Reaction Chemistry & Engineering

PAPER

View Article Online
View Journal | View Issue

Cite this: *React. Chem. Eng.*, 2018, **3**, 799

In-line separation of multicomponent reaction mixtures using a new semi-continuous supercritical fluid chromatography system†

Daniel E. Fitzpatrick, Robbie J. Mutton and Steven V. Ley *

A new bespoke semi-continuous parallel column supercritical fluid chromatography unit has been developed that solves the problem of effective separation of continuous, multicomponent reaction mixtures. It enables the rapid in-line separation of crude mixtures produced during batch and continuous flow reactions. It overcomes limitations inherent to other systems, enabling it to collect up to five individual fractions from mixtures with any number of constituent components, and adds new machinery to aid the modern synthetic chemist. The success of the system was exemplified using two Appel-type reactions, enabling the recovery of 81% of 1-bromoethylbenzene (with a first pass purity of 92%) and 89% of an intermediate to the anti-cancer drug candidate AZ82 (>99% purity) from batch mixtures. As a notable example, the system was also used in a telescoped flow process to separate an intermediate during the synthesis of isoniazid. In this case, the three-stage synthesis was operated at steady state for four hours during which time 96% recovery of the intermediate, isonicotinamide, was attained (with a purity of >99%).

Received 19th June 2018,
Accepted 18th July 2018

DOI: 10.1039/c8re00107c

rsc.li/reaction-engineering

Introduction

Isolating target products from multicomponent mixtures is a familiar challenge faced by chemists on a daily basis. In batch synthesis, techniques such as flash column chromatography, crystallisation and filtration are commonly employed to effect off-line purification. However, there are few such broadly versatile techniques to achieve the same outcome for continuous multi-step flow reactions. Indeed, in 2014 for example a particular problem we struggled with was the synthesis of the natural product Spirangien A in a fully flow-based manner due to the need for manual chromatographic separation of complex intermediates, suggesting new equipment needed to be developed.[1]

Currently, commonly used separation techniques for continuous synthesis include liquid–liquid extraction,[2–4] filtration[5,6] and solid-supported scavenging and reagents.[7,8] While these can be extremely useful, particularly when telescoping multiple reaction steps together,[9,10] where the output mixture from one reaction is linked directly with the input for the next, they are usually designed to *remove individual impurities* from mixtures, rather than isolating the compound of interest.

In order to overcome these issues, chemists will typically attempt to modify their reactions to remove the need for such downstream processing. A common method is the use of heterogeneous catalysis,[11,12] where one can avoid the energy-intensive requirement to separate homogeneous catalysts from reaction mixtures. Yet these techniques may not be suitable in many reaction scenarios or while one component of a crude reaction mixture may be removed there still exist others that need to be separated. Typically, manual intervention is required where flash or high-performance liquid chromatography is applied to isolate the desired compound.

Not surprisingly, there have been significant efforts to adapt chromatography to suit continuous processing regimes,[13] including applications targeting analytical high-throughput screening.[14] In their most part, reports describe simulated moving bed chromatography (SMBC)[15] systems where multiple columns are connected in a series arrangement and injections of crude mixture are staggered between them in a cyclic manner. While this technique works well for binary systems or multi-component mixtures where the target compound is either the first or last to elute from columns,[16,17] in most organic synthesis procedures these conditions are not necessarily satisfied and so the practical deployment of SMBC is somewhat limited to specific applications. SMBC separation of ternary and quaternary mixtures has been reported;[18,19] however, these required the use of multiple SMBC systems connected together, adding significant operational complexity and capital expense.[20]

Innovative Technology Centre, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. E-mail: svl1000@cam.ac.uk

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8re00107c

In this work we detail the first example of a parallel-column supercritical fluid chromatography unit that overcomes these limitations to achieve effective semi-continuous separations of crude reaction mixtures in both batch and continuous flow. This system also benefits from the ability to isolate up to five individual components from crude reaction mixtures at any one time.

Results and discussion

Process design

When operating a telescoped flow sequence, it is crucial that process disturbances are avoided such that steady state for all stages can be maintained for extended periods. This is particularly important in a pharmaceutical context where the GMP regime dominates and any fluctuations of process parameters may lead to operation outside acceptable variable windows and thus a significant waste of affected product material.

Accordingly, separation processes should be designed to accept a continuous flow of material as an input while discharging a continuous flow of purified material as an output to downstream operations. SMBC processes, as truly continuous operations, can conform to such a requirement in specifically designed cases. Semi-continuous processes, where batch procedures are run sequentially such that an apparent continuous operation is achieved, have also been deployed effectively when using, for example, affinity chromatography.[21,22]

In order to maintain the selectivity and broad usability offered by standard chromatography systems, we applied a semi-continuous mode of operation to columns arranged in parallel. Our operational design concept is shown in Fig. 1. In this example, a multi-component mixture is injected into a four-column array at α second intervals. Injections are spread evenly between the columns so that an injection occurs somewhere in the array every 0.25α seconds. The target compound is collected as it elutes from the column. In an ideal scenario, the target peak tail in one column would overlap with the peak rise in the next column such that an uninterrupted stream of target material can be diverted into collection reservoirs.

We chose supercritical fluid chromatography (SFC) as the separation technique for our system. SFC offers very rapid separations at semi-preparative and preparative scales and promotes excellent mixing between injected plugs of reaction mixture and column packing materials, rendering its resolution and general performance similar to ultra-high performance liquid chromatography (UHPLC).[23] Furthermore, it is common in SFC procedures to use carbon dioxide as the primary mobile phase,[24] removing to some extent reliance on environmentally-unacceptable and expensive solvents such as dichloromethane and hexane which are used regularly in standard liquid chromatography methods, and recognising that effluent carbon dioxide can be captured and recycled if necessary.

From a reaction telescoping perspective, using supercritical carbon dioxide as the primary mobile phase greatly

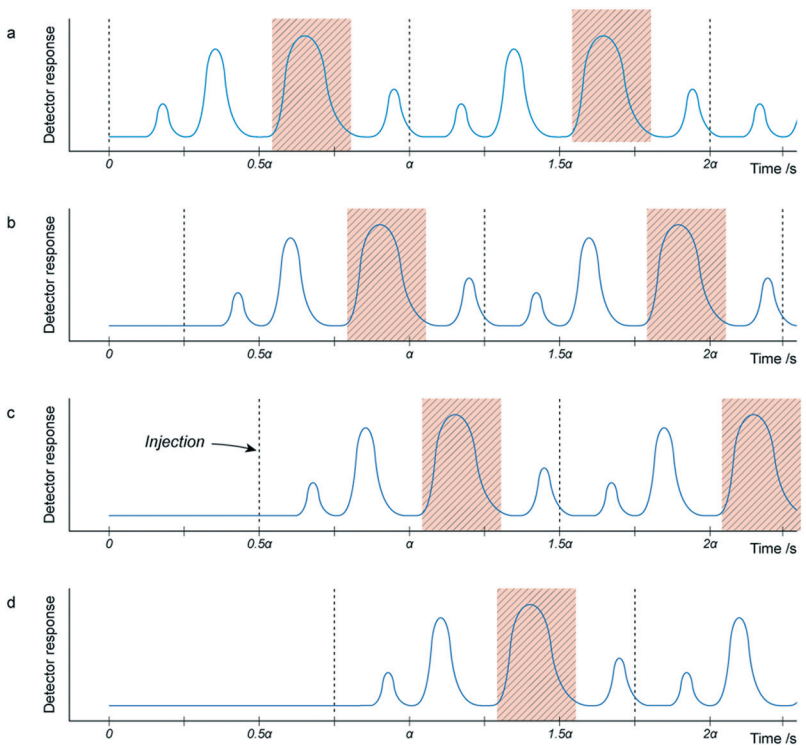


Fig. 1 Conceptual UV-vis traces for an example four-column system (a–d). Injections are staggered evenly between columns in an attempt to align collection windows (shown as hashed red above) to produce a constant output stream of target material.

reduces the energy requirements to remove solvent from collected fractions (*cf.* HPLC methods). In this scenario the pressure simply needs to be reduced to release gaseous carbon dioxide, leaving the compound of interest in a relatively small volume of modifier solvent.

Schematic

The equipment layout for the system is shown in Fig. 2. It consists of four independent column lines, fed by two large feed pumps (for CO_2 and modifier solvent supply). Multi-way switching valves placed downstream of each column allow isolation of up to five fractions (in addition to a sixth line for waste). Further discussion about hardware layout is included in the ESI.†

The injection system consisted of four loading loops connected in series (Fig. 3) and a recirculation pump. A cyclone separator was also included to separate the reaction mixture from depressurised carbon dioxide following injection.

The system consisted of 21 independent items of equipment, greatly increasing operational complexity and requirements over standard procedures. Accordingly, we harnessed an internet-based control system[25] to facilitate control and automation of apparatus at process start up, steady state operation and system shutdown. This arrangement was particularly useful when operating the unit in a telescoped flow process between two reaction stages, as described in the final example below, owing to its ability to integrate with additional reactor systems and inline detectors.

Multicomponent separations

Batch halogenation. Having constructed the SFC system, its separation performance was tested using two batch reactions. Crude mixtures from Appel-type transformations were chosen owing to their number of constituent components, and because we hoped to simplify the typically labour-intensive work up requirement to remove triphenylphosphine oxide produced during the reaction.

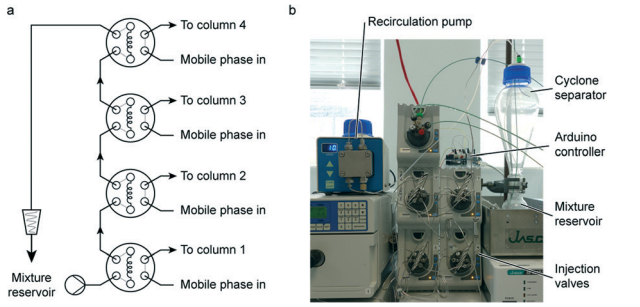


Fig. 3 Crude mixture was recirculated from a holding reservoir through four independent Rheodyne injection valves. A cyclone separator was included after the valves to aid with separation of gaseous CO_2 following depressurisation. (a) Schematic of equipment layout; (b) photography of injection system.

Synthesis of 1-bromoethylbenzene. We first focused on the simple transformation of 1-phenylethanol to its bromo-substituted product using carbon tetrabromide and triphenylphosphine (Scheme 1). Unlike standard Appel procedures, we modified reaction stoichiometry such that triphenylphosphine was the limiting reagent. This step was taken to ensure that there would be a range of compounds in the crude reaction mixture to be separated by the SFC unit, rather than an optimised reaction profile, to provide a more challenging test of the system.

SFC systems present a number of variables that can be used to tweak separation performance, such as the composition

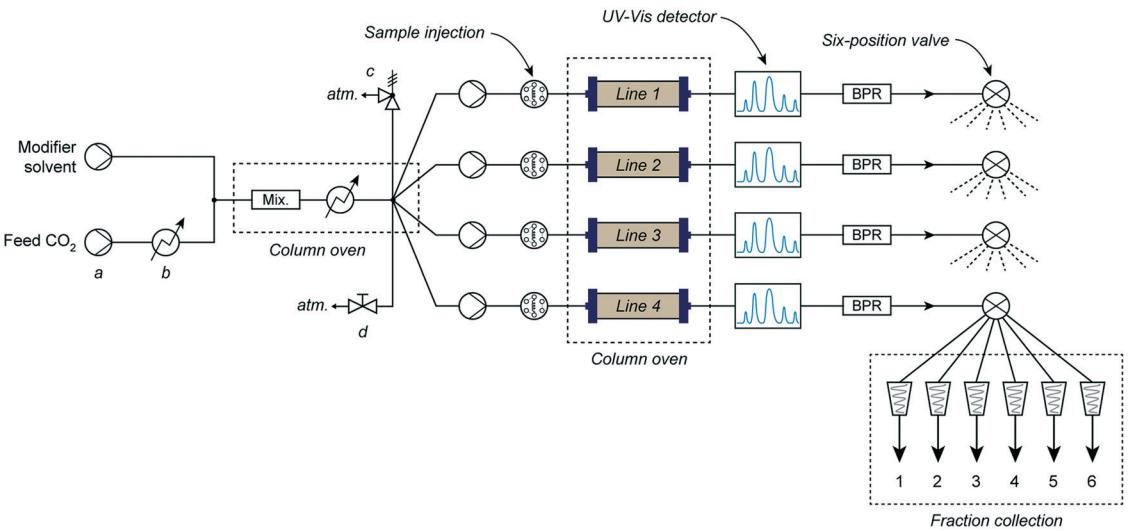
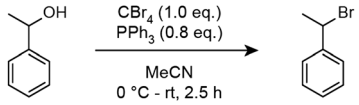


Fig. 2 Equipment schematic for a four-column system. Reaction mixture can be injected into each column line, independently of the remaining three. Each column has its own supply pump, UV-vis detector, automated back pressure regulator and six-position collection valve. Cyclone vessels are placed downstream of collection valves to aid with the separation of gaseous CO_2 from collected fractions following depressurisation. Notes: (a) pump head cooled to −12 °C; (b) heated to 60 °C; (c) pressure relief valve set to 34.35 MPa; (d) adjustable damper valve to reduce effects of pressure fluctuations caused by upstream pump movements. A photograph and more information about equipment have been included in the ESI.†



Scheme 1 Appel reaction to convert 1-phenylethanol to its bromo-substituted product in batch mode. The amount of triphenylphosphine in the reaction vessel was reduced so that it was the limited reagent.

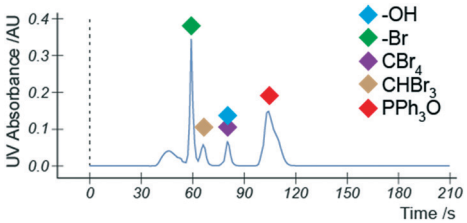


Fig. 4 Chromatogram obtained from one column line when using 12.5% MeOH in the mobile phase, a backpressure of 10 MPa and a column temperature of 65 °C.

and particle size of column packing materials, mobile phase pressure and system temperature (which affects density and solvating characteristics of supercritical fluids), selection of modifier solvent and mobile phase flow rates. Injection concentration and loading can also influence chromatography; however, for the examples presented in this paper, injection dilution was not required and so crude reaction mixtures were injected directly using 0.1 mL sample loops.

A systematic process was followed to identify the optimum conditions to isolate target products, as outlined in the ESI.† For this reaction, it was found that the use of methanol as modifier solvent (12.5% in CO_2) with a system temperature of 65 °C and back pressure of 10 MPa provided the best results (Fig. 4). 2-Ethylpyridine polymer (60 Å pore size, 5 μm particle size) was chosen as the stationary phase. Under these conditions, crude reaction mixture could be injected directly with each injection fully separated within two minutes. It is worth noting the excellent separation obtained in this reaction; while not applied here, unreacted starting materials could be diverted as they elute and recycled to improve overall reaction efficiency.

Having identified suitable separation conditions, attention was turned to the purification of the remaining crude reaction mixture. Given the consistent separation across each of the four column lines observed during trial runs and that this reaction was a relatively simple scenario, we decided to operate the product collection valve using a timing mechanism without incorporating UV detector feedback.

The UV traces for a 20-injection sequence is shown in Fig. 5. During this run the system was configured to collect only the product fraction, with all other peaks sent to waste. An 81% recovery of the target product was obtained with a purity of 92% (HPLC, ^{1}H NMR).

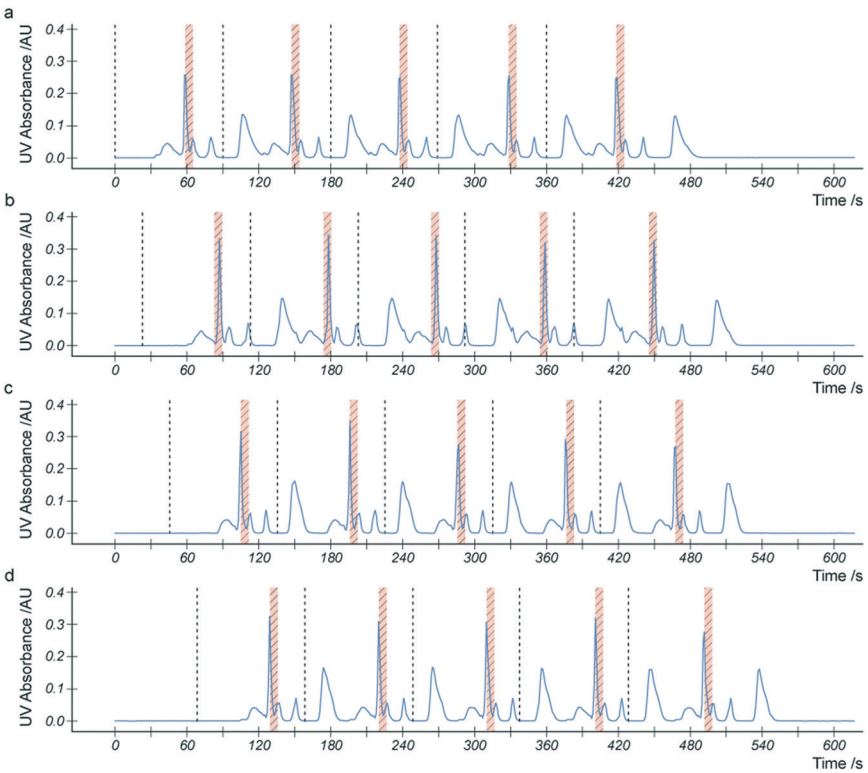
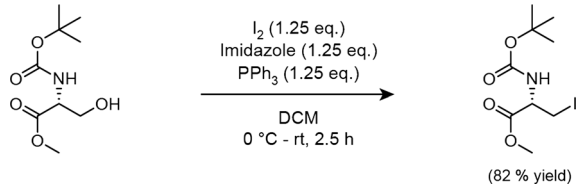


Fig. 5 UV-vis traces for a 20-injection sequence across the column lines (a–d). A timing mechanism was used to collect the target fraction (hashed red) for 8 seconds, set to trigger 56 seconds following injection. Injections are shown by dotted vertical lines.



Scheme 2 Appel-type reaction to produce an alkyl halide intermediate during the synthesis of drug candidate AZ82.

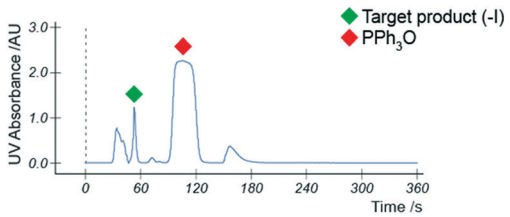


Fig. 6 UV-vis trace of the separation profile when using 10% methanol as modifier solvent, a back pressure of 10 MPa and a column temperature of 65 °C.

Synthesis of drug candidate intermediate. During a synthesis of the anti-cancer drug candidate AZ82,[26] issues arose during the workup following the formation of an alkyl halide intermediate (Scheme 2). As an Appel-type transformation, triphenylphosphine oxide formed during the reaction required a laborious and repetitive process to remove.[27] Even so, traces of the oxide could not be removed fully from fractions of our target compound; not an uncommon problem.

The use of polymer-supported triphenylphosphine alleviated these issues, as the oxide could be removed simply *via* filtration; however, the supported form of this reagent is expensive and thus its extensive use is undesirable. Given that no traces of the oxide were found in the first example above, we believed that the SFC unit could eliminate the requirement for polymer-supported triphenylphosphine while still allowing recovery of the target compound in high purity.

Having prepared a quantity of crude reaction mixture, we identified suitable operating conditions for the separation as described in the ESI.† It was interesting to observe that product breakdown occurred when diethylaminopropyl polymer (DEAP) columns were used. The basicity of the column packing material in this case led to elimination of the iodide, as confirmed with ^{1}H NMR (refer to ESI†).

For this example, separation over 2-ethylpyridine polymer columns with 10% methanol in the mobile phase, a column temperature of 65 °C and back pressure of 10 MPa led to the best separation profile (Fig. 6). Injected plugs of crude reaction mixture could be separated within three minutes.

We subsequently ran the system to separate remaining reaction mixture. Given the reduced purity when using a timing mechanism for fraction collection as in the previous example, we instead used UV detector feedback to trigger downstream valves. A five second delay was added after the target peak tail was detected before valves switched back to waste, in order to account to account for dead volume between detectors and collection valves.

The SFC unit provided 89% recovery of the target compound, with a purity of >99% (HPLC, ^{1}H NMR) and collection rate of 3.46 mmol h^{-1}. Moving from a timing mechanism for fraction collection, as in the initial example above, to UV-vis detection in this case led to significant improvement in fraction purity.

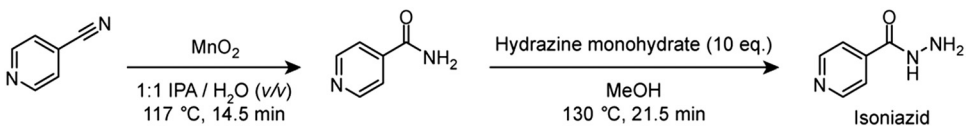
We were pleased to note that the relatively harsh and non-anhydrous supercritical conditions within the separation system did not lead to any degradation of product, allaying concerns about the carbonic acid catalysed breakdown of the methyl ester protecting group.[28,29]

Purification of a telescoped intermediate. One of the primary objectives of this project was to develop a system suitable for use as a downstream separation process in telescoped flow procedures. As a showcase final example of the utility of the system, we telescoped the synthesis of isoniazid, a treatment for tuberculosis, from 4-pyridinecarbonitrile (Scheme 3).

Previous work had yielded this flow route to this simple drug which consisted first of catalytic nitrile hydration over manganese dioxide, followed by a displacement with hydrazine monohydrate. As the first step yielded 91% of the intermediate amide together with 9% starting material, the SFC unit was harnessed to purify the product stream before the second transformation.

The full equipment schematic for the telescoped process is shown in Fig. 7. An inline infrared detector (IR) was positioned between the first reactor and SFC unit to aid with process start up (to detect the presence of amide in the crude stream from step one) and steady state monitoring.

This telescoped process consisted of three distinct control stages: start up, where the nitrile hydration step was commenced and allowed to reach steady state before the SFC unit was activated, followed by the initiation of the second reaction step; operation at steady state, where the system was allowed to run for extended periods while the target compound, isoniazid, was collected; and finally process shutdown where each telescoped step was directed to turn off in sequence. The logic used for the control system for these stages is included in the ESI.†



Scheme 3 Two-step telescoped synthesis of isoniazid, a drug used for the treatment of tuberculosis.

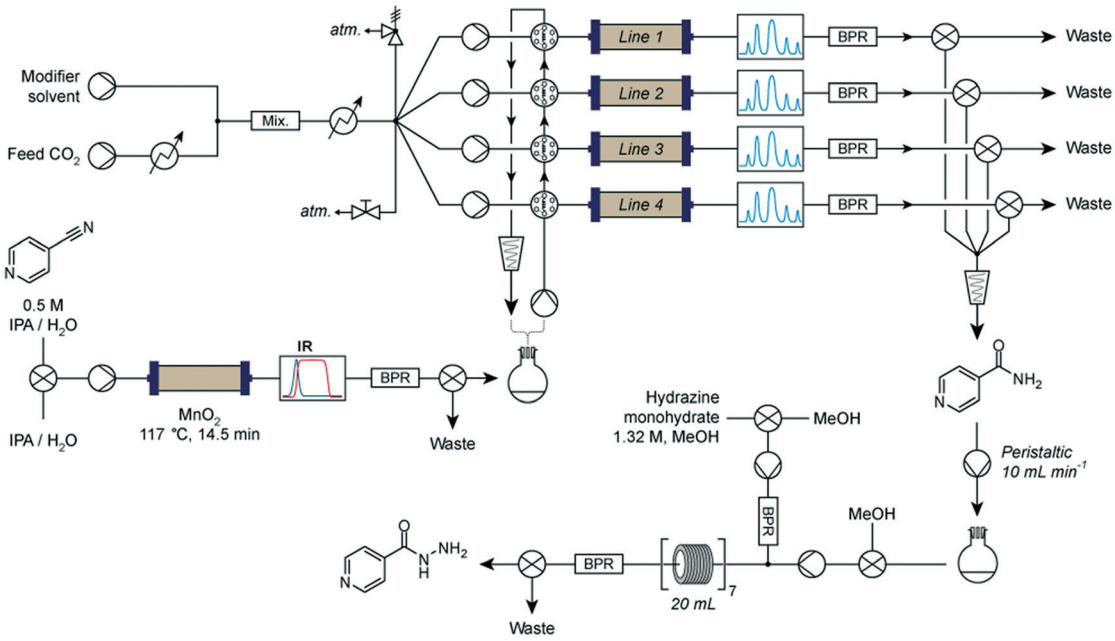


Fig. 7 Schematic showing the fully telescoped process, which involved 24 independent pieces of equipment. The SFC system was placed between two flow transformations, isolating the target product from the first step and feeding it directly into the second. The control system managed all stages of the process, eliminating the need for manual intervention following process initiation.

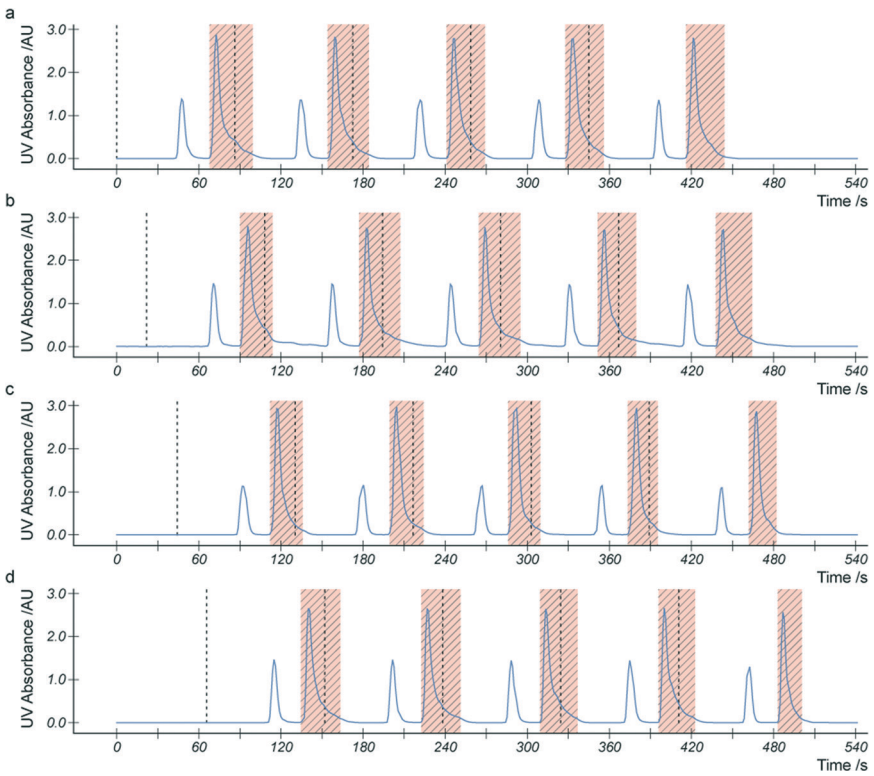


Fig. 8 Separation profiles in each column line (a–d) following 20 injections of crude reaction mixture from the first reaction step. The system was configured in this example to collect by UV detector feedback. Injections are shown by dotted vertical lines and collection windows shown by hashed red areas.

A manual test run of the SFC unit was conducted with crude reaction mixture from step one, yielding operational conditions that allowed 97% recovery of the amide with >99% purity (HPLC, ^{1}H NMR; refer to the ESI†). Under these

conditions (DEAP columns, 65 °C, 22.5% MeOH, back pressure 12.0 MPa), the total time for individual injection separation was 98 seconds.

Given the separation profile, we were able to stack injections into the column lines allowing us to tune parameters to match exactly the steady state flow rate of crude mixture exiting from the hydration step (Fig. 8). At steady state, the system was configured to make an injection into each column line at 1.44 minute intervals. Furthermore, peaks overlapped between the columns such that the collection of an unbroken stream of the amide product in modifier solvent (methanol) was directed into the second transformation step. Under these conditions, 7.6 mmol h^{-1} of the intermediate amide could be collected.

The telescoped process was operated at steady state for a four-hour period, during which time 2.25 mmol h^{-1} of isoniazid was collected to give an overall yield of 27% overall yield (91% from the first reaction, 96% average recovery from SFC and 31% yield from the second reaction). Pleasingly, the use of automation enabled a single researcher to operate the entire process with minimal manual intervention. Again, the separation profile in this example would enable operators to recycle unreacted starting material should it be desired.

Conclusions

In summary we have developed a highly modular supercritical fluid in-line chromatography system for the automated separation of reaction mixtures. The machinery can be employed in both batch and flow mode scenarios including a challenging telescoped flow reaction sequence.

The system stages sample injection to four separation columns and, following UV-vis detection, independent collection of up to five products through valve switching and cyclone depressurisation is achievable.

The equipment developed herein represents a first example of a parallel column SFC system for inline separation and provides the basis for further modification for continuous product processing. As the world of synthesis is becoming increasingly reliant on advanced equipment and machinery, the new device reported here adds significantly to the toolbox.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors gratefully acknowledge financial support from the Woolf Fisher Trust (DEF), EPSRC (SVL, RM; grant codes EP/K009494/1, EP/K039520/1 and EP/M004120/1) and ECH2020 (DEF, SVL; Future & Emerging Technologies (FET), "OneFlow", sponsor reference 737266).

Notes and references

1 S. Newton, C. F. Carter, C. M. Pearson, L. de C Alves, H. Lange, P. Thansandote and S. V. Ley, *Angew. Chem., Int. Ed.*, 2014, **53**, 4915–4920.
2 M. O'Brien, L. Konings, M. Martin and J. Heap, *Tetrahedron Lett.*, 2017, **58**, 2409–2413.
3 M. O'Brien, P. Koos, D. L. Browne and S. V. Ley, *Org. Biomol. Chem.*, 2012, **10**, 7031–7036.
4 S. V. Ley, R. J. Ingham, M. O'Brien and D. L. Browne, *Beilstein J. Org. Chem.*, 2013, **9**, 1051–1072.
5 R. J. Ingham, C. Battilocchio, D. E. Fitzpatrick, E. Sliwinski, J. M. Hawkins and S. V. Ley, *Angew. Chem., Int. Ed.*, 2014, **54**, 144–148.
6 S. Mascia, P. L. Heider, H. Zhang, R. Lakerveld, B. Benyahia, P. I. Barton, R. D. Braatz, C. L. Cooney, J. M. B. Evans, T. F. Jamison, K. F. Jensen, A. S. Myerson and B. L. Trout, *Angew. Chem., Int. Ed.*, 2013, **52**, 12359–12363.
7 R. I. Storer, T. Takemoto, P. S. Jackson, D. S. Brown, I. R. Baxendale and S. V. Ley, *Chem. – Eur. J.*, 2004, **10**, 2529–2547.
8 S. V. Ley, I. R. Baxendale, R. N. Bream, P. S. Jackson, A. G. Leach, D. A. Longbottom, M. Nesi, J. S. Scott, R. I. Storer and S. J. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3815–4195.
9 R. J. Ingham, C. Battilocchio, D. E. Fitzpatrick, E. Sliwinski, J. M. Hawkins and S. V. Ley, *Angew. Chem., Int. Ed.*, 2015, **54**, 144–148.
10 J. A. Newby, D. W. Blaylock, P. M. Witt, R. M. Turner, P. L. Heider, B. H. Harji, D. L. Browne and S. V. Ley, *Org. Process Res. Dev.*, 2014, **18**, 1221–1228.
11 T. Ouchi, C. Battilocchio, J. M. Hawkins and S. V. Ley, *Org. Process Res. Dev.*, 2014, **18**, 1560–1566.
12 S. Glöckner, D. N. Tran, R. J. Ingham, S. Fenner, Z. E. Wilson, C. Battilocchio and S. V. Ley, *Org. Biomol. Chem.*, 2015, **13**, 207–214.
13 S. V. Ley, D. E. Fitzpatrick, R. J. Ingham and R. M. Myers, *Angew. Chem., Int. Ed.*, 2015, **54**, 3449–3464.
14 S. Briem, B. Pettersson and E. Skoglund, *Anal. Chem.*, 2005, **77**, 1905–1910.
15 D. B. Broughton and C. G. Gerhold, *US Pat.*, 2985589, 1961.
16 A. G. O'Brien, Z. Horváth, F. Lévesque, J. W. Lee, A. Seidel-Morgenstern and P. H. Seeberger, *Angew. Chem., Int. Ed.*, 2012, **51**, 7028–7030.
17 J. W. Lee, Z. Horváth, A. G. O'Brien, P. H. Seeberger and A. Seidel-Morgenstern, *Chem. Eng. J.*, 2014, **251**, 355–370.
18 P. C. Wankat, *Ind. Eng. Chem. Res.*, 2001, **40**, 6185–6193.
19 J. W. Lee and P. C. Wankat, *J. Chromatogr. A*, 2010, **1217**, 3418–3426.
20 G. Strohlein, L. Aumann, M. Mazzottib and M. Morbidelli, *J. Chromatogr. A*, 2006, **1126**, 338–346.
21 E. Mahajan, A. George and B. Wolk, *J. Chromatogr.*, 2012, **1227**, 154–162.
22 M. A. Burns and D. J. Graves, *Biotechnol. Prog.*, 1985, **1**, 95–103.
23 A. Dispas, P. Lebrun, E. Ziemons, R. Marini, E. Rozet and P. Hubert, *J. Chromatogr. A*, 2014, **1353**, 78–88.
24 M. Saito, *J. Biosci. Bioeng.*, 2013, **115**, 590–599.

25 D. E. Fitzpatrick, C. Battilocchio and S. V. Ley, *Org. Process Res. Dev.*, 2016, **20**, 386–394.
26 J. Wu, K. Mikule, W. Wang, N. Su, P. Petteruti, F. Gharahdaghi, E. Code, X. Zhu, K. Jacques, Z. Lai, B. Yang, M. L. Lamb, C. Chuaqui, N. Keen and H. Chen, *ACS Chem. Biol.*, 2013, **8**, 2201–2208.
27 D. Crich and M. Y. Rahaman, *J. Org. Chem.*, 2009, **74**, 6792–6796.
28 D. Ruen-ngam, A. T. Quitain, M. Sasaki and M. Goto, *Ind. Eng. Chem. Res.*, 2012, **51**, 13545–13551.
29 T. Miyazawa and T. Funazukuri, *Biotechnol. Prog.*, 2005, **21**, 1782–1785.