Received: 30 April 2023 | Revised: 26 August 2023 | Accepted: 29 August 2023

DOI: 10.1002/jssc.202300300

RESEARCH ARTICLE

JOURNAL OF SEPARATION SCIENCE

Portable capillary LC for in-line UV monitoring and MS detection: Comparable sensitivity and much lower solvent consumption

Michael B. Hicks[1] | **Keith Mattern**[2] | **Jonathan Fine**[1] | **Shane Grosser**[2] | **Daya Patel**[1] | **Lauren Weisel**[1] | **Pankaj Aggarwal**[3]

[1]Analytical Research & Development, MRL, Merck & Co., Inc., Rahway, New Jersey, USA

[2]Process Enabling Technologies, MRL, Merck & Co., Inc., Rahway, New Jersey, USA

[3]Analytical Research & Development, MRL, Merck & Co., Inc., Massachusetts, Boston, USA

Correspondence
Michael B. Hicks, 126 East Lincoln Ave, Rahway, NJ 07065, USA.
Email: michael.hicks@merck.com.

Pharmaceutical development currently relies on quality separation methods from early discovery through to line-of-site manufacturing. There have been significant advancements made regarding the column particle packing, internal diameter, length connectivity, the understanding of the impact key parameters like void volume, flow rate, and temperature all that affects the resultant separation quality, that is, resolution, peak shape, peak width, run time, and signal-to-noise ratio. There is however a strong need to establish better alternatives to large bulky high-performance liquid chromatography racks either for process analytical reaction monitoring or mass spectrometry analysis in establishing product quality. Compact, portable high-pressure liquid chromatography can be a more efficient alternative to traditional ultra-high pressure liquid chromatography and traditional liquid chromatography. The compact versatile instrument evaluated here allows good separation control with either the on-board column with fixed ultra-violet wavelength cartridge or for use with a high-resolution mass spectrometry. Significant space reduction results in greener lab spaces with improved energy efficiency for smaller labs with lower energy demands. In addition, this compact liquid chromatography was used as a portable reaction monitoring solution to compare forced degradation kinetics and assess portable liquid chromatography-mass spectrometry capability for the analyses required for pharmaceutical drug product testing.

KEYWORDS
chromatography, compact, kinetics, miniaturization, portable

1 | INTRODUCTION

Miniaturized sample measurements are made routinely out of necessity in most pharmaceutical areas where sample sizes are limited, so high-throughput analyses allow getting the most information out of the least amount of material. For example, discovery microplates [1] are common for probing drug target-based activity [2, 3], for

Article Related Abbreviations: AMGS, analytical method greenness score; DILC^{TM}, patented direct inject liquid chromatography; ETC, Enabling Technologies Consortium; HVAC, heating ventilation and air conditioning; iCLC, in-line continous LC sampling; LED, light-emitting diode; MRL, the Research Labs at Merck & Co., Inc.; SQD, single quad mass spectral detection; SRM, single range monitoring mass spectrometry.

screening stem cells [4], profiling process synthesis stability [5]. There are even reported uses of miniaturization for the manufacturing stage with biosimilars [6]. However, while capillary electrophoresis (CE) is used as a typical analytical separation tool [7], there is much less focus on miniaturized liquid chromatography with fewer citable publications about portable liquid chromatography (LC) applications over the past decade [8, 9, 10-15], even though liquid chromatography is the most used separation technique in the industry.

Biologics and vaccines areas have historically relied very heavily on CE [16], and small molecules labs have routinely relied on capillary gas chromatography successfully for decades, especially with the appeal of gas chromatography-mass spectrometry (GC-MS) trending toward softer ionization methods [17]. However, when considering capillary LC, only over the last decade there has been major advances in the miniaturization of a flow cell and detector-in-one designs, for example, with an light-emitting diode (LED) light source and capillary column [18, 19]. These innovations have led to the clever use of syringe-metered pumps to deliver precise flow ratios of solvents through micro-flow tubing with a miniature valve, fitted with a nanoliter sample tube, for a compact LC experience [20, 21]. Recent systems by Axcend Ltd. were uniquely designed with inter-changeable column-containing cartridges fit with a miniature single wavelength Z-flow cell and LED source in one removable single wavelength cartridge. However, industry needs and the opportunity to expand the designs of these systems through the Enabling Technology Consortium (ETC) have led to very recent alterations to accept commercial capillary columns and provide column temperature heating capability. Axcend Ltd., through a collaborative partnership with the ETC, now accept more common, commercially available columns in the 150 μm ID, 50–150 mm length, and a 2.7–1.7 μm particle size ranges for their miniature LCs, while maintaining a cartridge-style column adapter [22]. One advantage that the Focus LC had over other past miniature LC systems was the higher pressure range of these systems up to 10,000 psi (689 bar), enabling the use of micro-LC columns with ultra-high-performance liquid chromatography (UHPLC) comparable column stationary phases using 1.7 μm particle sizes. Pressures and flow rates were therefore capable of running typical capillary-compatible flow rates successfully without damaging the system components. In the evaluation of a compact LC beta-test unit at MRL, we wanted to see how we could align the Axcend Focus LC unit to two areas of potential research need: one that focused on usage in the hood with on-line reaction monitoring tools, and the second for use as a sensitive single injection LC with mass spectrometry detection.

These studies tested the performance of the compact LC as a mobile separation option to generate data in direct comparison to a very typical ultra-high pressure LC industry standard conventional UHPLC system with ultraviolet (UV) detection. First, the Focus LC was used to compare the kinetics of a forced base degradation reaction of the drug substance typically used for method selectivity of a small molecule active pharmaceutical ingredient. Second, the Focus LC detection linearity and sensitivity was explored regarding the limit of quantitation and detection of the Focus LC coupled to a high-resolution mass spec were compared to a conventional LC-MS single quad mass spectral detection (SQD). We chose these two scenarios because reaction monitoring currently requires external UHPLC system stacks that are cumbersome, utilizing a lot of either bench or cart space. Similarly, high-resolution capillary or MS labs require most bench space for MS instrumentation, leaving less available space for both conventional and capillary LC systems. Having more portable solutions for each of these scenarios makes perfect sense for a portable separation solution for use by the current industry. These system designs are currently unlikely to replace the more qualified conventional LC systems for a quality control lab. However, with the evolution of most instrument design and innovation, as witnessed in the past with the transition from HPLC to UHPLC, we see a strong future opportunity for miniature LC systems to potentially progress and expand to many laboratories throughout the pharmaceutical research and development landscape.

2 | MATERIALS AND METHODS

2.1 | Chromatographic instrumentation

Standard chromatographic analyses were performed using an Infinity II, Agilent UPLC 1290 (Agilent, Inc.), equipped with a binary pump, column heater compartment column, and diode array detector. A Waters Acquity BEH C18, 2.0 × 50 mm, 1.7-μm column flowed to a diode array detector set to 254 nm, 2.5 Hz response time > 0.1 min, and 360 nm reference wavelength. All pre-column inlet and post detector outlet tubing were attached to an external six port switching valve (Valco Instruments Co. Inc.). This six port valve allowed a continuous flow of mobile phase either through the sample loop during injection or through the valve to a 20-μL injection loop, then to column and waste. The miniature Axcend Focus beta system (Axcend Technologies, LLC) was used for comparison equipped with internal binary syringe pumps, which runs through the same external six-port valve (as previously described) with a standard Agilent 0.2 μm in-line filter going into the internal microvalve system containing a 40-nL loop

to waste or into the onboard Waters X-Select HSS T3, 0.15 × 10 mm, 1.8 μm capillary column with Z-cell UV LED cell, 275 nm. An input and output from the reaction monitoring instrumentation allowed flow to either the Agilent or Axcend LC system.

2.2 | Reaction monitoring instrumentation

The reaction was carried out in a METTLER TOLEDO EasyMax 102 advanced synthetic workstation fitted with a 100-mL glass vessel, overhead pitched blade impeller, temperature probe, and pH probe (METTLER TOLEDO, GmbH). The base hydrolysis reaction was performed at a fill volume of 50 mL, and temperature was set to 60°C with a stir rate of 500 rpm using a pitched blade impeller. In-line reaction monitoring samples were taken using a prototype hardware version of Telescope Innovations' direct inject liquid chromatography (DILC) platform, which is now a commercially available product. This system was originally developed by the Hein Lab and utilized an external solvent valve, pump, and injection valve coupled to a METTLER TOLEDO EasySampler 1210 sampling probe to take and deliver samples to the LC system [23]. This system was designed to connect directly to a standard UHPLC system such as an Agilent 1290 but required modifications to connect to the capillary flow system of the Axcend Focus LC. The Agilent connection shown in Figure 1a utilized the standard DILC fluidic connections with a 100-μL sample loop. The Agilent system was triggered remotely to start the run once the sample was loaded in the injection loop. The Axcend Focus LC connection is shown in Figure 1b and bypasses the standard DILC injection loop flowing the sample directly to the Focus LC. The 1/16″ OD tubing of the DILC system is attached to the Focus LC capillary injection valve via a 1/16″−360 μm adapter and 360-μm stainless steel tubing. Solvent flow is directed to waste after the Focus LC injection valve. Sample analysis was remotely triggered from the DILC system via a single signal to ChemStation to trigger the initialization, pressurization, equilibration, and run on the Focus LC. Additionally, the capillary system of the Focus LC created a significant amount of backpressure that was too high for the DILC system; therefore, solvent flowrates were slowed down considerably when flowing through the Focus LC resulting in longer sampling times. Flushes of the DILC bypassed the Focus LC flowing directly to waste. Both systems utilized a 0.2-μm filter in-line at the inlet to the LC to prevent any solids from entering. See Figure 1 for the DILC setup for (a) the conventional LC and (b) the compact LC beta-test unit, also showing dimensions of each LC, with references to both.

2.3 | Base hydrolysis sample proportioning

The in-line reaction sampler requires ∼20 μL sample diluted with 1.6 mL of push volume solvent, approximately 80× with dispersion in the in-line flow tube to the LC. So, to deliver the appropriate volume (mg/mL) at the scale concentration, a reaction solution concentration of 5 mg/mL or 500 mg of the drug substance in 100 mL of the 90% water, 10% acetonitrile diluent was used. Trifluoroacetic acid was also purchased from Fisher Scientific. Acetonitrile HPLC Optima >99% grade, phosphoric acid 85 wt.% of ACS reagent grade, formic acid, and water were all Optima LC/MS grade reagents suitable for UHPLC-UV. Sodium hydroxide was ACS Reagent Grade from Fisher Chemical (Fair Lawn). The drug substance material used for the base degradation reaction was provided by MRL.

2.4 | Reaction control and monitoring software

iCLC Software, an earlier alpha-test version provided from Hein Lab (UBC), was used for sample and solvent movement to and from the probe to monitor the reaction coordinate. These data collections were then transferred to Agilent Open Labs for integration and processing for both the conventional and compact LC systems. For the Agilent system, data were acquired using ChemStation (Version C.01.05 or later).

2.5 | Mass spectral detection

We consider it most appropriate to test the compact LC where it would most likely be used for its portable advantages. A high-resolution MS lab with sizable high-resolution spectrophotometers but little space for HPLC racks made it an ideal lab environment. Hence, a Thermo Scientific Orbitrap Fusion Lumos (ThermoFisher Scientific) with a quadrupole mass analyzer was used based on availability with a micro-flow source. The MS mode was only used with scan range monitoring mode (150–350 amu) using Xcaliber (4.5.445.18) analysis to make a comparison to a more conventional LC-MS SQD. Compact LC effluent to the micro-source with electron transfer dissociation was collected using FreeStyle 1.8 SP1. For detector comparison to a non-capillary conventional HPLC Agilent SQD, LC-MS system was used for linearity and detection sensitivity comparison from scan mode (190–390) with mass extraction using Open labs on Windows 10 (Version C.01.05 or later).

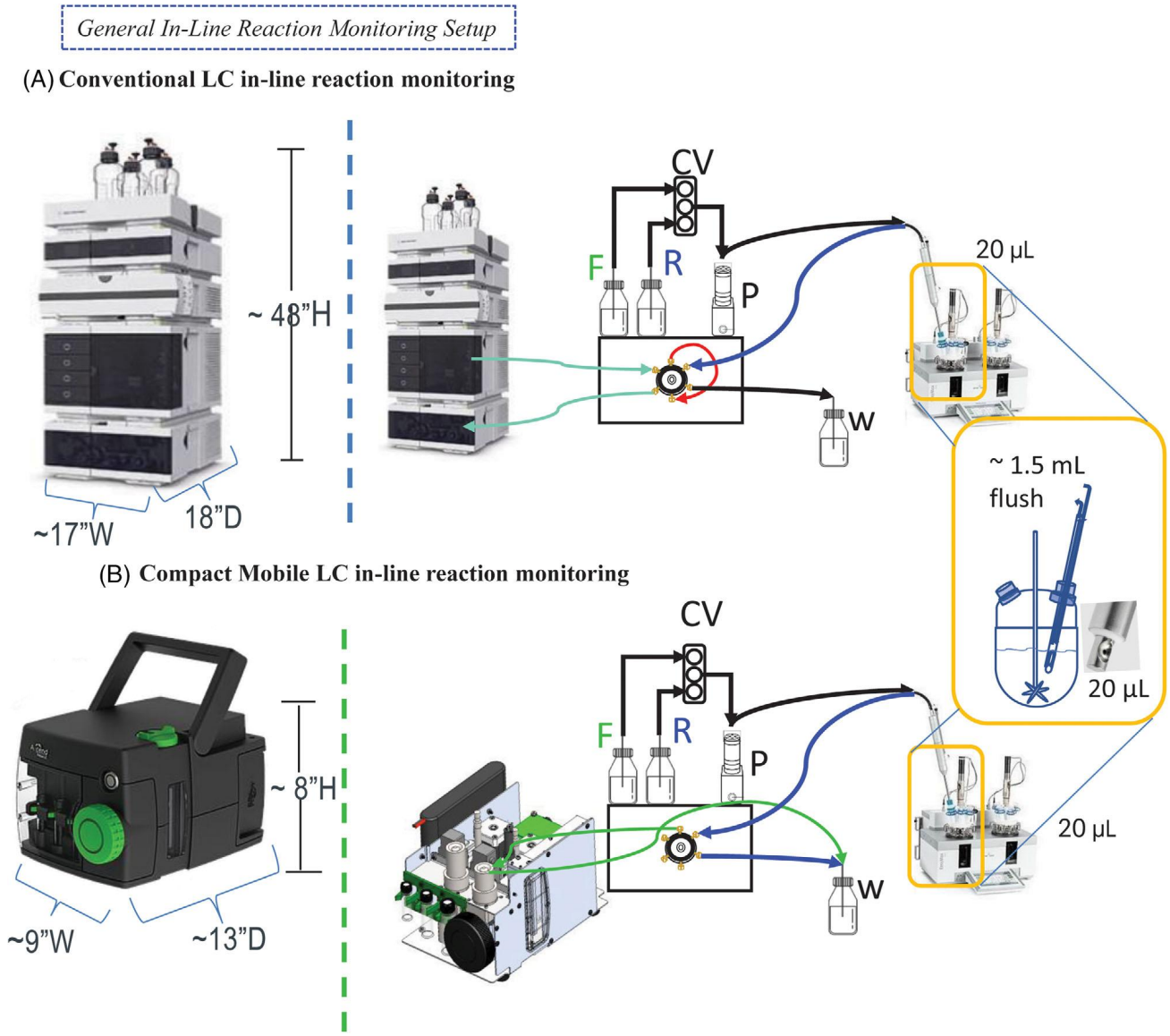

FIGURE 1 The reaction monitoring condition with (A) a conventional Agilent LC setup, where the solvent selection valve (CV) and pump (P) uses a dilution feed solvent (F) and a reaction rinse solvent (R) pumps through system with overage going to waste (W) (B) the portable compact LC using all the above in (a), except an internal LC-sample loop as part of the compact LC injection loop in place of the external selection loop (red loop in (a)).

3 | RESULTS AND DISCUSSION

3.1 | First-order dose-relevant degradation pathway

Drug substance and drug product responsibilities are shared with current pharmaceutical development teams in efforts to more holistically develop robust pharmaceutical products that are safe and reduce risks for patients, even through early phases of development. Analytical teams have expanded their roles to not only measure the overall quality of the active drug but also assess the active breakdown upon storage as a drug product, in formulations or even in the body. Understanding the primary degradation kinetics of the active pharmaceutical ingredient is essential and a significant undertaking.

Drug product degradation typically follows pseudo first-order kinetics, where the drug is in a suspension, capsule, or solid dosage drug product that degrades slowly to release the drug. Similarly, most hydrolysis/oxidation reactions in metabolism and in drug degradation follow first-order kinetics. Forced degradation procedures required for drug product method development determine the LC method performance [24]. The ability of a separation method to distinguish process impurities from degradation products is a critical attribute for assay/degradant (deg) drug product methods. The drug product "assay-degradant" LC method separates degradation products that can potentially be

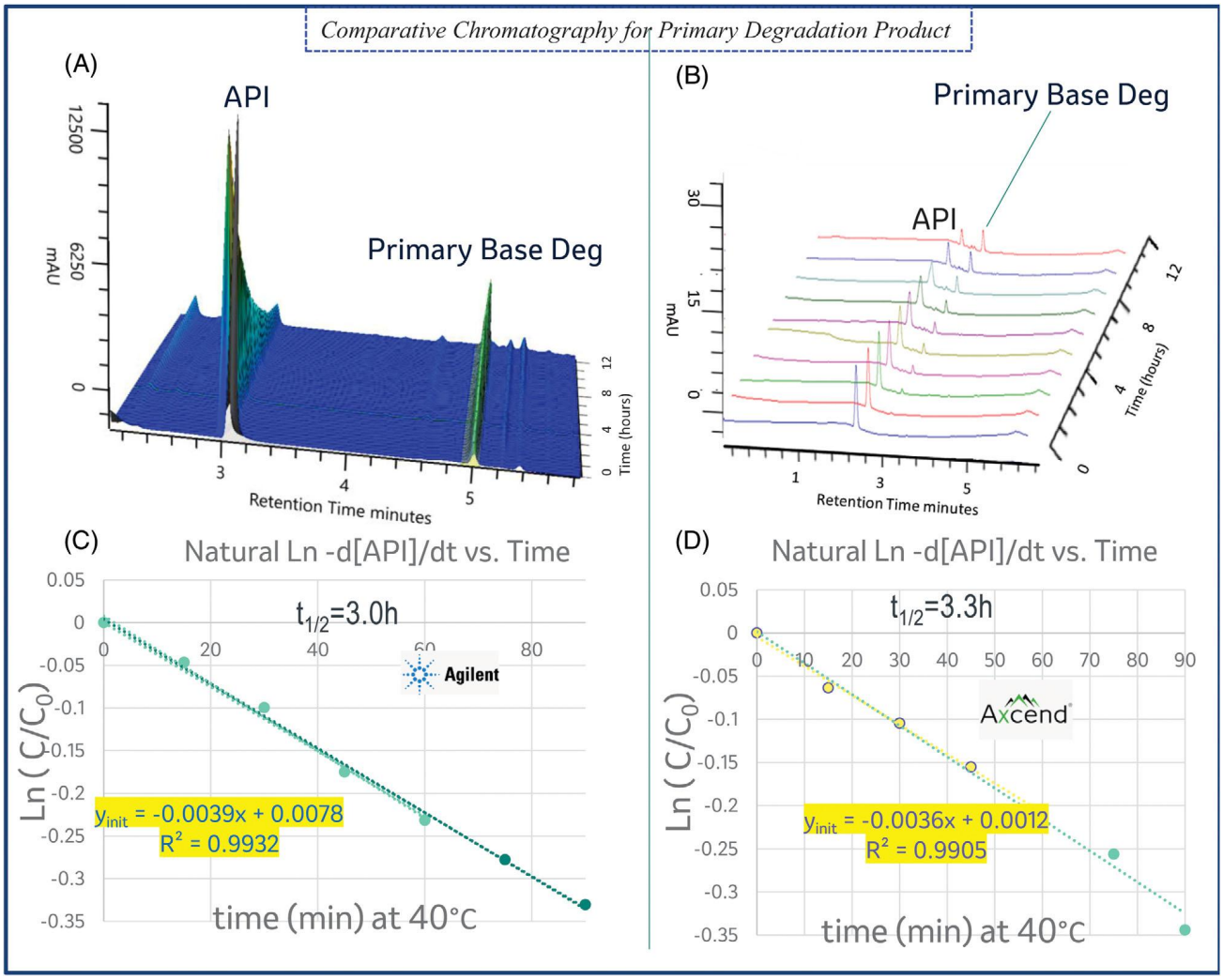

FIGURE 2 The reaction profiles during the degradation reaction: (A) the conventional ultra-high-performance liquid chromatography (UHPLC) using a Waters Acquity BEH C18 (2.0 mm × 50 cm, 1.8 μm) column at 30°C, 0.5 mL/min, 260 nm and (B) the portable compact LC using a Waters HSS T3 (150 μm × 10 cm, 1.8 μm) column, ambient temperature, 0.002 mL/min, 275 nm. Natural log of loss of the drug substance with time using ultraviolet (UV) with (C) the conventional LC 254 nm (D) the compact LC with pre-set detection at 275 nm.

formed with formulation storage, which is related to either packaging defects or off-label storage excursions. The key is to ensure that the forced treatment, in this case by base, is related to primary degs and not secondary degs that are not likely to occur during drug product use. In these studies, we chose to demonstrate that the compact LC can provide similar results for the degradation of the primary degradants that are collected using conventional LC.

Figure 2 shows (a) side-by-side comparison of a conventional LC to the compact LC chromatography during the reaction with base (b) conventional to compact first-order plot comparisons of log versus time for the hydrolysis reaction studies. We thought that this reaction-focused approach for understanding the degradation kinetics is much better than the current hit-or-miss strategy currently used for regular-stressed time interval samplings. Typically, in MRL drug product, intervals are selected at 12 and 24 h and then scaled back when over-stress degradation is observed. This latter strategy is appropriate to identify general stability liabilities. However, once a specific degradation-forming condition is known, kinetic monitoring at these stress-sensitive conditions will prevent a lot of repeat experiments to identify dose relevant critical degradants. Both types of LC show very similar first-order degradation half-lives to indicate that a portable instrument can be used in the hood of the reaction to achieve comparable results.

In addition to using the compact LC for looking at reactions in the hood, the team also wanted to investigate instrument performance as a compact separation tool for use with MS detection. We connected the instrument to a high-resolution instrument but used first-order MS detection methods. Note that the Lumos was selected based on availability and the micro-spray source capability.

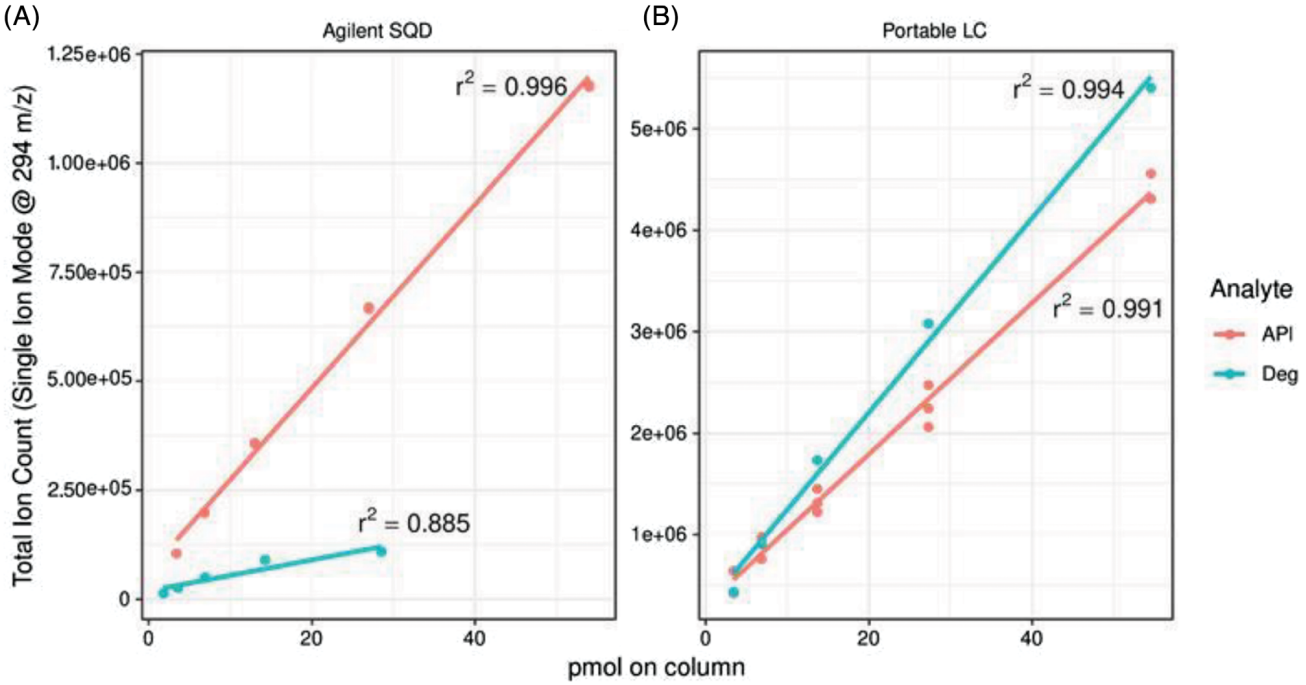

FIGURE 3 Comparative linearity of the drug substance isolated base degradation product (~95% pure) using response from (A) Agilent LC-MS SQD, a 5 μL injection with a BEH Acquity C18 100 mm × 2.1 mm, 1.7 μm comparison from scan mode (190–390 [*m/z*]) with mass extraction; (B) compact LC-Focus 2 μL/min, 40 nL loop with a Waters CSH-C19 150 μm × 50 mm, 1.7 μm capillary column at 40°C and Orbitrap, 50,000 resolution, scan 180−325 (*m/z*).

3.2 | Linearity for mass spectral data

Linearity is a critical parameter for the early to late-stage quantitation with any LC separation method to ensure that both low and high responses will report amounts based on similar signal response factors. The relative response ratios and ranges depend on critical peak attributes like peak width, tailing, and analyte absorbance extinction coefficients. These are particularly important at lower concentration levels. Good chromatographic method attributes will enhance peak shape to be clearly noise-distinguishable at the detection limit and provide reproducible results at both low and high concentrations, leading to higher signal-to-noise ratios at the low end and proper non-tailing and non-shifting peak shape at the high end of quantitation. These peak characteristics are all necessary elements in addition to excellent linear response. Figure 3 illustrates the linear response range plot Agilent LC-MS SQD in single range monitoring mass spectrometry (SRM) mode (left), compared to the linear response range plot for compact LC (right) with a LUMOS using only first mass analyzer in SRM mode. We found that while the capillary system had better response for the degradant peak, perhaps based on chromatography differences, both systems achieved a wide range linear response for the same very low level concentrations.

3.3 | Response sensitivity for a conventional UHPLC and a compact LC with MS

The analyte response near the limit of quantitation is critical for any methods' ability to detect and potentially quantitate degradation products from the atmospheric pressure ionization (API) in the formulated drug product. In addition to detector sensitivity, chromatography peak shape is often under-estimated regarding peak sharpness and the general ability of the chromatography to minimize peak broadening. There are many advantages with changing the larger stacked LC capillary systems to external, portable LC solutions in terms of saving space. A high-resolution mass spec lab already has minimal available space due to many high-resolution mass spectral detectors. These labs could benefit from a portable system that could be brought from MS system to MS system. The Thermo LUMOS was available and often used with an external Waters capillary system. This system was temporarily replaced with the compact LC to understand operations and determine challenges. It was very encouraging that in light of several challenges getting the tubing as close as possible to the spray source and achieving reproducible flow rates, a comparable if not slightly improved signal to noise was obtained. Figure 4 shows the relative signal to

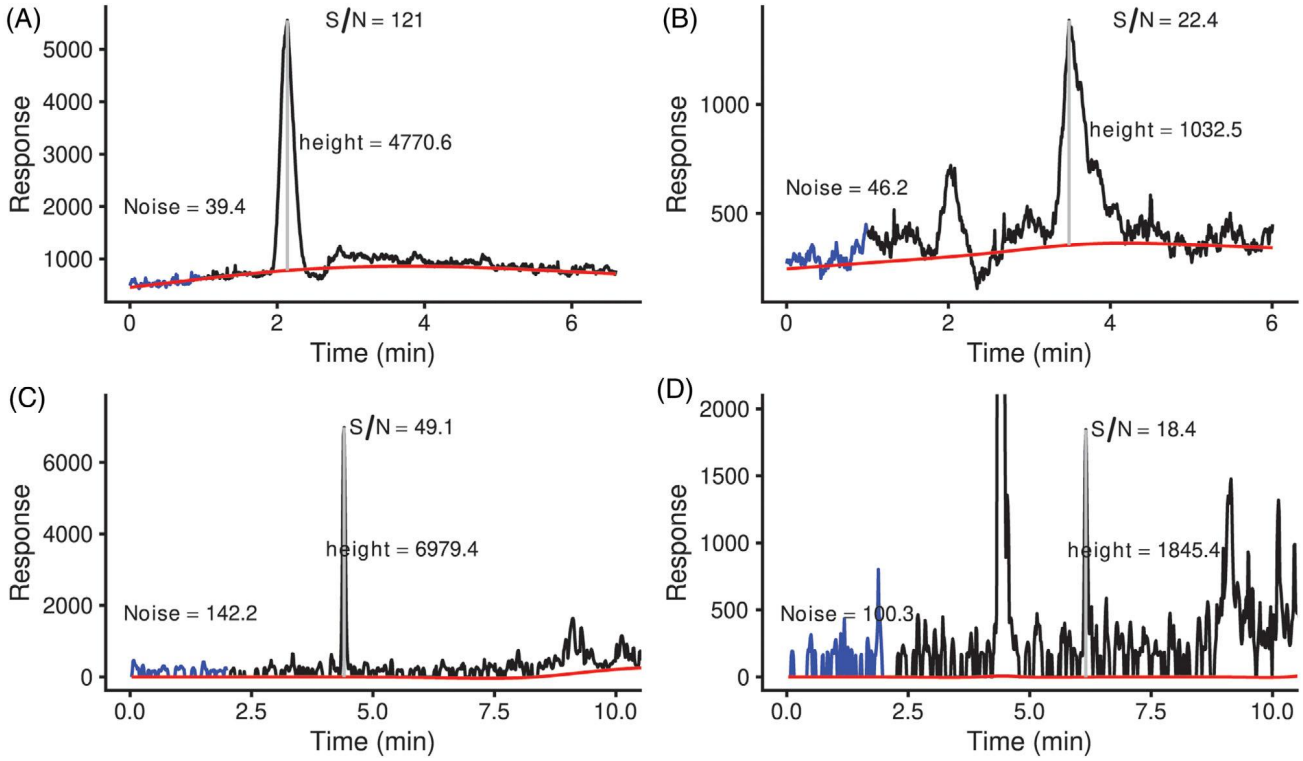

FIGURE 4 Sensitivity comparisons for the drug substance at 2 min (A) and the major base degradation product at 3.8 min (B) using the compact-LC-MS detection to the drug substance at 4.7 min (C) and the major base degradation product 6.8 min (D) with conventional LC-MS, SQD. The signal-to-noise (S/N) ratios were calculated using similar mass spectrometry (MS) scan ranges for both instruments.

noise ratio of the Agilent compared to the compact LC. The noise was calculated using the initial start of the chromatogram as shown in blue. Peak at baselines shown in red was calculated using the asymmetric least squares algorithm as implemented in the ALS baseline package [25], and noise was calculated as the average absolute difference between the signal and the baseline. Signal-to-noise ratio, as calculated here, was the ratio between the peak height and calculated noise. The portable system shows very good signal-to-noise ratio for both the active drug substance and the base degradant.

3.4 | Spiked placebo blend linearity and relative signal to noise ratio

Reduction to practice for both linearity and sensitivity involves the use of the drug substance in actual extracted placebo samples. Often, depending on the polar or non-polar separation nature for the API in the DP, mass spectral linearity and sensitivity can be an issue, where potential co-extracted interferences could lead to matrix suppression or other detection-altering scenarios. There is no better way to demonstrate these differences than to work with the placebo sample. Figure 5 illustrates both the drug substance linearity and peak shape in the presence of the placebo blend despite the presence of a closely eluting tablet-based excipient for the compact LC chromatography.

4 | FURTHER DISCUSSION

4.1 | Why do we want to miniaturize liquid chromatography HPLC/UHPLC?

LC has been well established for over five decades and is undisputedly the separation method of choice for all regulatory methods and standardized test methods. The use of HPLC/UHPLC to quantitatively monitor process impurities, establish potency, confirm drug product content uniformity, and determine either %label claim or dissolution release rates is critical for any drug portfolio. A portable compact version of HPLC/UHPLC that will bring this powerful separation tool to many labs that normally could not afford it would be a formidable advantage. The reduced footprint is the first advantage. For instance, shifting real estate demand looking toward a post pandemic future has resulted in re-thinking how we work. There is a push toward more modular laboratories and the need for much more flexibility with lab space. More than any single instrument, reaction raw material,

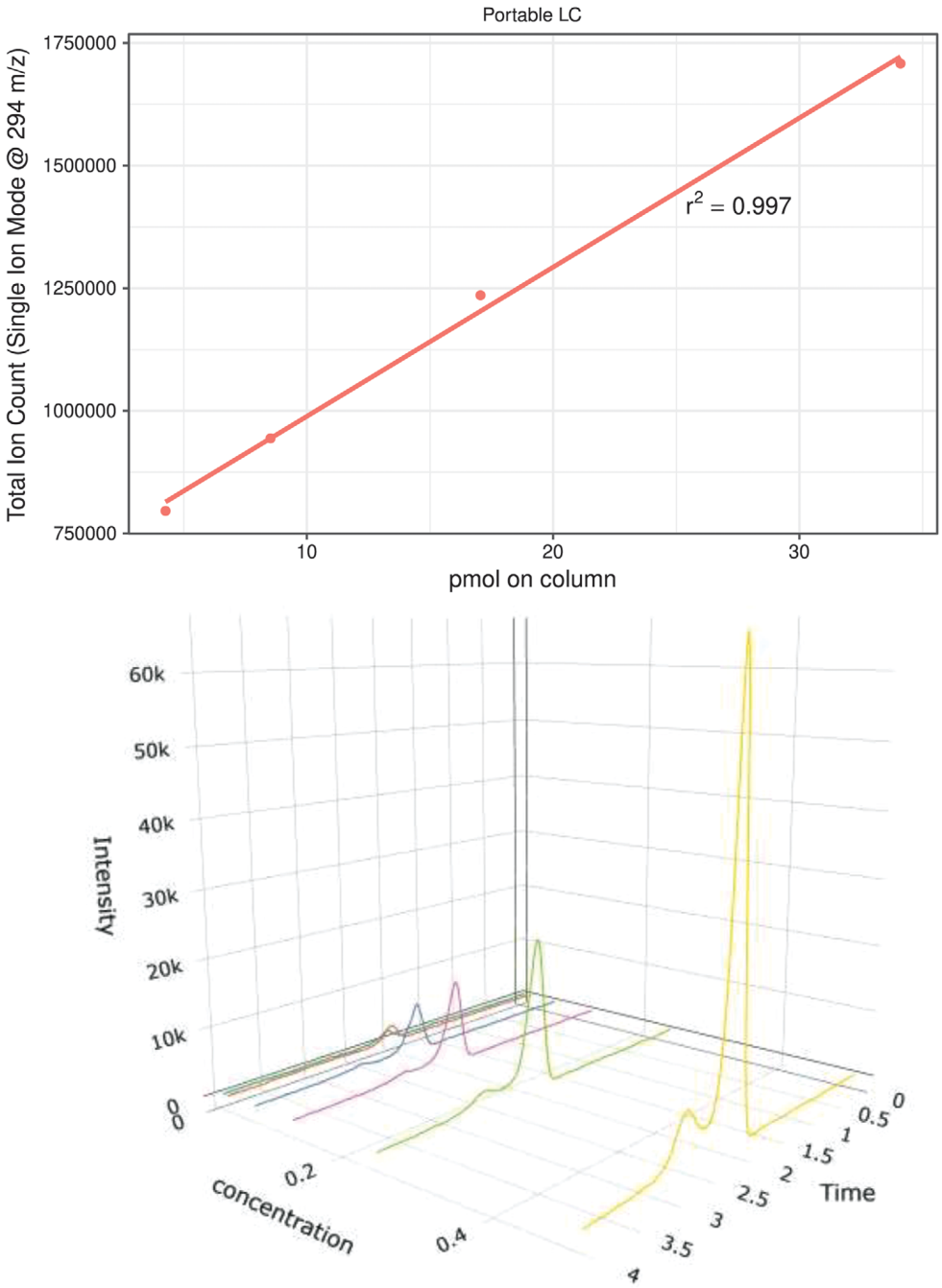

FIGURE 5 Spiked blend linearity of the drug substance in the presence of the placebo blend from the compact LC-Focus with a Waters LUMOS 2 μL/min, 40 nL loop with a Waters CSH-C18 150 μm × 50 mm, 1.7 μm capillary column at 40°C and Orbitrap, 50,000 resolution, scan 180–325 (*m/z*) (top); chromatogram showing the drug substance at several picomolar concentrations spiked into the placebo blended material (bottom).

or process, the costliest energy demand associated with any laboratory is heating ventilation and air conditioning (HVAC). So as future-minded open lab spaces become more flexible and task focused, miniaturization is a natural direction to ensure the best utilization of lab space. A second major advantage is the reduction in the solvent and energy demand typical of traditional chromatography instruments.

What does reducing solvent amounts mean from a sustainable solvent use perspective? Recently, analytical departments at Merck & Co., Inc. (Rahway, NJ) and other pharmaceutical companies practicing methods development have used the analytical method greenness score (AMGS) metric to track solvent and energy consumption of methods that are used thousands of times in a drugs' lifecycle. The metric gave practical and measurable

TABLE 1 The analytical method greenness score (AMGS) and relevant method parameter comparisons for conventional high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) with the compact liquid chromatography (LC).

Metric/parameter	HPLC method	UHPLC method	Compact LC
AMGS	**1682**	**754**	**80**
Instrument energy score	850 (50%)	335 (44%)	3 (4%)
Solvent energy score	349 (21%)	260 (34%)	47(58%)
Solvent EHS score	483 (29%)	159 (22%)	31 (38%)
Flow rate (mL/min)	1.5	0.4	0.002
Run time (min)	45	30	30
Sample preparation volume (mL)	45	45	45
Total (mL), 13 runs	878	156	0.8

statistics that one could benchmark and work to improve when considering development [26]. One thing that miniaturized LC methods brought is a reduction in solvent and energy consumption by several orders of magnitude. Table 1 shows comparison between conventional LC types (HPLC/UHPLC) and compact LC. The compact LC does not include the energy of having an onboard autosampler since it uses a single syringe load port, where both conventional LC systems included energy scores with external stack autosamplers. However, it is quite evident that the flow rate makes the greatest impact in solvent energy and the portable all-in-one unit has considerably lower energy consumption. All these factors contribute to a low score that implies a portable solution to LC that could provide comparable data and would be a very sustainable alternative to current conventional systems. In addition, laboratory spaces tend to raise costs and result in a larger carbon footprint. The large number of analytical development, process chemistry and biologics labs globally, contribute to the overall CO_2 emissions footprint with very high HVAC and electrical energy demands [27]. So, miniaturization tools are a much more sustainable trend for more sustainable labs of the future with a smaller space and instrument footprint demand.

Table 1 illustrates that there is both a solvent usage element and an instrument energy burden toward separation method sustainability. So, the number of injections, the flow rate, the run time, the energy consumption, while the instrument is running, and the solvent selection with its energy demands for disposal are all considered with the AMGS metric. The resultant method sustainability values are an order of magnitude lower with the use of this miniaturized LC alternative.

4.2 | Implementation of portable LC solutions for online reaction monitoring

Process analytical technologies and data-rich experimentation teams at Merck & Co., Inc. rely on innovative tools to analyze reactions in real time. There are many methods of monitoring reactions from this group that involve spectroscopic, solution pH, viscometrical, electrochemical, and thermal detection measurements, all available in real time with reaction probes. Among these tools is the use of online sampling with tools like the in-line reaction sampler described previously. The team relies on full LC racks on carts outside of the reaction hoods. These carts require that engineers roll the cart in front of the hood to align with probe sampling methods. Over the past 5 years, the Hein Lab (UTC BC) has developed a unique and precise means of automating the 20 μL sampling tool to bring the sample via external pump through a convention LC loop in a six-port sampling valve. This procedure has been used for effectively studying reactions [28, 29]. In our efforts, we collected data from a typical forced degradation of the drug substance that required a better understanding of the degradation kinetics and compared that with the same reaction but replacing the conventional UHPLC with a compact LC. The sampling procedure for the online conventional analysis at MRL has been published prior. The optimal volume needed to carry the 20 μL sample from the probe to the LC injection loop, referred to as the push volume, was optimized by varying the push volumes and measuring the peak response. Once this maximum response is known, the push volume can be determined from the slope to ensure more reproducibility between injections. This system setup resulted in push volumes of 1.2 and 1.4 mL for the conventional LC and the compact LC, respectively. Figure 1 shows the typical setup to complete online sampling and a comparison of the two systems.

4.3 | Specificity: focus shift from drug process to drug product

Drug substance impurity methods require, among other method attributes, linearity, sensitivity, and analyte resolution for constantly changing chemical processes. Drug product LC methods also require the additional separation of difficult formulation excipient interferences. While many drug substance purity methods are adequate for drug product analysis, in many cases these methods are not always sufficient and can involve the greatest separation method challenges for drug-product stability indicating methods. This is particularly the case for active pharmaceutical ingredients that degrade in the presence of the formulation, such as under accelerated temperature and

humidity conditions, for example, open-dish 40°C/75% relative humidity. Drug substance forced- degradation experiments become very important to identify potential degradation pathways for example photodegradation, hydrolysis, oxidation, epimerization, whether specific to base or acid and temperature. These same drug substance pathways hold true for the drug product formulation and identify formulation or packaging issues early.

4.4 | Comparative alternatives to conventional LC for mass spectral detection

While separations are a necessary lab cost, capital is best spent on other lab resources, for example, NRM, MS, automation capabilities for either process capabilities or other specialized detection methods, for example, charged aerosol detection, electrochemical, or ionization detection to enhance and advance the lab capability. Most MS laboratories will spend millions on acquiring the most current high-resolution spectrophotometers. This leaves little for large HPLC stacks that require dedicated lab bench space or large movable racks for moving among MS detectors. The compact LC is the future for many where chromatography is not the sole focus but where the need for robust methods with high sensitivity and reproducibility is also essential.

Many high-resolution MS groups develop optimized conditions to isolate peptide or protein ions with particular focus on the strategy to ensure daughter ion stability, consistent sheath gas flow, and focusing the spray to enable optimal detection. While there has been a lot of efforts to provide a microflow or nanoflow stream to a microflow or nanoflow sources using conventionally sized UHPLCs, there will be a lot more effort to improve compact LC capability and options for the advantages discussed previously. Medina et al. in a recent review [30] highlight the different miniaturized source developments and innovations like lab on a chip and compact mobile instruments that could be the future for MS laboratories.

5 | CONCLUDING REMARKS

In these studies, we determined that a mobile/portable LC system can provide comparable data to conventional LC-PDA systems as a solution for in-line kinetic reaction monitoring measurements for base hydrolysis and similar off-line LC-MS measurements. The key degradant was easily separated and able to be tracked to show the same hydrolysis reaction half-life. Similarly, the compact LC was able to obtain very good linearity and signal-to-noise ratio when used as a portable LC solution for MS analysis. There were some issues regarding minimizing system tube lengths for setup with the microspray source MS at the lower flow rates. Getting the compact system as close to the spray source as possible provided optimal results to achieve the needed performance for this beta-test Compact LC unit. These compact mobile separations systems lay the groundwork for a better understanding of how compact LC systems can be employed. These advances and innovations can only lead to future system improvements resulting in more sustainable methods for the future labs. These portable solutions will also help provide an improved solution to couple with higher resolution mass spec instruments, providing comparable performance to conventional LCs currently employed.

ACKNOWLEDGMENTS

We thank ETC and our partners from Axcend Ltd. that provided the system and opportunity to use and provide feedback for the beta unit testing. We also want to thank and acknowledge the Merck & Co., Inc. innovation team for their contributions to funding the research and supporting efforts with this new compact separation technology. Also special thanks to Alena Bensussan Preclinical Development Rahway and Olivier Mozziconacci currently in MRL discovery San Francisco for allowing access and providing data insight on MS data handling and export. The beta unit for use with kinetics analysis was purchased and owned by MRL Process Enabling Technologies group at the time of the testing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Supporting information regarding signal-to-noise calculations will be made available upon request.

ORCID

Michael B. Hicks https://orcid.org/0000-0003-1005-4953

REFERENCES

1. Mayr LM, Bojanic D. Novel trends in high-throughput screening. Curr Opin Pharmacol. 2009;9:580–88.
2. Holbrook-Smith D, Durot S, Sauer U. High-throughput metabolomics predicts drug–target relationships for eukaryotic proteins. Mol Syst Biol. 2022;18:e10767.
3. Ye C, Ho DJ, Neri M, Yang C, Kulkarni T, Randhawa R, et al. DRUG-seq for miniaturized high-throughput transcriptome profiling in drug discovery. Nat Commun. 2018;9:1–9.
4. Carpentier A, Nimgaonkar I, Chu V, Xia Y, Hu Z, Liang TJ. Hepatic differentiation of human pluripotent stem cells in miniaturized format suitable for high-throughput screen. Stem Cell Res. 2016;16:640–50.

5. Krska SW, DiRocco DA, Dreher SD, Shevlin M. The evolution of chemical high-throughput experimentation to address challenging problems in pharmaceutical synthesis. Acc Chem Res. 2017;50:2976–85.
6. M Wooding K, Peng W, Mechref Y. Characterization of pharmaceutical IgG and biosimilars using miniaturized platforms and LC-MS/MS. Curr Pharm Biotechnol. 2016;17:788–801.
7. Fanali C, Fanali S. Chiral separations using miniaturized techniques: state of the art and perspectives. Isr J Chem. 2016;56:958–67.
8. Aturki Z, Rocco A, Rocchi S, Fanali S. Current applications of miniaturized chromatographic and electrophoretic techniques in drug analysis. J Pharm Biomed Anal. 2014;101:194–220.
9. Shaaban H. New insights into liquid chromatography for more eco-friendly analysis of pharmaceuticals. Anal Bioanal Chem. 2016;408:6929–44.
10. Berlioz-Barbier A, Baudot R, Wiest L, Gust M, Garric J, Cren-Olivé C, et al. MicroQuEChERS–nanoliquid chromatography–nanospray–tandem mass spectrometry for the detection and quantification of trace pharmaceuticals in benthic invertebrates. Talanta. 2015;132:796–802.
11. Ishida A, Fujii M, Fujimoto T, Sasaki S, Yanagisawa I, Tani H, et al. A portable liquid chromatograph with a battery-operated compact electroosmotic pump and a microfluidic chip device with a reversed phase packed column. Anal Sci. 2015;31:1163–69.
12. Blue LE, Franklin EG, Godinho JM, Grinias JP, Grinias KM, Lunn DB, et al. Recent advances in capillary ultrahigh pressure liquid chromatography. J Chromatogr A. 2017;1523:17–39.
13. Coates LJ, Lam SC, Gooley AA, Haddad PR, Paull B, Wirth H-J. Modular, cost-effective, and portable capillary gradient liquid chromatography system for on-site analysis. J Chromatogr A. 2020;1626:461374.
14. Foster SW, Xie X, Pham M, Peaden PA, Patil LM, Tolley LT, et al. Portable capillary liquid chromatography for pharmaceutical and illicit drug analysis. J Sep Sci. 2020;43:1623–27.
15. Lam SC, Coates LJ, Hemida M, Gupta V, Haddad PR, Macka M, et al. Miniature and fully portable gradient capillary liquid chromatograph. Anal Chim Acta. 2020;1101:199–210.
16. Suntornsuk L. Capillary electrophoresis in pharmaceutical analysis: a survey on recent applications. J Chromatogr Sci. 2007;45:559–619.
17. Gruber B, David F, Sandra P. Capillary gas chromatography-mass spectrometry: current trends and perspectives. TrAC, Trends Anal Chem. 2020;124:115475.
18. O'Toole M, Lau K-T, Shazmann B, Shepherd R, Nesterenko PN, Paull B, et al. Novel integrated paired emitter-detector diode (PEDD) as a miniaturized photometric detector in HPLC. Analyst. 2006;131:938–43.
19. Zhao X, Xie X, Sharma S, Tolley LT, Plistil A, Barnett HE, et al. Compact ultrahigh-pressure nanoflow capillary liquid chromatograph. Anal Chem. 2017;89:807–12.
20. Nazario CED, Silva MR, Franco MS, Lanças FM. Evolution in miniaturized column liquid chromatography instrumentation and applications: An overview. J Chromatogr A. 2015;1421:18–37.
21. Lynch KB, Chen A, Liu S. Miniaturized high-performance liquid chromatography instrumentation. Talanta. 2018;177:94–103.
22. Breitbach Z. Collaboratively advancing compact liquid chromatography for supporting process understanding and automating analytical instrumentation in pharmaceutical and biopharmaceutical R&D. Pittcon 2023, Session S23-04. 2023.
23. Malig TC, Koenig JDB, Situ H, Chehal NK, Hultin PG, Hein JE. Real-time HPLC-MS reaction progress monitoring using an automated analytical platform. React Chem Eng. 2017;2:309–14.
24. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—a review. J Pharm Anal. 2014;4:159–65.
25. Eilers PH, Boelens HF. Baseline correction with asymmetric least squares smoothing. Leiden University Medical Centre Report. 2005.
26. Hicks MB, Farrell W, Aurigemma C, Lehmann L, Weisel L, Nadeau K, et al. Making the move towards modernized greener separations: introduction of the analytical method greenness score (AMGS) calculator. Green Chem. 2019;21:1816–26.
27. Dong Y, Coleman M, Miller SA. Greenhouse gas emissions from air conditioning and refrigeration service expansion in developing countries. Annu Rev Environ Resour. 2021;46:59–83.
28. Chung R, Hein JE. The more, the better: Simultaneous in situ reaction monitoring provides rapid mechanistic and kinetic insight. Top Catal. 2017;60:594–608.
29. Christensen M, Adedeji F, Grosser S, Zawatzky K, Ji Y, Liu J, et al. Development of an automated kinetic profiling system with online HPLC for reaction optimization. React Chem Eng. 2019;4:1555–58.
30. Vargas Medina DA, Maciel EVS, de Toffoli AL, Lanças FM. Miniaturization of liquid chromatography coupled to mass spectrometry: 2. Achievements on modern instrumentation for miniaturized liquid chromatography coupled to mass spectrometry. TrAC, Trends Anal Chem. 2020;128:115910.

How to cite this article: Hicks MB, Mattern K, Fine J, Grosser S, Patel D, Weisel L, et al. Portable capillary LC for in-line UV monitoring and MS detection: Comparable sensitivity and much lower solvent consumption. J Sep Sci. 2023;2300300. https://doi.org/10.1002/jssc.202300300