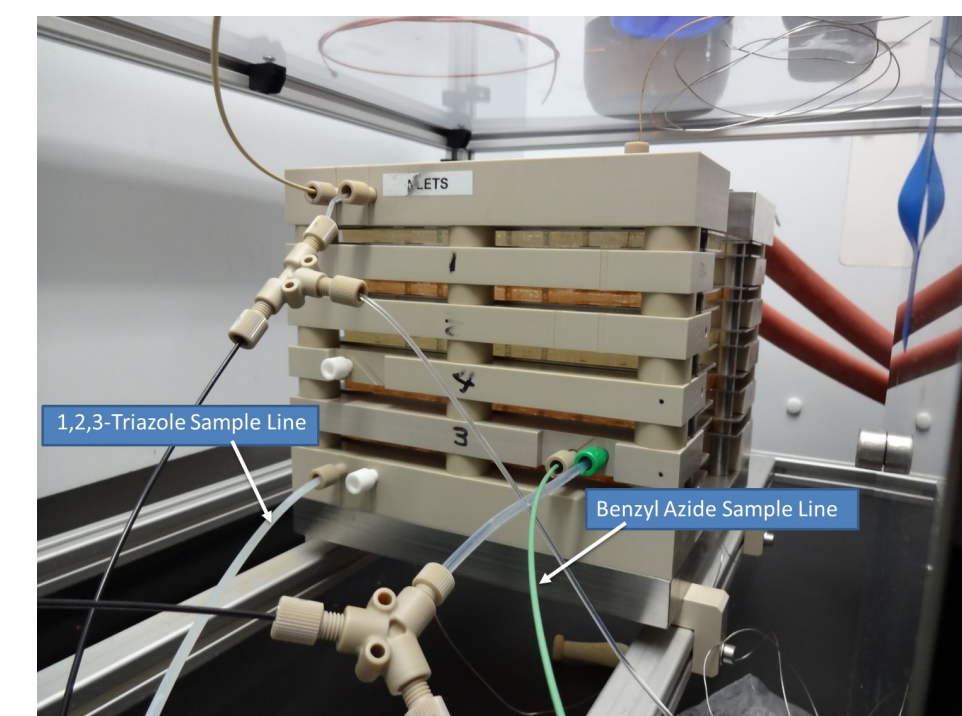


Figure 5. Two connections of the UPLC system to the Chemtrix KiloFlow reactor.

		Injection Sequence for Design of Experiment										
Injection Number	Benzyl Azide Synthesis (Replicate Injections, concentration mg/mL)				Triazole Synthesis (Replicate Injections, concentration mg/mL)							
	1	2	3	4	1	2	3	4	5	6	7	8
Triazole					5.13	5.18	5.08	5.04	5.23	5.57	4.84	4.85
Benzyl Azide	36.64	37.28	37.33	37.37	15.79	15.38	15.26	15.41	15.42	15.23	15.40	15.04
Benzyl Bromide	0.036	0.066	0.072	0.06	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Table 1. Example of a single set of data collected for the Design of Experiments. Specific data illustrated is for the concentration of sodium hydroxide and cyanoacetamide at 0.75 M at 80°C. Quantitative results shown for all three compounds of interest.

RESULTS

Seven combinations of sodium hydroxide / cyanoacetamide concentrations and reactor temperatures were evaluated in the DoE. The constant flow rates of the reagent pumps maintained a constant residence time in the reactor. Each set of reaction conditions was evaluated for 1 hour. To start, the controlled state for the benzyl azide reaction was confirmed with four online samples pulled from the reactor and analyzed (see Figure 5). After the fourth benzyl azide injection, the PSM was switched to pull samples from the 1,2,3-triazole sample line (Figure 5) and eight online 1,2,3-triazole samples were pulled from the reactor and analyzed. Figure 6 shows examples of the UPLC analysis of each reaction. The first chromatogram shows an excess of benzyl bromide, demonstrating that the benzyl azide formation had not reached controlled state. The second chromatogram shows that the controlled state of the benzyl azide reaction has been achieved; where all benzyl bromide has been converted to benzyl azide. The third chromatogram is an example of the 1,2,3-triazole formation at controlled state. With the use of its internal diluter to make accurate and precise dilutions, the PSM was used to generate calibration curves from individual standard vials to quantify all reaction components. Figure 7 shows an example of a typical calibration curve used to quantitate the results for this experiment. Each point on the curve was injected in triplicate. Empower Chromatography Data Software was used to integrate and quantitate the concentration for each injection generating real time reports. Quantitative replicate results for a single set of Design of Experiments for benzyl bromide, benzyl azide and triazole synthesis are shown in Table 1. The summary of all the mean quantitative results for the seven DoE for the 1,2,3-triazole is shown in Table 2. An increasing conversion of benzyl azide to the 1,2,3-triazole product was obtained as both temperature and concentration of sodium hydroxide and cyanoacetamide were increased. However, under these flow reaction conditions, significant levels of benzyl azide remained and benzyl bromide was not detected. Figure 8 shows an expanded view of the UPLC traces showing the impurities detected in the various reaction steps as well as the clearance of the potential genotoxic impurities (benzyl bromide and benzyl azide) in the final isolated product.

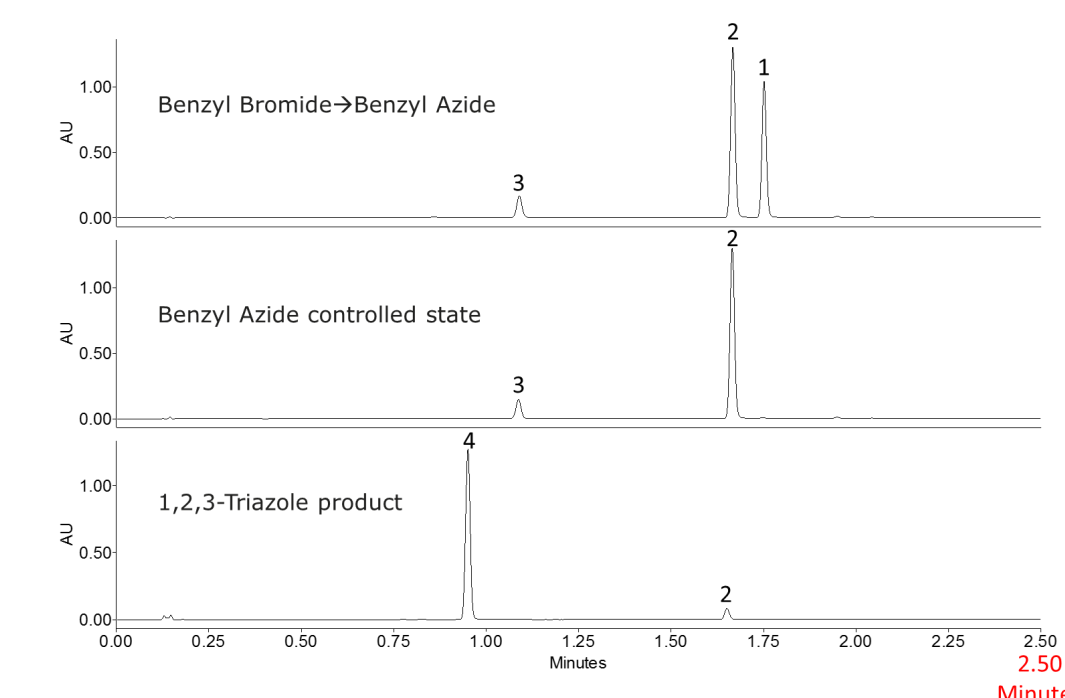


Figure 6. Ultra Performance LC analysis of reaction components. 1 benzyl azide, 2 benzyl bromide, 3 unknown, 4 1,2,3-triazole

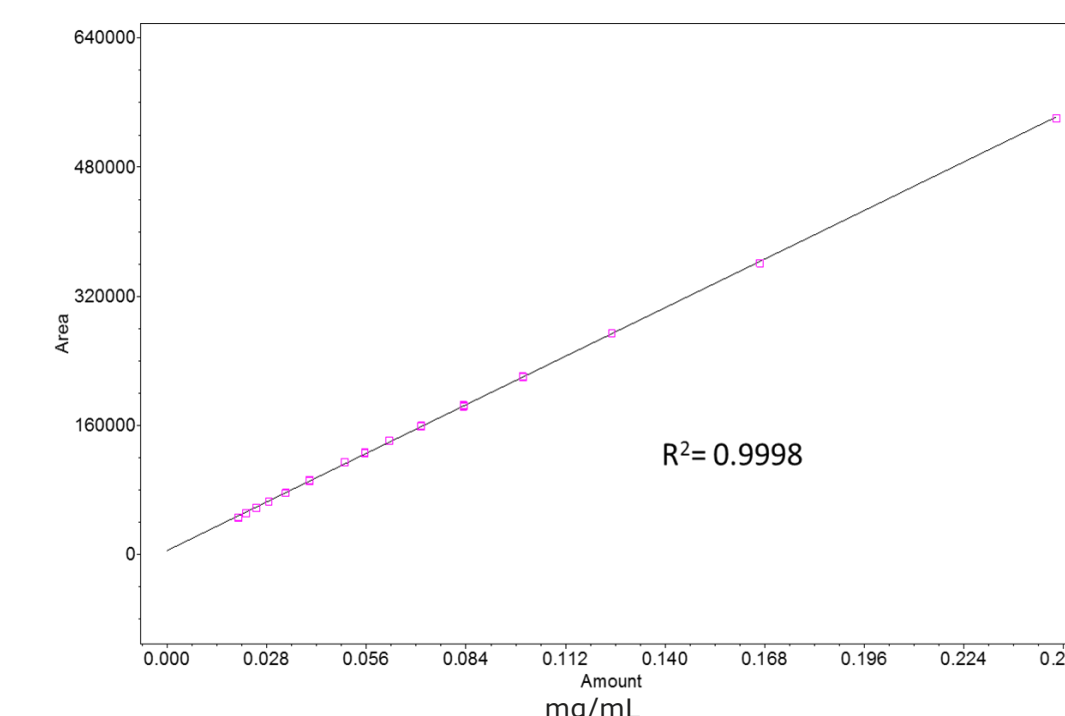


Figure 7. Calibration curve generated from a single vial of 1,2,3-triazole at 1 mg/mL. Each point generated from three replicate injections using dilutions (1:4 to 1:50) with the PSM.

Run Sequence	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4	Response 5
	Concentration of Sodium Hydroxide and Cyanoacetamide	Reaction Temperature	Area Percent of Triazole	Concentration of Triazole	Area Percent of Remaining Benzyl Azide	Concentration of Remaining Benzyl Azide	Concentration of Remaining Benzyl Bromide
	Molarity	Degrees C	%	mg/mL	%	mg/mL	mg/mL
6	0.5	90	91.78	5.184	8.22	10.224	Not Detected
7	0.5	80	87.67	3.474	12.33	10.836	Not Detected
1	0.5	70	81.6	2.304	18.39	17.256	Not Detected
5	0.75	90	94.37	7.11	5.63	9.336	Not Detected
3	0.75	80	91.63	5.112	8.37	15.372	Not Detected
2	0.75	70	87.18	3.456	12.82	16.836	Not Detected
4	1.00	90	95.92	9.018	4.08	8.496	Not Detected

Table 2. Mean quantitative and area percent results of the DoE for the synthesis of the 1,2,3-triazole show optimal yield with sodium hydroxide and cyanoacetamide at 1M and reactor temperature set at 90°C.

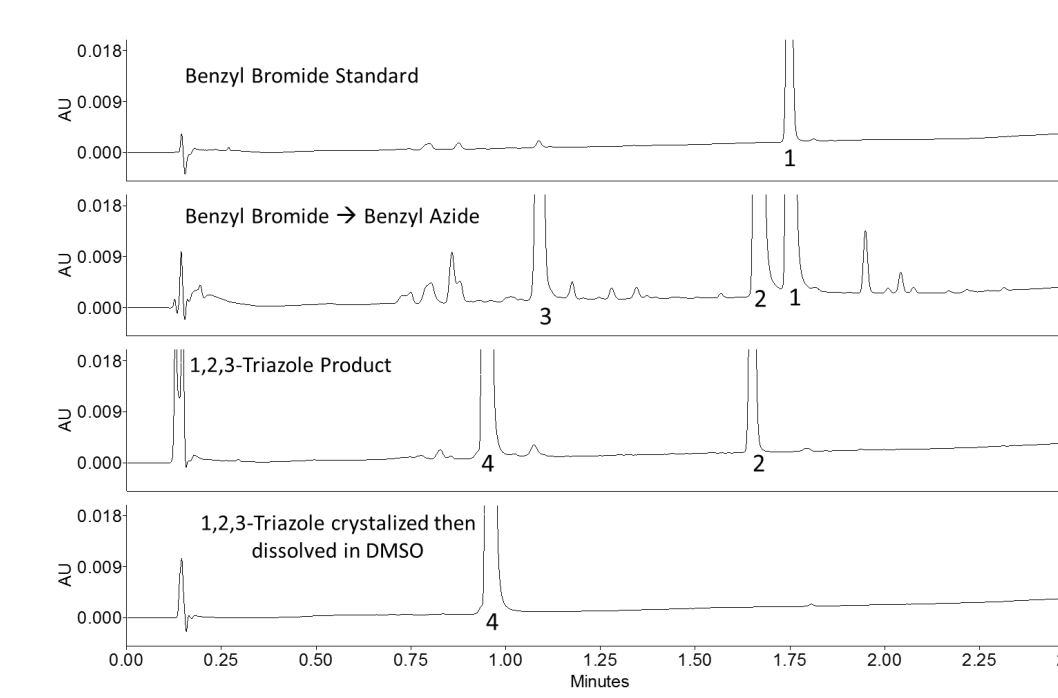


Figure 8. Using Ultra Performance LC to monitor low level impurities. 1 benzyl bromide, 2 benzyl azide, 3 unknown, 4 1,2,3-triazole

CONCLUSIONS

- The PATROL UPLC Pilot Process Analysis System enabled a DoE to investigate a continuous flow synthesis of a 1,2,3-triazole
- Direct online sampling from multiple points of the flow reactor combined with real-time UPLC analysis provides greater analytical confidence in each stage of the synthesis
- Increased sensitivity from the UPLC system enabled the monitoring of low level impurities that could be potential genotoxic impurities (i.e benzyl bromide and benzyl azide)
- Accurate and precise calibration curves generated from a single standard vial provides simple and automated real time quantitation of reaction samples
- Automated dilution of concentrated process samples provided flexible analysis keeping the detector within its linear dynamic range
- The risk of benzyl azide explosion is minimized by automated online sampling and the small reaction volume of the continuous flow process

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