Novel Application of Direct Inject Liquid Chromatography (DILC™) as Real-time Process Analytical Technology for Flow Reactions

Yangzhong Qin,*,‡[a] Umme Ayesa,[a] François Lévesque,[b] Yining Ji*,‡[a]

ABSTRACT: We report advanced applications of the DILC™ system as a powerful automated tool for monitoring reaction conversions in flow processes for the first time. By applying the DILC™ system to a photocatalytic flow reaction, we systematically mapped the reaction conversions across various residence times and temperatures. The DILC™ system has demonstrated significant effectiveness in flow reactions by facilitating reaction optimization, enhancing sustainability, and minimizing human intervention. Furthermore, the DILC™ has proven to be an excellent online tool for calibrating spectroscopic data. This innovative methodology not only streamlines analytical workflows but also lays the groundwork for future advancements in automated in-process control (IPC) technologies.

Introduction

Process Analytical Technology (PAT) techniques are employed to improve manufacturing processes through the integration of real-time analytical measurements of critical quality attributes.[1] These methods can be utilized at various stages, from initial process development at the laboratory scale to process characterization and control at the manufacturing scale.[2-6] Traditional spectroscopic techniques, such as Raman and Fourier Transform Infrared (FTIR) spectroscopy, play a major role in PAT, which aims to enhance process understanding and control in manufacturing, particularly in the pharmaceutical and chemical industries.[6-8] However, several challenges are associated with achieving In-Process Control (IPC) using these tools. These challenges include insufficient sensitivity for accurately detecting analytes at low concentrations. Additionally, the high susceptibility to changes in reaction conditions and variations in instrumentation across different sites and scales complicates the development of robust quantitative models. High-Performance Liquid Chromatography (HPLC) is widely regarded as the gold standard in analytical technology within the pharmaceutical and chemical industries for several reasons: 1) it offers excellent resolution and sensitivity, enabling the detection of low concentrations of active pharmaceutical ingredients (APIs) and impurities; 2) it facilitates precise quantitative analysis, making it an ideal method for determining the concentrations of compounds in a mixture; and 3) HPLC methods are generally robust and can be reliably reproduced across different laboratories and instruments.

[a]Analytical Research and Development, Merck & Co., Inc., Rahway, New Jersey 07065, United States
[b]Process Research and Development, Merck & Co., Inc., Rahway, New Jersey 07065, United States
Electronic Supplementary Information (ESI) available. See
DOI: 10.1039/x0xx00000x

Given these advantages, HPLC could serve as an excellent PAT tool, with traditional online HPLC being a notable example.[9, 10] However, these techniques utilize sipping tubes to collect samples, which often requires large sample volumes, have limited sample processing capabilities, and are restricted to homogeneous reactions. Importantly, in the field of small molecule chemistry, reactions typically require sampling, quenching, and dilution steps prior to HPLC analysis. These requirements present significant challenges for integrating chromatography as a real-time, in-situ PAT in this field. Recently, Telescope Innovation's proprietary Direct Injection Liquid Chromatography (DILC™) technology has emerged as a solution to this challenge. DILC™ fully automates the processes of sample extraction, quenching, and dilution, allowing for the real-time transmission of reaction samples to the HPLC.[11] This capability enables the identification and quantification of critical reaction species throughout the progression of the reaction, offering valuable insights into reaction kinetics, mechanisms, and the effects of various parameters on the reaction outcome. Collectively, these capabilities position this technology as an effective online PAT tool.

To date, DILC™ has primarily been employed in batch reactions, where the sampling probe (Figure 1) is directly immersed in the reaction solution.[11-15] Over the years, the pharmaceutical industry is increasingly adopting continuous manufacturing strategies, often in conjunction with the use of flow reactors.[16-18] Flow chemistry allow for precise control over critical parameters such as temperature, pressure, and stoichiometry at the mixing point.[19] This enhanced control contributes to improved yields, reduced impurities, and increased productivity in pharmaceutical production. We anticipate that integrating DILC™ with flow reactions will enable real-time monitoring and analysis of the reaction stream exiting the flow reactor, thereby streamlining analytical procedures and reducing the need for human intervention. Importantly, this technique will provide timely insights into steady-state conditions and reaction conversions, which can help to: 1) significantly streamline the optimization process by promptly providing information on the next optimization conditions without the need to halt and restart the reaction; 2) minimize waste and reduce reagent usage, aligning with sustainable practices in chemical manufacturing; and 3) ensure that the reaction performs as expected at larger volumes, facilitating smoother transitions and more predictable outcomes.

To further enhance the applications of DILC™, this report aims to demonstrate its effectiveness as a PAT tool in flow reactions for the first time (Figure 1). This technique emphasizes its ability to analyze chemical reaction progress in real time, providing insights into reaction conversions and enabling us to make immediate adjustments to optimize conditions. Additionally, when integrated with spectroscopic techniques, DILC™ could also serve as a complementary, orthogonal, automated, and online method for quantifying and validating spectroscopic measurements.

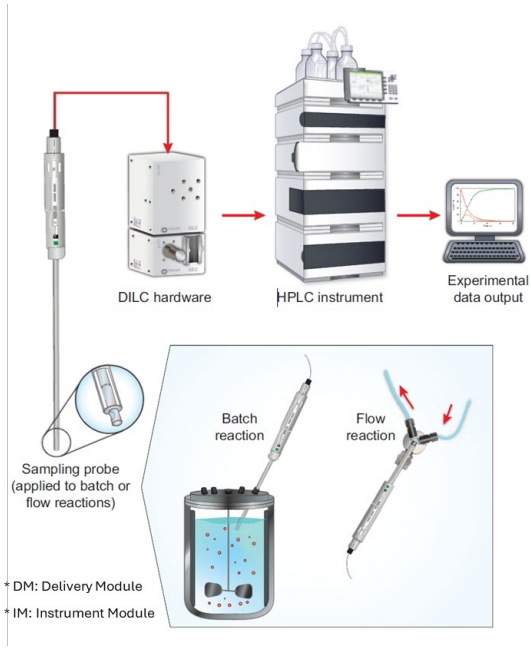
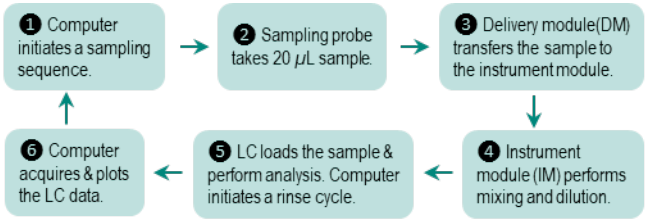


Figure 1. Schematic representation of DILC™ applied to both batch and flow reactions.

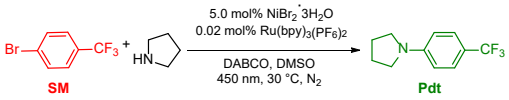
Results and discussion

The DILC™ integrates an EasySampler (Mettler Toledo), an Agilent LC system, and real-time analytical software (iCLC 7.2 from Mettler Toledo) (Figure 1). Operationally, the automated sampling and analysis procedure begins with the control computer initiating a sampling sequence (1), as illustrated in Scheme 1, sending a command to the sampling probe to collect a 20 μL sample from the reaction mixture (2). Upon successful collection, the delivery module promptly transfers the sample to the instrument module (3). Here, the instrument module performs the necessary mixing and dilution according to predefined protocols to prepare the sample for analysis (4). Subsequently, the liquid chromatography system loads the prepared sample, executes the analytical procedures, and follows a rinse cycle to ensure the system is ready for subsequent samples (5). Following each analysis, the iCLC software retrieves the LC data from the HPLC system and plots it in real time, allowing for the visualization of reaction kinetics over time (6). In the meanwhile, the control computer reinitiates the sampling sequence (1), creating a continuous loop of automated sampling and analysis.



Scheme 1. Schematic representation of the operational procedure for the DILC™ system.

We initially performed our model reaction — a photoredox C–N coupling reaction[20, 21] between 4-bromobenzotrifluoride and pyrrolidine, catalyzed by $Ru(bpy)_3(PF_6)_2$ and $NiBr_2{\cdot}3H_2O$ (Scheme 2) — in an EasyMax batch reactor. This setup was equipped with both an EasySampler probe and a ReactIR probe (see Figure S1 in the SI), enabling the simultaneous collection of FTIR and HPLC data.



Scheme 2. Photoredox C–N coupling between pyrrolidine and 4-bromobenzotrifluoride. $[4\text{-bromobenzotrifluoride}]_0$ = 0.25 M; $[pyrrolidine]_0$ = 0.375 M; $[DABCO]_0$ = 0.45 M; $NiBr_2{\cdot}3H_2O$ (5.0 mol%); $Ru(bpy)_3(PF_6)_2$ (0.02 mol%); 450 nm (Kessil lamp, 100% intensity).

The reaction was initiated by light irradiation, facilitated through the window of the EasyMax reactor (see Figure S1 in the SI). To monitor the progress of the reaction using HPLC, we conducted sampling with DILC™ at 5-minute intervals, followed by HPLC analysis using a 2.5-minute method (see Figure 2A and Table S1 for the HPLC method). This method enabled us to successfully obtain detailed reaction profiles that illustrate the kinetics of both the reactants and products in real time (Figure 2C, dots). Concurrently, in situ FTIR actively monitored both the reactants and products by tracking their characteristic FTIR signals at 1172 cm^{-1} and 1157 cm^{-1}, respectively (Figure 2B and Table S2), with a 30-second interval. Importantly, we observed a strong correlation between HPLC and FTIR data (Figure 2C and Figure S2). This further demonstrates that DILC™ is not only an effective standalone PAT tool but also an excellent online method for calibrating spectroscopic measurements.[22]

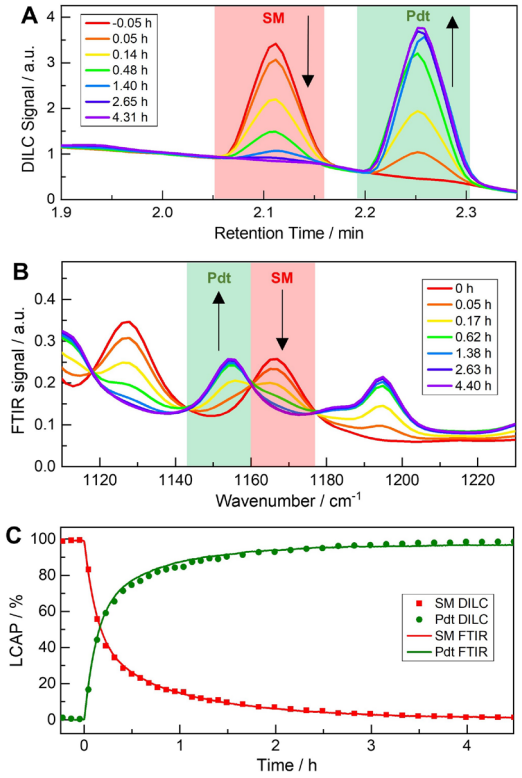


Figure 2. A) HPLC chromatogram illustrating the decrease in the starting material (SM, red box) and the increase in the product (Pdt, green box) over time. B) FTIR spectra depicting the decrease in the SM signal (red box) and the increase in the Pdt signals (green box) over time. C) Illustrating the strong correlation between DILC data (dots) and FTIR data for both SM (red) and Pdt (green).

Following the successful demonstration of the DILC™ system's capabilities in batch mode, we proceeded to conduct the flow reaction. We utilized a plug flow reactor, specifically the commercially available 10-mL Vapourtec UV-150 photoreactor (Vapourtec Ltd., U.K.), which was equipped with a 405 nm light-emitting diode (LED) array producing an output of 9 W of light (Figure S3). To integrate the EasySampler probe into the flow setup, we proposed utilizing a flow cell (Figure 3).

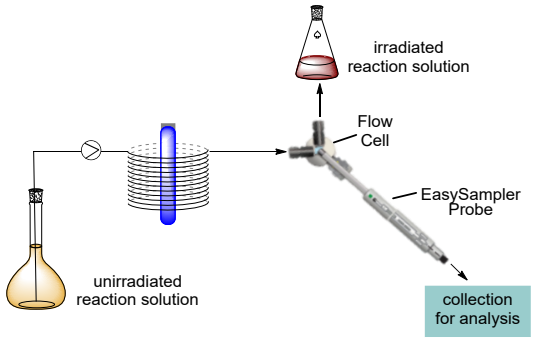


Figure 3. Diagram illustrating a 10 mL plug flow reactor setup integrated with an EasySampler probe through a flow cell.

The setup allows for the evaluation of both temperature and residence time effects on the reaction outcome. We first attempted to evaluate the reaction performance at different residence time. Using a fixed temperature of 35 °C, we increased the residence time from 1.0 minutes to 60 minutes. Upon the solution exiting the flow cell, the DILC™ system extracts samples for analysis at five-minute intervals. It is important to note that the initial data points collected at each change in residence time may be affected by potential diffusion from the preceding solution within the flow cell. Consequently, we decided to transition to the next residence time only after the conversion measured by DILC™ had reached a steady state, which typically occurred after the collection of the third or fourth sample (Figure 4A). As the data was collected, the software reported the conversions of the flow reaction at different residence times (Figure 4A). This data enabled us to map the conversion at various residence times (Figure 4B, blue) within a short timeframe. Meanwhile, the steady-state conversions at each residence time were also confirmed by offline NMR measurements (Figure 4B, red) of irradiated samples collected from the exit of the flow cell (Figure 3). Notably, residence times exceeding 30 minutes resulted in conversion rates greater than 95%. Importantly, these results were obtained continuously without the need to stop and resume the flow, due to the integration of online HPLC, which facilitated real-time monitoring of reaction conversion for each tested condition. This capability allowed for the prompt identification of subsequent conditions to evaluate. The elimination of the need to halt and restart the process is particularly significant, as achieving a steady state for each startup can be resource-intensive in terms of both time and materials.

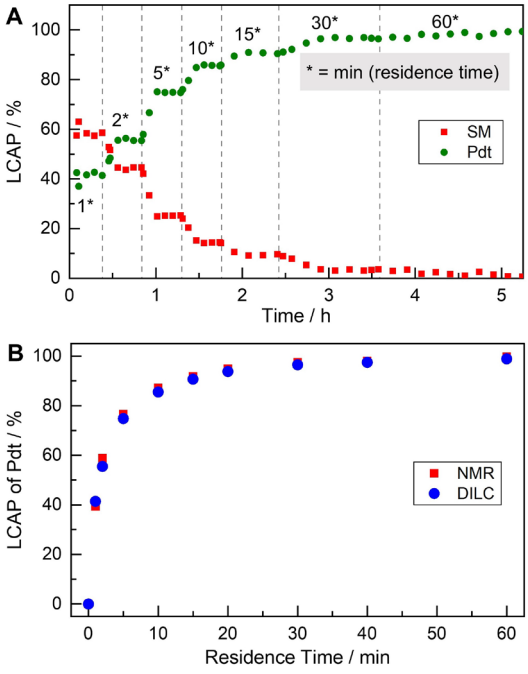


Figure 4. A) Real-time automated tracking of the photocatalytic conversion (Scheme 2) using DILC™ at varying residence times with a fixed temperature of 35 °C. B) Illustrating the final conversions at different residence times.

Following similar procedure, we subsequently investigated the effect of temperature on the reaction. The results indicated that, at

a fixed residence time of 5 minutes, increasing the temperature from 35 °C to 60 °C significantly enhanced the reaction rate. However, further increasing the temperature to 75 °C resulted in only minimal additional improvement in conversion (Figure 5).

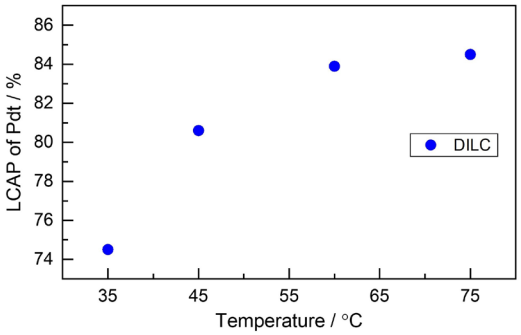


Figure 5. Illustrating the final conversions at different temperatures with a fixed residence time of 5 minutes.

As demonstrated with the model reaction presented, the use of DILC™ has the potential to significantly streamline the optimization of reaction conditions for any given flow reaction. The ability to monitor real-time conversion is essential, as it enables the identification of subsequent experimental conditions without the need for interruption and resumption of the process, thereby enhancing overall workflow efficiency and sustainability. Importantly, integrating DILC™ into flow reactions could help eliminate the need for offline sampling and the associated yield loss from diversions to waste prior to confirming that steady state has been reached. This benefit becomes increasingly significant at larger scales and in scenarios where analytical analyses require extended durations, particularly during manufacturing.

Conclusions

In conclusion, we have demonstrated the enhanced capabilities of the DILC™ system as an automated online tool for tracking the reaction progress in real-time, making it a powerful PAT tool. Additionally, we successfully showcased the first demonstration of DILC™ within a flow reaction framework, enabling precise monitoring of reaction conversion under each tested condition, thereby streamlining the optimization process and enhancing opportunities for further improvements in sustainability.

Author Contributions

‡ Y. Ji and Y. Qin contributed equally.
Y. Ji and Y. Qin wrote the manuscript.
Y. Qin, Y. Ji, U. Ayesa and F. Lévesque conducted the experimental work.

Data Availability

The data supporting this article have been included as part of the Supplementary Information.

Conflicts of interest

The authors declare no competing financial interests.

Acknowledgements

We greatly acknowledge Wenjun Liu and Bhumit Patel for helpful discussions and Keith A. Mattern for assistance with setting up the DILC system.

Notes and references

1. Guidance for Industry: PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance; U.S. Food and Drug Administration: Rockville, MD, 2004.
2. A. Chanda, A. M. Daly, D. A. Foley, M. A. LaPack, S. Mukherjee, J. D. Orr, G. L. Reid, D. R. Thompson and H. W. Ward, *Org. Process Res. Dev.*, 2015, **19**, 63-83.
3. K. A. Esmonde-White, M. Cuellar, C. Uerpmann, B. Lenain and I. R. Lewis, *Anal. Bioanal. Chem.*, 2017, **409**, 637-649.
4. A. P. Ferreira and M. Tobyn, *Pharm. Dev. Technol.*, 2015, **20**, 513-527.
5. Y. Miyai, A. Formosa, C. Armstrong, B. Marquardt, L. Rogers and T. Roper, *Org. Process Res. Dev.*, 2021, **25**, 2707-2717.
6. Y. Qin, K. A. Mattern, V. C. R. Zhang, K. Abe, J. Kim, M. Zheng, R. Gangam, A. Kalinin, J. N. Kolev, S. Axnanda, Z. E. X. Dance, U. Ayesa, Y. N. Ji, S. T. Grosser, E. Appiah-Amponsah and J. P. McMullen, *Org. Process Res. Dev.*, 2024, **28**, 432-440.
7. G. Gerzon, Y. Sheng and M. Kirkitadze, *J. Pharmaceut. Biomed.*, 2022, **207**.
8. K. A. Esmonde-White, M. Cuellar and I. R. Lewis, *Anal. Bioanal. Chem.*, 2022, **414**, 969-991.
9. B. A. Patel, N. D. S. Pinto, A. Gospodarek, B. Kilgore, K. Goswami, W. N. Napoli, J. Desai, J. H. Heo, D. Panzera, D. Pollard, D. Richardson, M. Brower and D. D. Richardson, *Anal Chem*, 2017, **89**, 11357-11365.
10. T. Graf, L. Naumann, L. Bonnington, J. Heckel, B. Spensberger, S. Klein, C. Brey, R. Nachtigall, M. Mroz, T. V. Hogg, C. McHardy, A. Martinez, R. Braaz and M. Leiss, *J Chromatogr A*, 2024, **1729**.
11. T. C. Malig, L. P. E. Yunker, S. Steiner and J. E. Hein, *Acs Catal*, 2020, **10**, 13236-13244.
12. Y. Sato, J. L. Liu, A. J. Kukor, J. C. Culhane, J. L. Tucker, D. J. Kucera, B. M. Cochran and J. E. Hein, *J Org. Chem.*, 2021, **86**, 14069-14078.
13. A. J. Kukor, M. A. Guy, J. M. Hawkins and J. E. Hein, *React Chem Eng*, 2021, **6**, 2042-2049.
14. M. C. Deem, J. S. Derasp, T. C. Malig, K. Legard, C. P. Berlinguette and J. E. Hein, *Nat Commun*, 2022, **13**.
15. A. J. Kukor, N. Depner, I. Cai, J. L. Tucker, J. C. Culhane and J. E. Hein, *Chem Sci*, 2022, **13**, 10765-10772.
16. C. Bottecchia, F. Lévesque, J. P. McMullen, Y. Ji, M. Reibarkh, F. Peng, L. S. Tan, G. Spencer, J. Nappi, D. Lehnherr, K. Narsimhan, M. K. Wismer, L. K. Chen, Y. P. Lin and S. M. Dalby, *Org. Process Res. Dev.*, 2022, **26**, 516-524.
17. Y. Ji, C. Bottecchia, F. Lévesque, K. Narsimhan, D. Lehnherr, J. P. McMullen, S. M. Dalby, K. J. Xiao and M. Reibarkh, *J Org. Chem.*, 2022, **87**, 2055-2062.
18. D. L. Hughes, *Org. Process Res. Dev.*, 2020, **24**, 1850-1860.
19. M. B. Plutschack, B. Pieber, K. Gilmore and P. H. Seeberger, *Chemical Reviews*, 2017, **117**, 11796-11893.
20. E. B. Corcoran, M. T. Pirnot, S. S. Lin, S. D. Dreher, D. A. DiRocco, I. W. Davies, S. L. Buchwald and D. W. C. MacMillan, *Science*, 2016, **353**, 279-283.
21. F. Lévesque, M. J. Di Maso, K. Narsimhan, M. K. Wismer and J. R. Naber, *Org. Process Res. Dev.*, 2020, **24**, 2935-2940.
22. Y. Ji, Z. H. Lin, L. Lawson, F. Levesque, D. A. Foley, R. Espina and H. Robert, *React Chem Eng*, 2023, **8**, 2270-2274.