

# Shimadzu Journal

Vol **08**  
ISSUE1

Pharmaceutical

# Dear Readers,



**Shuzo Maruyama**

General Manager,  
Analytical & Measurement Instruments Division



The new coronavirus (COVID-19) has now become a global pandemic. Shimadzu Corporation would like to express our greatest sympathies to those affected by COVID-19. We would also like to offer our deepest condolences to the loved ones of those who lost their lives from the disease.

In order to prevent further spread of the new coronavirus, we intend to implement measures in accordance with government instructions for the safety of our customers, the local community, business partners, employees, and others. At the same time, as a company with the corporate philosophy of contributing to society through science and technology, we intend to develop products and technologies that can contribute to ameliorating the situation.

We recently announced the release of the "2019 Novel Coronavirus Detection Kit", with sales beginning on 20th April in Japan. The "2019 Novel Coronavirus Detection Kit" uses our unique Ampdirect technology, which allows the PCR reaction solution to be added directly to the biological sample without the need for extracting and purifying RNA. Therefore, this detection kit significantly reduces the amount of work required to prepare samples and, moreover, halves the overall time required

for PCR detection. Preparations are underway to export these kits overseas. We will make every effort to combat this crisis, applying the full capabilities of the Shimadzu Group.

In this issue, we describe the application of Shimadzu products in the pharmaceuticals market. It's highlighted by an interview with Dr. Mirlinda Biba, Principal Scientist at Merck & Co., Inc. Dr. Biba was a member of the semi-preparative SFC project team at the Enabling Technologies Consortium. Shimadzu was selected by them as a collaborative partner, a partnership that resulted in the development of the Nexera UC Prep SFC system. This issue also introduces multiple applications using various analytical techniques. These include the use of LC-MS/MS for the identification of the genotoxic impurity NDMA in ranitidine and metformin, and the identification of an impurity related to the ICH Q3D elemental impurities guideline using ICP-MS and EDX.

At Shimadzu, we are integrating techniques from analytical instruments, medical systems and other fields, aiming to contribute globally at an even deeper level to the advanced health-care field. We hope that this issue will prove to be a great help to all of you. Your generous feedback is always appreciated.

**Yours Sincerely,**

## INSIGHT FROM CUSTOMER

04



### Interview

#### Interview with Dr. Mirlinda Biba

We interviewed Dr. Mirlinda Biba, a Principal Scientist in the Analytical R&D department at Merck in the United States. She has been working with Supercritical Fluid Chromatography (SFC) for almost 20 years. She is also a member of the Enabling Technologies Consortium (ETC) and joined the project to develop a new preparative SFC instrument.

08



### Report

#### Cross-Pharma Collaboration on the Development and Evaluation of a New Mid-Scale Preparative Supercritical Fluid Chromatography Instrument

Precompetitive collaborations focused on the development of new technologies for conducting pharmaceutical research and development are becoming increasingly popular. This article reports on one of the first development projects from the recently formed Enabling Technologies Consortium (ETC): the creation of a new instrument for carrying out mid-scale preparative supercritical fluid chromatography purifications.

## REPORT

19



### Report 1

#### Application of HPLC in Quality Analysis of Hydroxychloroquine Sulfate

In this study, methods were established for analysis of hydroxychloroquine sulfate and related substances following European Pharmacopoeia (EP10.0) and for determination of hydroxychloroquine sulfate content in tablets following US Pharmacopoeia (USP 43). Using a Shimadzu UHPLC system, excellent separation was achieved between hydroxychloroquine sulfate and impurity C, as well as between impurity B and impurity C referring to the EP assay.

23



### Report 2

#### Determination of genotoxic impurity NDMA in Ranitidine by LCMS-8050 and LCMS-9030

Based on the conditions recorded in FDA documents, we developed a method for the analysis of NDMA in ranitidine drug substance and capsule preparations using the Shimadzu LCMS-8050 Liquid Chromatograph-Tandem Mass Spectrometer, and a method using the LCMS-9030 Q-TOF Mass Spectrometer for the reference of relevant testers.

28



### Report 3

#### Determination of genotoxic impurity NDMA in Metformin by LCMS-8050 and GCMS-TQ8050 NX

This study presents the development of three methods to detect NDMA in metformin using the Shimadzu LCMS-8050, GCMS-TQ8050 NX and GCMS-QP2020 NX, based on the recipe published by the National Institutes for Food and Drug Control and Health Sciences Authority of Singapore.

33



### Report 4

#### The Necessity of Analyzing Elemental Impurities in Drug products, and Approaches to Their Measurement

This article introduces approaches to analyzing elemental impurities by ICP-MS or EDX-RF based on ICH Q3D (ICH: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Q3D: Guideline for Elemental Impurity).

## SHIMADZU SELECTION

35



These articles were selected by Shimadzu. Relating pharmaceutical analysis and development, they are from posters presented at ASMS 2019 and from application notes. They feature a variety of instruments we produce and include cutting-edge technologies. Please obtain the articles of your interest through the links on the titles.

## LATEST TOPICS

37



**Latest Topics 1**

### Global Pharma Summit 2019 Report

On November 14th, 2019, Shimadzu (Asia Pacific) hosted The Global Pharma Summit in Singapore. This was the seventh chapter of the event, with the theme of "Transforming the Future of Pharma". The event brought 103 pharmaceutical industry leaders, health-care providers and researchers to Singapore from 20 countries around the world.

39



**Latest Topics 2**

### Shimadzu Scientific Instruments Partners with Northern Michigan University to Support Medicinal Plant Chemistry Program

Shimadzu Scientific Instruments (SSI) and Northern Michigan University (NMU) have partnered to establish the Shimadzu Analytical Core Laboratory for Medicinal Plant Sciences. Through this collaboration, SSI has donated instrumentation for the laboratory to support NMU's first-in-the-nation medicinal plant chemistry program.

40



**Latest Topics 3**

### Shimadzu and Providence Cancer Institute Partner to Advance Cancer Immunotherapy Research

Shimadzu Corporation (Shimadzu) and the Earle A. Childs Research Institute, a division of Providence Cancer Institute (Providence) in Portland, Oregon, have entered into a joint research agreement to apply mass spectrometry technology to develop tools for personalized cancer immunotherapeutics.

41



**Latest Topics 4**

### Celebrating 50 Years of Mass Spectrometry Innovation

The year 2020 marks 50 years since the release of Shimadzu's first mass spectrometry device, the LKB-9000. To celebrate this occasion, Shimadzu is planning a number of activities throughout the year.

## NEW PRODUCTS

43



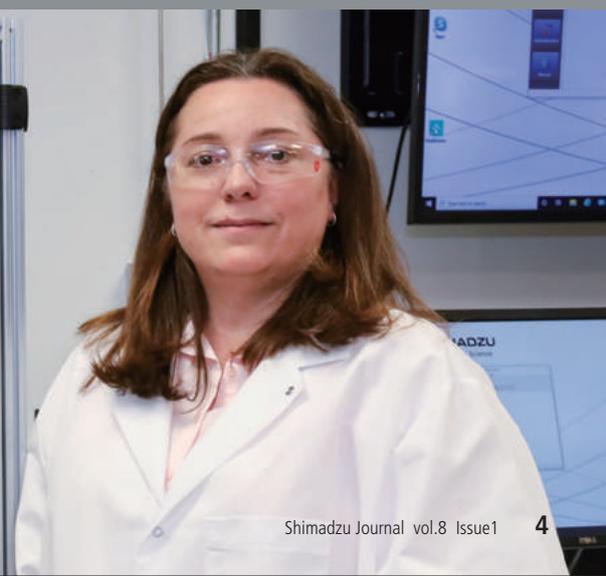
LCMS-8060NX, UV-i Selection, Shim-pack Bio HIC

# Supercritical fluid chromatography

INTERVIEW

## Dr. MIRLINDA BIBA

Mirlinda Biba is a Principal Scientist in the Analytical R&D at Merck. She has almost 20 years of experience working with analytical and preparative supercritical fluid chromatography. She joined Merck in 2001 in the Separations Lab where she worked with some of the first preparative SFC instruments. She also led the SFC team and helped with advancing the SFC technology for GMP chiral analysis in commercial space. Over the years she has partnered with many industrial and academic collaborators to advance the supercritical fluid chromatography technology and applications. Her research work in this area has resulted in over 40 publications and numerous presentations at various conferences.



**Dr. Biba, thank you very much for spending some time for this interview. Please tell us a little bit about yourself and how you got involved in the project?**

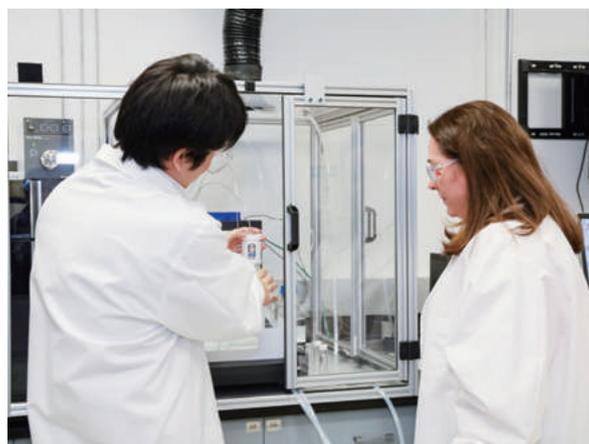
Thank you for the opportunity to share my experience. I have been working with supercritical fluid chromatography (SFC) for almost 20 years. I joined Merck in the Process Chemistry department in 2001 and worked in Dr. Chris Welch's Separations lab. The group had just acquired one of the first Multigram II preparative SFC systems by Dr. Terry Berger and this became my first project. During that time, chiral chromatography was key for delivering enantiomerically pure compounds for pharmaceutical development and clinical studies. Due to its unique selectivity for chiral resolution, speed, and 'greener' approach compared to preparative HPLC, preparative SFC quickly became the technique of choice for preparative chiral separations. Over the years, the use of preparative SFC has expanded to achiral separations, impurity isolations, and more recently for peptides and proteins. Preparative SFC is now widely used in the pharmaceutical industry for small scale purifications to support drug discovery and development.

The rapid growth of preparative SFC area led to a few startup companies entering the marketplace, and smaller companies merging

or continuously being acquired by larger ones which ultimately led to established products becoming unavailable. Therefore, replacing aging or obsolete instruments was difficult with the few suitable options available. As a result, preparative SFC users who were participating members of Enabling Technologies Consortium (ETC) got together to solve this problem.

**Could you outline the project and let us know why the project was started? Why was Shimadzu selected as the partner?**

Preparative SFC users across the pharmaceutical industry were faced with the challenges to replace aging and obsolete instruments and felt that the available instruments on the market had some limitations and did not meet their requirements. In order to address this problem, participating members of ETC got together and created a request for information (RFI) for developing a new preparative SFC instrument that would meet their list of requirements. The team generated a list of desired features, including both hardware and software instrument specifications. The RFI was published through the ETC website in July, 2016 and received interest from a number of potential suppliers. After careful review and discussions with several vendors, the



team selected Shimadzu Corporation as the partner for this collaboration and the project was initiated in April, 2017. The ETC team selected Shimadzu Corporation as the partner for this collaboration project based on considerations of technical expertise, anticipated project timeline, and overall collaborative spirit.

**As the leader of the project, was there any difficulty in managing the project?**

This was one of the very first projects that was initiated by the new Analytical and Purification Working Group of ETC. So some of the general guidelines were not well established yet and this project became the pilot for this working group. There was also a lot of interest from all participating ETC members to join this project and ultimately we had a large group of over 30 participating members from 10 major pharmaceutical companies.

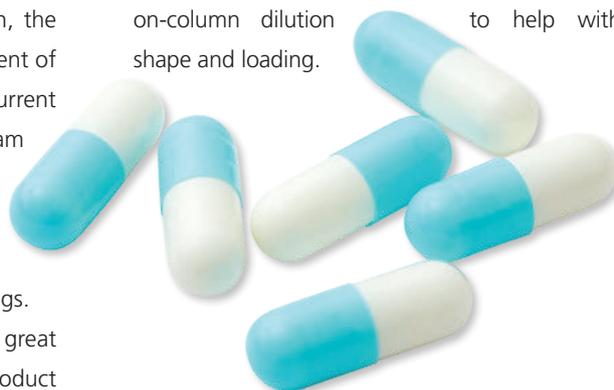
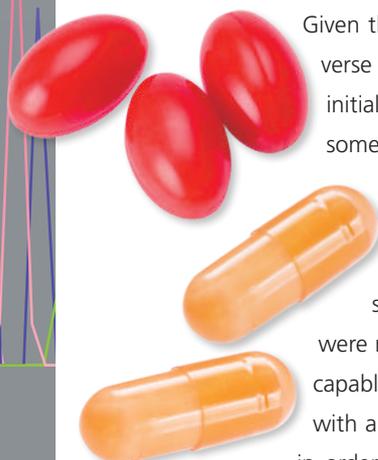
Given this large and diverse SFC user group, initially there were some differences of opinion for the desired system specifications, where some team members preferred a larger scale prep SFC instrument while others were more interested in a smaller scale system capable of high-throughput milligram scale with an open bed fraction collector. However, in order to have a successful collaboration, the team needed to align and focus on the development of a prep SFC instrument with the most important current need for all participating companies, so the team agreed on the development of a semi-preparative scale SFC instrument. This was a highly collaborative team which met regularly for monthly teleconference meetings and a few in-person meetings. All participating members provided regular and great feedback to the Shimadzu teams the product

development progressed and overall it was a very successful collaboration.

**Now that the Nexera UC Prep system has been released, could you provide a review of the instrument and explain the key features that are important and unique to the Nexera UC Prep? Are there any additional features that you would like to see in the future?**

The new Nexera UC Prep system was developed based on SFC user feedback and desired instrument features and specifications. The ETC project team was very pleased with the provided features and overall design of the system. The overall footprint of the new instrument is considerably smaller than most conventional prep SFC systems. The system also appears to be quiet, which is very important for labs that have multiple prep SFC instruments. The new Nexera UC Prep system also does not contain an external chiller, which can be large and take up a lot of valuable laboratory space,

create a lot of noise, and can be prone to leaks and spills. Some of the other key features of the Nexera UC Prep system include the novel gas-liquid separator for improved recovery and ease of use and cleaning, a newly designed fraction collector that can be customized per customer needs, and an innovative injector flow path for on-column dilution to help with peak shape and loading.



Software control that is simple and user-friendly is also very important and the newly developed Prep Solution software provides some key important features. The method editor function allows the user to quickly build a method and input all key method parameters, including fraction collection by slope, threshold, and time. The instrument monitor function allows the user to view the complete status of the system and be able to make on-the-fly changes, which is very important for a prep SFC instrument to minimize downtime and maximize recovery and productivity. The data viewer function allows the user to visualize previously collected chromatograms and compare data files.

Some additional features to see in the future that might be of interest to many preparative SFC users include the mass spectrometry directed preparative purifications,

ability to install and run multiple columns, and open bed fraction collection for high-throughput purifications.

**Finally, could you share Shimadzu's strengths compared to other vendors (not limited to the instruments)?**

Shimadzu has an amazing team of hardware and software engineers that are highly innovative and professional, collaborative, and great to work with! More importantly, they listened to every user feedback, were flexible and open to any suggestions, and tried to provide solutions based on customer needs.

**It was significant to know what you think of us and our collaboration. We will strive to meet your expectation more than ever. Thank you very much.**



Learn more about Nexera UC Prep:

[https://www.shimadzu.com/an/hplc/nexera/prep\\_uc/index.html](https://www.shimadzu.com/an/hplc/nexera/prep_uc/index.html)

Semi-Preparative SFC project team members representing Shimadzu and Enabling Technologies Consortium





Insight from customer / Report

# Cross-Pharma Collaboration on the Development and Evaluation of a New Mid-Scale Preparative Supercritical Fluid Chromatography Instrument

Mirlinda Biba,\* Mengling Wong,\* Anne Akin,\* Eric T. Manning,\* Lisa Schaffter, Larry Miller, Yingru Zhang, William Farrell, Jimmy O. DaSilva, Lisa Nogle, Benjamin Hritzko, Frank Riley, Robert P. DePianta, Kevin Barry, Donghong A. Gao, Eric Seest, Meenakshi Goel, Loanne Chung, James Paulson, Heewon Lee, Douglas B. Moore, Shuping Dong, William Leister, Nobumitsu Fukushima, Andy Sasaki, Timothy Lee, Takayuki Iriki, Masayuki Nishimura, Masami Tomita, Michiaki Owa, Katsuhiko Tanaka, Tomohiro Shagawa, Thomas J. Moran, Takeshi Bamba, and Christopher J. Welch

**Abstract** Precompetitive collaborations on the development of new enabling technologies for conducting pharmaceutical research and development are becoming increasingly popular, as pharmaceutical companies recognize the economic and practical benefits of cross-industry collaborations. We report here one of the first new technology development projects from the recently formed Enabling Technologies Consortium, the creation of a new instrument for carrying out mid-scale preparative supercritical fluid chromatography purifications. Input from a team of experienced separation scientists from 10 pharmaceutical companies helped to define a set of critical product attributes that provided guidance to a team of instrument developers in the creation of a new instrument prototype, with testing and feedback from the group helping to improve and refine the final product features. Two beta test units were placed in laboratories at two ETC member companies, allowing for an in-depth performance testing, following an extensive testing procedures developed by the project team. The evaluation results showed that the system met all required specifications. We describe here the development and testing of the new instrument and provide several examples of its use in carrying out chromatographic purifications in support of pharmaceutical discovery and development research.

**Keyword** preparative supercritical fluid chromatography, Enabling Technologies Consortium, cross-pharma collaboration, chiral separation

## Introduction

Precompetitive collaborations on the development of new enabling research technologies are becoming increasingly popular in the pharmaceutical industry.<sup>1</sup> In addition to the financial benefit of cost sharing across multiple participants, the collective identification and articulation of technological needs can give potential solution providers a clear understanding of what is needed, with joint technology evaluation facilitating the creation of new research tools that better fit the demands of a broader group of end users. The Enabling Technologies Consortium (ETC) was founded in 2015 to enable the rapid, collaborative development of new enabling technologies for pharmaceutical development and manufacturing.<sup>2-4</sup> Although several information-sharing projects have already been reported,<sup>5,6</sup> we now report the first ETC project, which has resulted in the development of a new commercial instrument for pharmaceutical research.

The creation of a semipreparative supercritical fluid chromatography (SFC) purification instrument addresses an important, long-standing, and somewhat unusual capability gap identified by the Analytical and Purification Working Group of the ETC.



Table 1. Instrument Conditions for Protocol Tests

Test	Constant Instrument Parameters	Varied Instrument Parameters	Test Notes
Cosolvent Pump Linearity	No column; Flow rate: 100 mL/min Cosolvent: 1% acetone in methanol BPR: 100 bar; Heat exchanger: 40°C; Wavelength: 272 nm	% of co-solvent 0, 20, 40, 60, 80, 100	Equilibrated system for 5 minutes after each increase of cosolvent before measuring the absorbance value Repeat test at 150 bar
Injector Linearity and Precision	Standard: ibuprofen 10 mg/mL in methanol Column: 2-EP (150 mm x 21 mm i.d., 5 µm) Cosolvent: 15% methanol with 0.1% ammonium hydroxide Column oven: 40°C Heat exchanger: 40°C; Heat exchanger: 40°C; BPR: 100 bar; Wavelength: 254 nm	Injection volumes: 0.5, 2, 2.5, 4, 5, 6, 7.5, 8 mL	Linearity at flow rate: 125mL/min Loop sizes of 2, 5, and 10 mL
		Injection volumes: 150, 250, 350, 500, 750, and 1000 µL	Linearity at low flow rate: 70 mL/min Loop size 2 mL.
		Injection volumes: 0.05, 0.2, 2.0, 8 mL	Injector precision at low flow rate: 70mL/min Made 7 injections at each volume, calculated RSD of last 5 injections
Cosolvent Pump Volumetric Flow Test	No column; CO <sub>2</sub> supply turned off Cosolvent: 100% methanol; BPR: 150 bar	Flow rates: 2, 25, 50, and 100 mL/min	Equilibrated system for 5 minutes at each flow rate and then a graduated cylinder was used to collect the flow for 1 min at each flow rate
UV Detector Accuracy Test	No column; Flow rate: 30 mL/min; Cosolvent: 100% methanol; Column oven: 40°C	Caffeine and anthracene at 10 mg/mL (2 mL injection)	Spectrum of each compound was observed and spectral max and min were recorded
BPR Accuracy	No column; Flow rate: 100 mL/min Cosolvent: 20% methanol; Column oven: 40°C	BPR at 100 and 200 bar	Evaluated using calibrated pressure gauge after equilibrating at each set point for 5 min and with one single measurement at each setpoint
Oven Temperature Accuracy	No column; Flow rate: 0 mL/min; Mobile phase: 100% CO <sub>2</sub>	Temperatures: 16, 20, 40 and 80°C	Evaluated using a temperature probe inside the oven after equilibrating at each set point for 5 min and with one single measurement at each setpoint.
Injector Carry-over Test	Column: 2-EP (250 mm x 21 mm i.d., 5 µm) Flow rate: 70 mL/min; Cosolvent: 10% methanol Injection volume: 0.5mL; BPR: 100 bar; Column oven: 40°C; Wavelength: 220 nm	Using two standards, caffeine and anthracene, both at 10 mg/mL	The injection sequence included two methanol blank injections, a caffeine injection, an anthracene injection, then 3 methanol blank injections. Collect each injection as a separate fraction and assay each fraction with HPLC for caffeine and anthracene. Repeated test with 2 mL injection volume
Fraction Collector Cross Talk	Standards: ibuprofen and ketoprofen each at 50 mg/mL in methanol Column: 2-EP (150 mm x 21 mm i.d., 5 µm) Flow rate: 70 mL/min; Cosolvent: 15% methanol BPR: 100 bar; Column oven: 40°C; Wavelength: 220 nm	Injection volume: 2 mL	Equilibrated system for 10 minutes. Injected ibuprofen and collected entire 5-minute run in fraction 2. Injected methanol and collected in fraction 3 for 5 minutes. Injected ketoprofen and collected in fraction 4 for 5 minutes. Repeated sequence until all 10 fractions are collected. Collected fractions 2-10 two more times using the same sequence. Diluted collected fractions with methanol to 250 mL and analyzed for cross talk.
Fraction Recovery Test	Standard: ibuprofen ketoprofen each at 50 mg/mL in methanol Column: 2-EP (150 mm x 21 mm i.d., 5 µm) Flow rate: 70 mL/min; Cosolvent: 15% methanol BPR: 100 bar; Column oven: 40°C; Wavelength: 220 nm	Fraction collection based on time windows, slope, and threshold	Made 5 injections collecting peak A in fraction 2 and peak B in fraction 4 for each run. Diluted each fraction to 100 mL with methanol and re-injected each diluted fraction 7 times using the last 5 injections to determine mean values for retention time and peak area. Diluted the 50 mg/mL ibuprofen/ketoprofen standard to 5mg/mL and injected 7 times using the last 5 injections to calculate average peak area. Calculated recovery by comparing the peak areas of the collected fractions to the peak areas of the diluted standard
Peak Area Reproducibility			
Retention Time Reproducibility			

Gram-scale preparative SFC purifications have been widely used in the pharmaceutical industry for nearly 20 years and have become an important enabling technology for the rapid exploration of stereochemical complexity in drug discovery and development.<sup>7-21</sup> The rapid growth of the field led to a tumult in the industry supplying instrumentation to carry out this task, with startup companies entering the arena, small companies merging or being acquired by larger ones, new products entering the marketplace, and established products becoming unavailable. An upshot of this flux was the disappearance of one of the standard instruments for carrying out mid-scale SFC purifications, leaving few suitable options

in the marketplace for the replacement of aging units. To address this problem, the participating ETC members created a request for information (RFI) for a project aimed at development of a new preparative SFC instrument meeting a prescribed list of specifications. The group publicized this request through the ETC website and received expressions of interest from a number of potential suppliers. Subsequent review and discussions with several vendors led to the commission of a collaborative development project with Shimadzu Corporation. We now describe the results of this collaboration on the development and testing of this new preparative SFC instrument, providing several examples of its use in carrying



out chromatographic purifications in support of pharmaceutical discovery and development research.

## Experimental section

Two beta test models of the Shimadzu semipreparative SFC instruments were used for this work and installed on-site at two ETC member companies. Each unit consisted of a CO<sub>2</sub> pump with an integrated chiller, cosolvent pump, make-up pump, column oven, back-pressure regulator (BPR), diode array detector, syringestyle injection system, and a very versatile fraction collection system with a novel gas-liquid separation (GLS) system. The system is controlled through PrepSolution and LabSolutions software.

Table 2. Desired Prep SFC System Features and Specifications

instrument feature	desired instrument specifications <sup>a</sup>
column size	2–3 cm i.d. × 25 cm length
flow rate	CO <sub>2</sub> pump: 25–200 mL/min modifier pump: 1–100 mL/min
modifier pump	minimum modifier percentage of 5% across the entire flowrate range (25–200 mL/min) solvent selection valve on modifier pump allowing selection and mixing multiple modifiers
outlet pressure	programmable up to at least 200 bar
column temperature	efficient temperature control up to at least 60°C
Injector	modifier or mixed-stream mode loop/syringe pump (0.5–10 mL) total time for injection less than one minute stacked injection capability
fraction collection	6–8 fractions + waste low-pressure with visible GLS (allow fast visual assurance that system is operating correctly and for cleaning verification) separate ventilated collection cabinet to allow up to at least 2 L collection vessels
fraction collection settings	point and click time point settings from saved chromatogram
fraction collection triggering	UV and/or MS using time, slope, and/or threshold manual fraction collection option
method editing	simple, graphical (visual), “on-the-fly” editing
Detection	UV (190–700 nm) other: MS, CAD, ELSD, polarimeter, and so forth.(optional)
CO <sub>2</sub> recycling option	available as an option
GMP qualification	available as an option
Safety	shutdown triggers: leak detection in collection cabinet (both liquid and CO <sub>2</sub> ), high pressure on CO <sub>2</sub> and modifier pump and on column, high temperature on column secondary containment in collection cabinet
Footprint	Benchtop modular components to allow flexibility in stacking separate collection cabinet
Service	response time in less than 2 days local service technician reasonably priced service contracts
system control	Windows 7 or higher operating system external PC preferred

<sup>a</sup>The desired instrument specifications listed here are the minimum requirements from the SFC users for the development of a new prep SFC instrument, which were listed in the RFI. All detailed instrument specifications for the final product can be found in the product brochure.<sup>23</sup>

A commercial version of the instrument is now available as the Nexera UCPrep SFC.<sup>22,23</sup>

Testing of the instrument was conducted in two phases. In the first phase, the prep SFC instrument was tested against a protocol developed and agreed upon between the participating ETC members and Shimadzu Corporation. Details of the protocol tests are found in Table 1. In the second phase, the system was tested using “real-world” development samples to see how it compared to other instruments currently used in the two labs.

Model compounds, caffeine, anthracene, ibuprofen, ketoprofen, and trans-stilbene oxide (TSO), were obtained from Sigma-Aldrich, St. Louis, MO. The solvents, methanol and acetone, were obtained from EMD, Temecula, CA. Carbon dioxide was obtained from Praxair, San Francisco, CA. Compounds depicted in Figures 7–9 are from ongoing projects under investigation in these laboratories.

## Results and discussion

The preparative SFC project originated in the analytical and purification working group of ETC. Interested members in the working group from several pharmaceutical companies formed a project team and met to decide on target specifications. Given the large and diverse user group, some differences of opinion on desired specifications and features of the targeted prep SFC instrument were perhaps inevitable. Some project team members wanted larger scale prep SFC systems that could accommodate preparative columns up to 10 cm in diameter while others were interested in systems capable of high-throughput milligram-scale mass-directed purification with an open-bed fraction collector. The project team soon settled on the development of a semipreparative-scale SFC system with features illustrated in Table 2 as representative of the most important current need for the participating companies.

In addition to the features outlined in Table 2, the ETC project team agreed that safety, affordability, ruggedness, dependability, and ease of use would be critical attributes for success of the project. Interestingly, a group dynamic soon evolved that coalesced around a shared vision for a successful outcome. Individual requests to add new features or capabilities were considered, when consistent with this shared vision, but were overruled when jeopardizing cost, development of timeline, safety, or ease of use.

When clear on the project requirements, the group issued a RFI, which was announced via publication on the ETC website and a press release in July 2016. The project team reviewed several responses and selected three suppliers to submit a formal proposal. The ETC group ultimately chose Shimadzu for the project partner based on considerations of technical expertise, anticipated

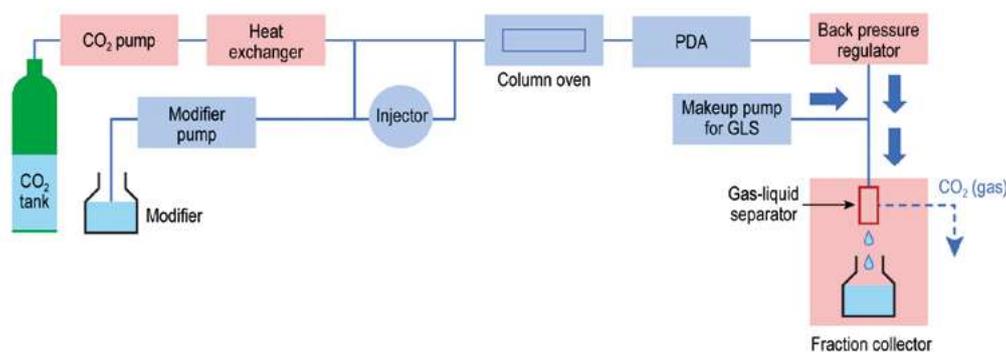


Figure 1. Flow diagram of the new preparative SFC instrument.

project timeline, and overall collaborative spirit. In April 2017, ETC signed a collaboration agreement with Shimadzu, and the project began in earnest. One of the key benefits of the ETC mechanism is that it allows for multiple ETC member companies to engage in a collaboration with a third party as a single entity. It was noted by Shimadzu that the execution of the legal framework defining this collaboration was streamlined by the ability to negotiate with ETC versus the effort that would have been required negotiating with all of the participating ETC members on an individual basis. Furthermore, another key benefit of the ETC mechanism is that from an individual company perspective, the shared funding mechanism afforded by ETC allows for more work to be accomplished for only a fraction of the necessary monetary investment relative to accomplishing the same work alone.

The participating ETC members next met with Shimadzu scientists and engineers to discuss many of the key hardware and software design details. In addition to face-to-face meetings and teleconferences, ongoing discussions between seasoned prep SFC users and instrument designers were very important. When the project team reached general agreement on the design specifications, the Shimadzu design team undertook the creation of a new instrument prototype. Ongoing discussions between participating ETC project team members took place over the next few months, as the project progressed. In particular, the software development team spent a lot of time learning about detailed workflows from the experienced SFC users to better understand why certain features were needed and how to best implement them into the software.

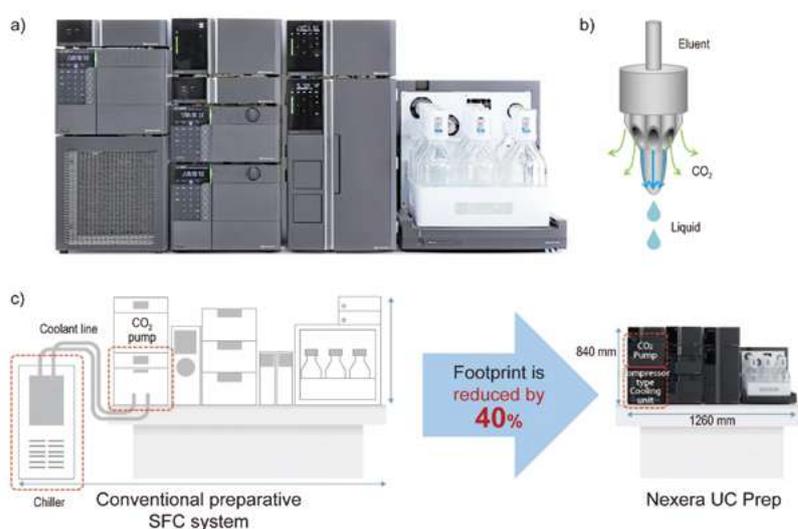


Figure 2. Key hardware features of the new mid-scale preparative SFC instrument. (a) Photo of final product design, (b) innovative gas-liquid separator with branched channel design improves sample recovery and reduces formation of aerosols, (c) an internal chiller within the instrument eliminates the requirement for a large, noisy, and spill-prone external chiller, reducing the overall instrument footprint.

A precommercial prototype instrument embodying most of the anticipated features of the commercial instrument was first evaluated by participating ETC members in May 2018 at Shimadzu headquarters in Columbia, MD (Figure 1). An overall assessment was conducted, and feedback and requested improvements were collected by Shimadzu hardware and software engineers. Subsequently, beta test units incorporating these design changes were placed within laboratories at two ETC member companies, allowing for in-depth performance testing and evaluation under real-world conditions.

The ETC project team was pleased with the overall design and functionality of the new instrument (Figure 2a). The newly designed preparative SFC instrument also included some improved hardware features. A new patented technology for the GLS provided increased recovery as well as ease of use and cleaning (Figure 2b).

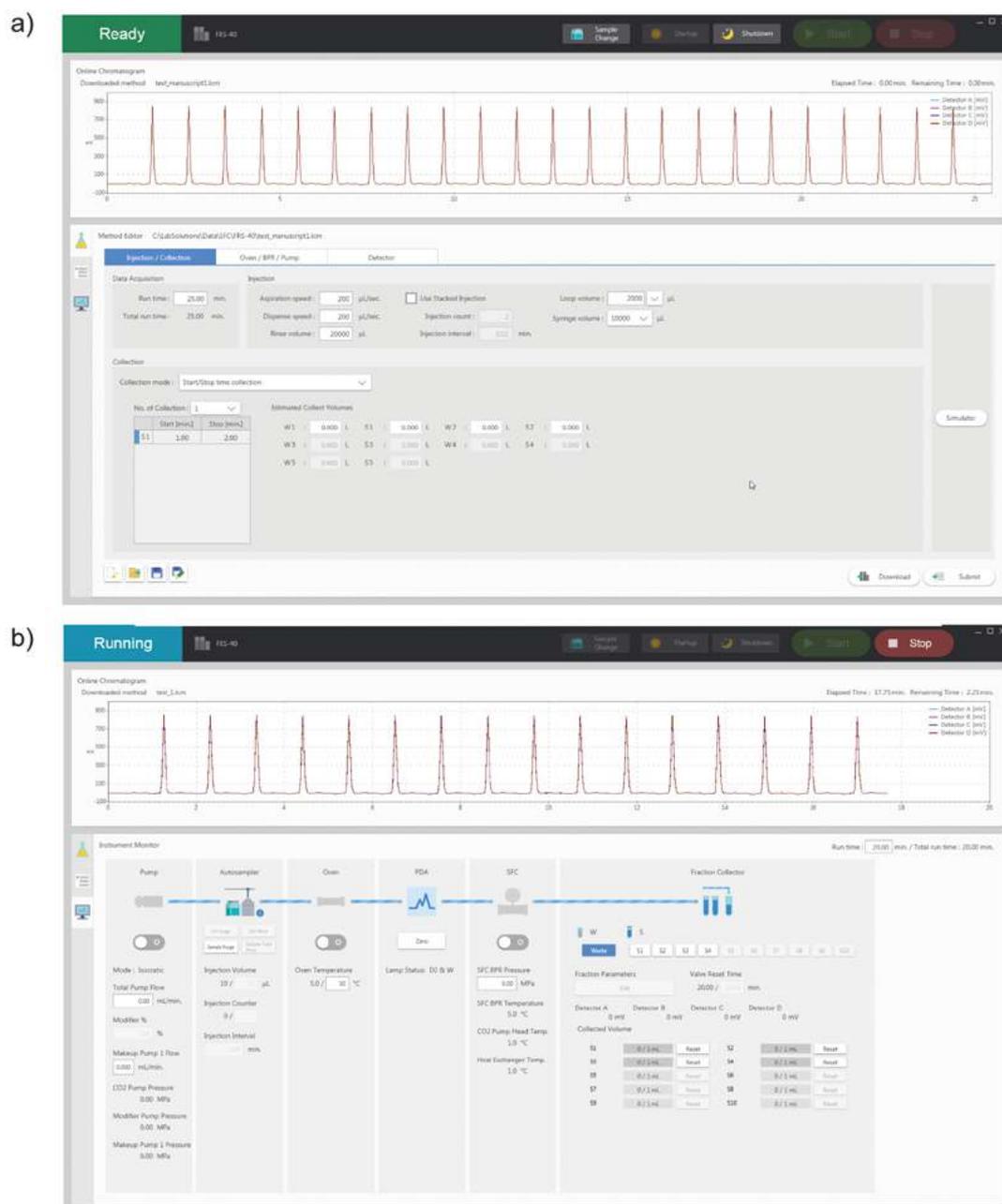


Figure 3. Snapshots from the newly developed PrepSolution software including (a) method editor and (b) instrument monitor. Data viewer is not shown.

An innovative injector flow path for on-column dilution was added to help with the peak shape and sample loading on the column. Finally, an internal chiller was used, instead of the typical external chillers found with conventional preparative SFC instruments, which provided a more compact bench-top design with a reduced footprint (Figure 2c). The system was also quiet, an important consideration, especially when a number of SFC instruments are placed in a single room.

Similarly, software was a very important part of the project,

where the user input was critical to achieve the desired level of user-friendliness. Following an agile approach allowed participating members to collaborate every month to view the progress of the software and to provide insightful feedback. Active discussions between the participating members allowed the developers to create a user experience that combined expected software features with ease of use. Three independent software components were integrated in the final design for the instrument including method editor, instrument monitor, and data viewer functions (Figure 3).

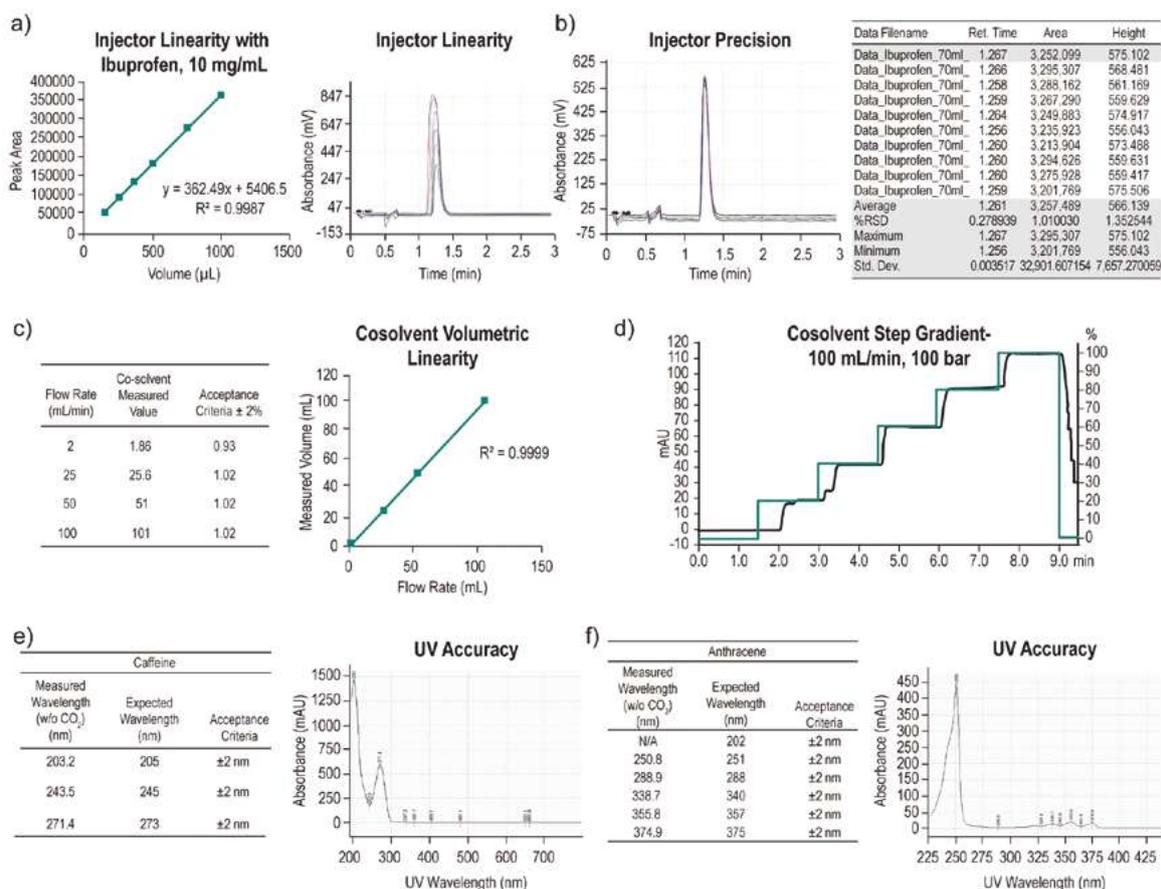


Figure 4. Performance evaluation of the beta test instrument showed excellent performance for injection, mobile phase pump, and detection features. (a) Injector linearity at 70 mL/min was evaluated, and an  $R^2$  of 0.998 was determined with the injection volumes ranging from 150 to 1000  $\mu$ L. (b) Injection precision with 0.28% RSD of retention time, and 1.0% RSD of area was achieved for a 750  $\mu$ L of injection. The table on the right was generated directly from the LabSolution software. (c) Cosolvent pump volumetric flow test was carried out at four different flow rates at 2, 25, 50, and 100 mL/min. The linearity  $R^2$  is 0.999. (d) Cosolvent step gradient was tested at 0, 20, 40, 60, 80, and 100% cosolvent, which is 1% acetone in methanol, at a 100 mL/min total flow rate. (e,f) UV detector accuracy tests were carried out for low and high wavelengths using caffeine and anthracene, respectively. The photodiode array detector wavelength accuracy is  $\pm 1$  nm, and spectral resolution is  $\pm 1.4$  nm. All details for these tests can be found in the Experimental Section, as shown in Table 1.

The method editor function (Figure 3a) allows user-defined construction of preparative chromatographic methods under a variety of injection, detection, collection, and gradient scenarios including stacked injection, manual sample collection, and sample collection *via* defined stop-start times or by the detector threshold or detector slope within time windows. In addition, a simulator subroutine allows the use of a previously collected chromatogram to facilitate the creation of new method injection and collection parameters.

The instrument monitor function (Figure 3b) provides the user with a complete picture of the status of the operating instrument, providing readings on the flow rate, modifier percentage, back pressure, temperature, absorbance, fraction collector valve position, and so forth. Key instrument settings are also adjustable on-the-fly by the user, including total pump flow, modifier percentage, make-up pump flow rate, injection parameters, oven temperature,

BPR setting, fraction parameters, and fraction collector valve position. On-the-fly adjustment of these parameters allows users to fine-tune instrument settings during prolonged stacked injection sequences, thereby minimizing downtime and maximizing productivity. Ongoing interactions between participating ETC project team members and Shimadzu engineers over a number of months were important in creating capabilities and a user interface that enabled top performance.

Finally, along with the PrepSolution control software, a new data viewer was created that allows the user to visualize previously collected chromatograms, including the ability to load and compare several different data files to enable comparisons over time. The data viewer allows the user to easily compare collected peaks across injections of a single stacked injection data file or across multiple single-injection data files.

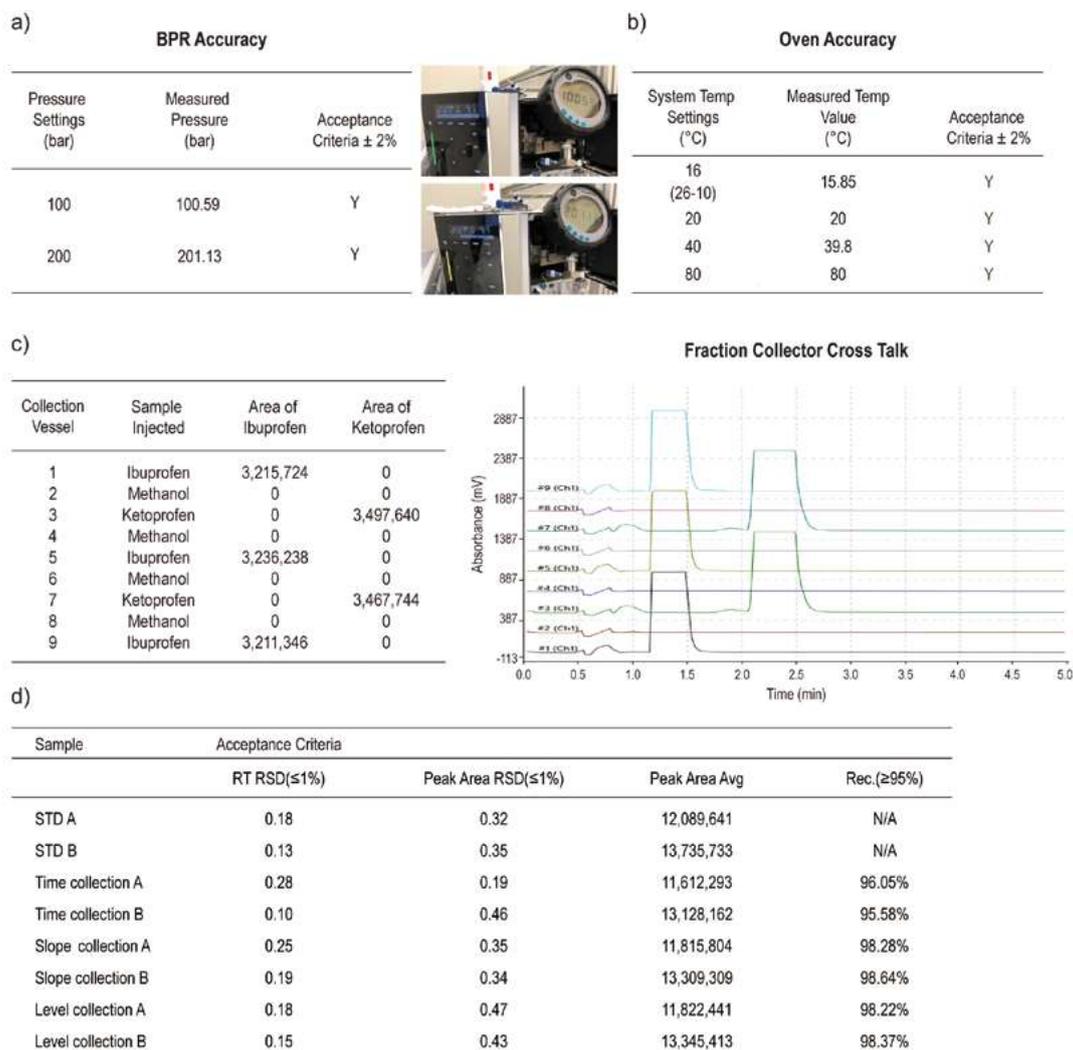


Figure 5. Performance evaluation of the BPR, oven, and injection carryover: (a) the BPR accuracy was determined using a calibrated pressure gauge at 100 and 200 bars. (b) Oven accuracy was evaluated using a temperature probe inside the oven at the following temperatures: 16, 20, 40, and 80 $^{\circ}\text{C}$ . (c) Fraction collector cross-talk was evaluated to determine carryover from fraction-to-fraction using ibuprofen and ketoprofen. There was no observed cross-talk between each fraction collected. Additionally, there was no observed carryover from injection-to-injection (data not shown). (d) System performance was evaluated for overall recovery, peak area, and retention time reproducibility for fractions collected based on retention time windows, threshold, and slope values. Details for these tests can be found in the Experimental Section.

With these hardware and software features in place, the project team's next objective was to test the performance of the instrument. Initial testing focused on the simple protocols to ensure the proper functioning of the various hardware and software components of the instrument. Suitable performance was observed for a number of parameters, including detector linearity, minimal injection carryover, suitable sample recovery and precision, variability of flow, back pressure, temperature, and injection volume (Figures 4 and 5).

System stability was investigated by making a series of injections with each run consisting of 50 stacked injections of TSO. Over the course of 48 h of continuous running, more than 1750 injections were made. Overlaying chromatograms from the beginning,

middle, and end of the sequence, showed no drift in retention times or changes in peak shape (Figure 6), indicating that the instrument is rugged enough to run for several days without issue.

Given the satisfactory performance of the prototype instrument, we next investigated instrument performance in the purification of a number of "real-world" samples. Figure 7 shows representative chromatograms from the resolution of the enantiomers of 385 mg of a proprietary development compound. A single injection of 32 mg of racemate (Figure 7a) affords baseline resolution. Automated injection and fraction collection (Figure 7b) enable rapid enanti-separation with excellent purity and recovery. The instrument provided outstanding ease of use and maneuverability in carrying

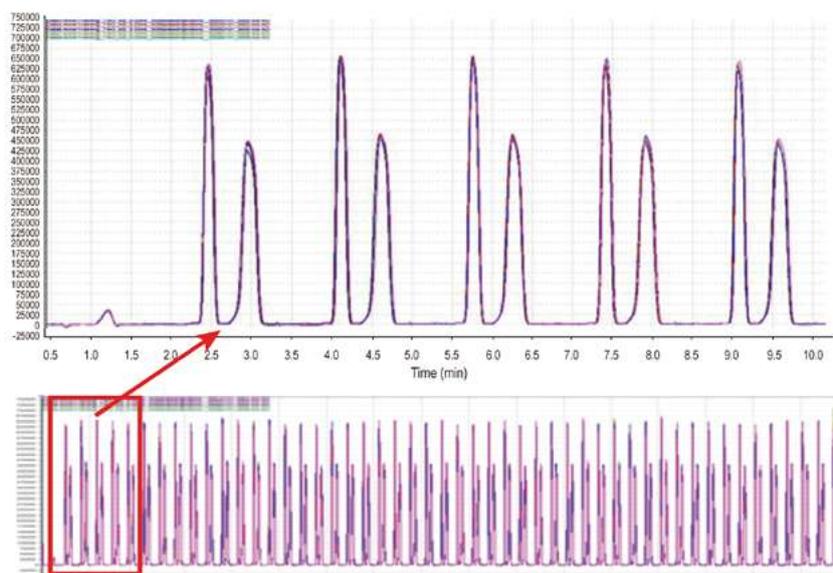


Figure 6. System robustness evaluation by continuously running stacked injections over 2 days. Preparative separation of TSO enantiomers. Conditions: CHIRALPAK AS column (250 mm × 21 mm i.d., 5 μm); 50 mL/min 10% MeOH/90% CO<sub>2</sub>; 100 bar outlet pressure; 40°C column temperature; and injection of 250 μL of 10 mg/mL solution at every 1.65 min.

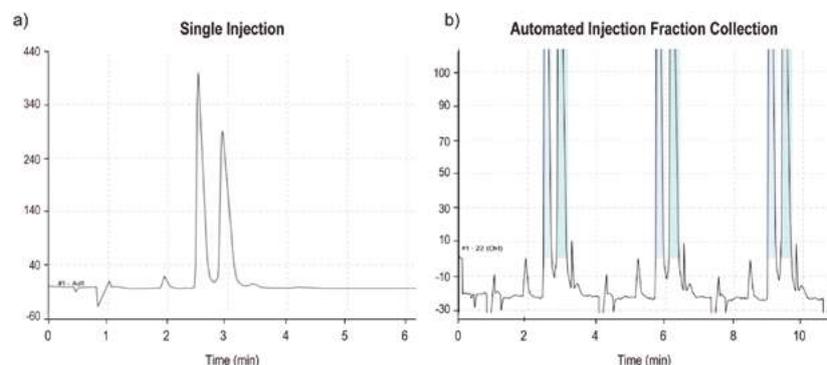


Figure 7. Preparative chiral separation of a racemic development candidate. (a) Single injection and (b) detail from “stacked” injection series with automated injection and fraction collection. Conditions: (S,S) Whelk-O 1 column (250mm × 21mm i.d., 10 μm); 80 mL/min 10% (0.1% NH<sub>4</sub>OH) in MeOH/CO<sub>2</sub>; 100 bar outlet pressure; 25°C column temperature; injection of 500 μL of 64 mg/mL solution of racemate (32 mg) every 3.25 min; time-based fraction collection; and recovery 97.5% (154.3 mg enantiomer 1 with 100% purity, and 152.2 mg enantiomer 2 with 99% purity).

out such small-scale enantioseparations, thereby powerfully enabling synthetic route investigations, a critical need in pharmaceutical discovery and development.

In addition to the resolution of racemic mixtures, preparative chromatography is often used for rescue purifications in pharmaceutical research and development. Thus, preparative chromatography can play a valuable role as a “safety net” for chemical synthesis, providing valuable upgrades in chemical or enantiopurity when needed. Figure 8 shows a typical example, where preparative SFC using the beta instrument was used to remove an

unwanted regioisomer from a development compound.

The isolation and characterization of chemical impurities is another important function of pharmaceutical research that is powerfully enabled by preparative SFC. Although analytical chromatography with MS detection can be useful for developing hypotheses regarding the identity of impurities, preparative chromatographic enrichment is often essential for unambiguous structure elucidation. In addition, assessing the biological activity of impurities is greatly facilitated by preparative chromatographic purification. In the example presented in Figure 9, isolation of a

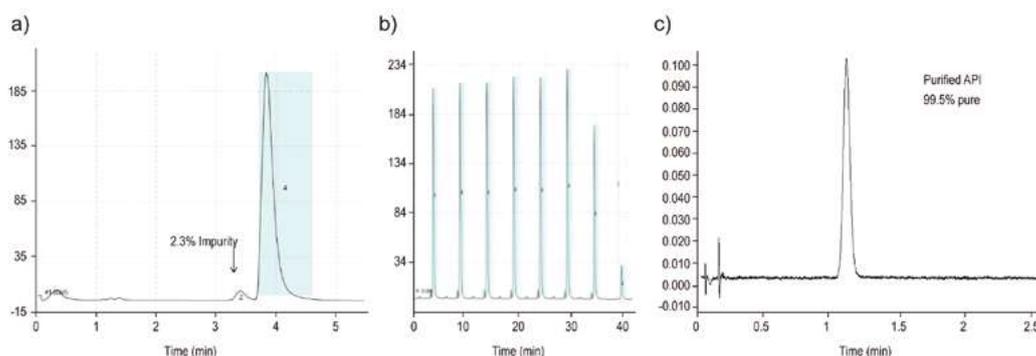


Figure 8. Preparative SFC purity upgrade to remove an unwanted regioisomeric impurity from a development candidate. (a) Single preparative injection of the crude product containing 2.3% regioisomeric impurity and (b) detail from “stacked” injection series with automated injection and fraction collection. Preparative conditions: Lux Cellulose-3 column (250 mm × 21 mm i.d., 5 μm); 70 mL/min, 35% (0.1% NH<sub>4</sub>OH in MeOH)/CO<sub>2</sub>; 100 bar outlet pressure; 40°C column temperature; UV 260 nm; 500 μL injection of 6.25 mg/mL solution of crude product in 1:2 MeOH, ACN every 4 min; and recovery 15 mg of R2, >99.5% purity. (c) Analytical chromatogram of purified API, >99.5% pure. Analytical conditions: Lux Cellulose-3 column (50 mm × 4.6 mm i.d., 3 μm); 4 mL/min, 25% (0.1% NH<sub>4</sub>OH in MeOH)/CO<sub>2</sub>; 120 bar outlet pressure; 40°C column temperature; UV 254 nm; and 2 μL of injection of 1 mg/mL solution of API.

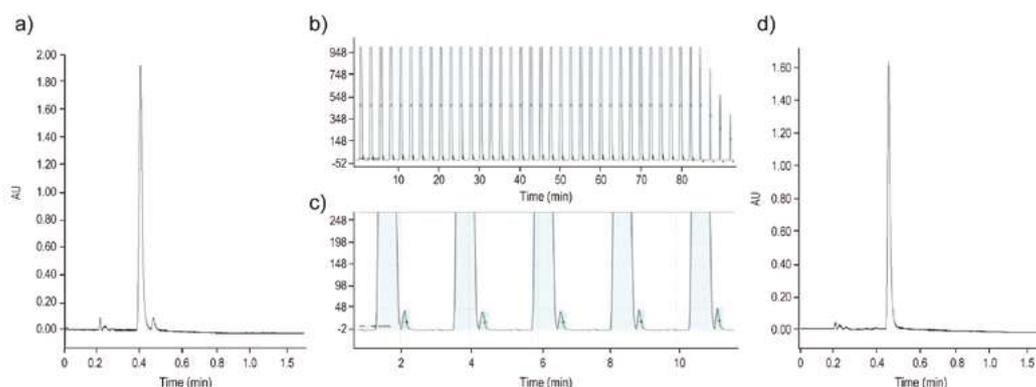


Figure 9. Preparative SFC purification of a minor impurity to facilitate chemical and biological characterization. (a) Analytical injection showing the crude development compound containing ~1% impurity and (b) “stacked” injection series with automated injection and fraction collection; preparative conditions: Synergi Polar-RP column (150 mm × 21 mm i.d., 5 μm); 70 mL/min 10% (0.1% NH<sub>4</sub>OH in MeOH)/CO<sub>2</sub>; 100 bar outlet pressure; 25°C column temperature; injection of 1 mL of 25 mg/mL solution of 10:1 API: impurity (1 g) every 2.25 min; time-based fraction collection; and recovery 98% (971 mg API, and 9.1 mg impurity). (c) Expanded view of stacked injection sequence and (d) analytical chromatogram showing isolated impurity. Analytical conditions: Synergi Polar-RP column (50 mm × 2.0 mm i.d., 2.5 μm); 1.5 mL/min 10% (0.1% NH<sub>4</sub>OH in MeOH)/CO<sub>2</sub>; 120 bar outlet pressure; 40°C column temperature; and injection of 2 μL of injection of 1 mg/mL solution of impurity.

minor impurity from 1 g of a developmental compound was facilitated by preparative SFC separation.

## Conclusions

A new paradigm for the creation of enabling technologies for pharmaceutical research and development was employed in the successful creation of a commercial gram-scale preparative SFC instrument. The project brought together, under the auspices of

the Enabling Technologies Consortium, a team of preparative SFC experts from across the pharmaceutical industry and a team of seasoned instrument designers and software engineers. Ongoing discussions, candid feedback, and a close working relationship within the project team led to rapid progress and convergence on instrument and software features that are well suited to the practical preparative SFC workflows that are in current use within the pharmaceutical industry. The instrument developed through this collaboration more than met the SFC user’s expectations. The new system was easy to use, rugged, reliable, and not only did it



have most of the physical features requested by the team but there were additional and unexpected improvements such as the internal chiller, the novel gas–liquid separator, and the ability to perform on-column sample dilutions. We believe that this form of collaborative instrument development represents an important new paradigm for facilitating the creation of new research technologies that better address the collective needs of researchers within the pharmaceutical industry.

## Author information

### Corresponding Authors

**Mirlinda Biba** – Merck & Co., Inc., Rahway, New Jersey 07065, United States; [orcid.org/0000-0002-9888-0821](https://orcid.org/0000-0002-9888-0821); Email: [mirlinda\\_biba@merck.com](mailto:mirlinda_biba@merck.com)

**Mengling Wong** – Genentech, Inc., South San Francisco, California 94080, United States; Email: [wong.mengling@gene.com](mailto:wong.mengling@gene.com)

**Anne Akin** – Pfizer Central Research, Groton, Connecticut 06340, United States; Email: [anilineone@gmail.com](mailto:anilineone@gmail.com)

**Eric T. Manning** – Shimadzu Scientific Instruments, Columbia, Maryland 21046, United States; Email: [etmanning@shimadzu.com](mailto:etmanning@shimadzu.com)

### Authors

**Lisa Schaffter** – Abbvie, Worcester, Massachusetts 01605, United States

**Larry Miller** – Amgen Research, Cambridge, Massachusetts 02142, United States

**Yingru Zhang** – Bristol Myers Squibb, Princeton, New Jersey 08540, United States

**William Farrell** – Pfizer, San Diego, California 92121, United States

**Jimmy O. DaSilva** – Merck & Co., Inc., Rahway, New Jersey 07065, United States; [orcid.org/0000-0001-7044-0640](https://orcid.org/0000-0001-7044-0640)

**Lisa Nogle** – Merck & Co., Inc., Boston, Massachusetts 02115, United States

**Benjamin Hritzko** – Bristol Myers Squibb, Princeton, New Jersey 08540, United States

**Frank Riley** – Pfizer Central Research, Groton, Connecticut 06340, United States

**Robert P. DePianta** – Pfizer Central Research, Groton, Connecticut 06340, United States

**Kevin Barry** – Biogen, Cambridge, Massachusetts 02142, United States

**Donghong A. Gao** – Boehringer-Ingelheim, Ridgefield, Connecticut 06877, United States

**Eric Seest** – Eli Lilly & Co., Indianapolis, Indiana 46285, United States

**Meenakshi Goel** – Genentech, Inc., South San Francisco, California 94080, United States

**Loanne Chung** – Pfizer, San Diego, California 92121, United States

**James Paulson** – Bristol Myers Squibb, Princeton, New Jersey 08540, United States

**Heewon Lee** – Boehringer-Ingelheim, Ridgefield, Connecticut 06877, United States

**Douglas B. Moore** – Bristol Myers Squibb, Princeton, New Jersey 08540, United States

**Shuping Dong** – GSK, Upper Providence, Pennsylvania 19426, United States

**William Leister** – GSK, Upper Providence, Pennsylvania 19426, United States

**Nobumitsu Fukushima** – Shimadzu Corporation, Kyoto 604-8511, Japan

**Andy Sasaki** – Shimadzu Scientific Instruments, Columbia, Maryland 21046, United States

**Timothy Lee** – Shimadzu Scientific Instruments, Columbia, Maryland 21046, United States

**Takayuki Iriki** – Shimadzu Scientific Instruments, Columbia, Maryland 21046, United States

**Masayuki Nishimura** – Shimadzu Scientific Instruments, Columbia, Maryland 21046, United States

**Masami Tomita** – Shimadzu Corporation, Kyoto 604-8511, Japan

**Michiaki Owa** – Shimadzu Corporation, Kyoto 604-8511, Japan

**Katsuhiro Tanaka** – Shimadzu Corporation, Kyoto 604-8511, Japan

**Tomohiro Shagawa** – Shimadzu Corporation, Kyoto 604-8511, Japan

**Thomas J. Moran** – Shimadzu Scientific Instruments, Columbia, Maryland 21046, United States

**Takeshi Bamba** – Kyushu University, Fukuoka 812-8582, Japan; [orcid.org/0000-0002-7442-2738](https://orcid.org/0000-0002-7442-2738)

**Christopher J. Welch** – Indiana Consortium for Analytical Science & Engineering (ICASE), Indianapolis, Indiana 46202, United States; [orcid.org/0000-0002-8899-4470](https://orcid.org/0000-0002-8899-4470)

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acs.oprd.0c00136>

### Funding

This research was financially supported by Shimadzu Corporation.

### Notes

The authors declare no competing financial interest.

### Acknowledgments

We are grateful to Regina Black and Glenn Wilcox for valuable input at the outset of this project and to James Vergis, Alexis Myers and Ann Marie Stanley for valuable contributions in the administration of the functions of the Enabling Technologies Consortium. We are also



grateful to Philip Searle, Justin Simanis, Christian Mueller, Erin Jordan, Chandan Barhate, Dauh-Rung Wu, Jingfang Cutrone, Shiuhan Yip, Kanaka Hettiarachchi, Michael Hayes, Min Liu, and Amber Guillen for their valuable suggestions, Peter Ratsep, Michael Parks, Charlie Wang, Kelly de la Torre, and Tessa Burt for their help with the beta instrument installations and testing, and to Sue Wilks and Merck Creative Studios for their help with the manuscript figures.

## Dedication

Dedication: This paper is dedicated to the memory of Atsu Apedo, an excellent scientist, esteemed colleague, and a valuable team member in the early days of the project.

## References

- (1) Welch, C. J.; Hawkins, J. M.; Tom, J. Precompetitive Collaboration on Enabling Technologies for the Pharmaceutical Industry. *Org. Process Res. Dev.* **2014**, *18*, 481–487.
- (2) Welch, C. J.; Faul, M. M.; Tummala, S.; Papageorgiou, C. D.; Hicks, F.; Hawkins, J. M.; Thomson, N.; Cote, A.; Bordawekar, S.; Wittenberger, S. J.; Laffan, D.; Purdie, M.; Boulas, P.; Irdam, E.; Horspool, K.; Yang, B.-S.; Fernandez, J.; Fernandez, P.; Ferretti, A.; May, S.; Seibert, K.; Wells, K.; McKeown, R. The Enabling Technologies Consortium (ETC): Fostering Precompetitive Collaborations on New Enabling Technologies for Pharmaceutical Research and Development. *Org. Process Res. Dev.* **2017**, *21*, 414–419.
- (3) Arnaud, C. H. The Enabling Technologies Consortium fills in pharma technology gaps: Consortium works with outside vendors to improve supporting technologies for drug development. *Chem. Eng. News* **2018**, *96*, 22–23.
- (4) Enabling Technologies Consortium. <https://www.etconsortium.org/> (accessed on March 28, 2020).
- (5) Conder, E. W.; Cosbie, A. S.; Gaertner, J.; Hicks, W.; Huggins, S.; MacLeod, C. S.; Remy, B.; Yang, B.-S.; Engstrom, J. D.; Lamberto, D. J.; Papageorgiou, C. D. The Pharmaceutical Drying Unit Operation: An Industry Perspective on Advancing the Science and Development Approach for Scale-Up and Technology Transfer. *Org. Process Res. Dev.* **2017**, *21*, 420–429.
- (6) Bahr, M. N.; Damon, D. B.; Yates, S. D.; Chin, A. S.; Christopher, J. D.; Cromer, S.; Perrotto, N.; Quiroz, J.; Rosso, V. Collaborative Evaluation of Commercially Available Automated Powder Dispensing Platforms for High-Throughput Experimentation in Pharmaceutical Applications. *Org. Process Res. Dev.* **2018**, *22*, 1500–1508.
- (7) Berger, T. A.; Fogleman, K.; Staats, T.; Bente, P.; Crockett, I.; Farrell, W.; Osonubi, M. The development of a semi-preparatory scale supercritical-fluid chromatograph for high-throughput purification of 'combi-chem' libraries. *J. Biochem. Biophys. Methods* **2000**, *43*, 87–111.
- (8) Welch, C. J.; Leonard, W. R.; DaSilva, J. O.; Biba, M.; Albanese-Walker, J.; Henderson, D. W.; Laing, B.; Mathre, D. J. Preparative Chiral SFC as a 'Green' Technology for Rapid Access to Enantiopurity in Pharmaceutical Process Research. *LC-GC Eur.* **2005**, *23*, 16–29.
- (9) Welch, C. J. Chiral Chromatography in Support of Pharmaceutical Process Research. In *Preparative Enantioselective Chromatography*; Cox, G., Ed.; Blackwell: London, 2005; pp 1–18.
- (10) White, C. Integration of supercritical fluid chromatography into drug discovery as a routine support tool. *J. Chromatogr. A* **2005**, *1074*, 163–173.
- (11) White, C.; Burnett, J. Integration of supercritical fluid chromatography into drug discovery as a routine support tool. *J. Chromatogr. A* **2005**, *1074*, 175–185.
- (12) Phinney, K. W. Enantioselective separations by packed column subcritical and supercritical fluid chromatography. *Anal. Bioanal. Chem.* **2005**, *382*, 639–645.
- (13) Leonard, W. R.; Henderson, D. W.; Miller, R. A.; Spencer, G. A.; Sudah, O. S.; Biba, M.; Welch, C. J. Strategic use of preparative chiral chromatography for the synthesis of a preclinical pharmaceutical candidate. *Chirality* **2007**, *19*, 693–700.
- (14) Miller, L.; Potter, M. Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical discovery environment. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2008**, *875*, 230–236.
- (15) Taylor, L. T. Supercritical fluid chromatography for the 21st century. *J. Supercrit. Fluids* **2009**, *47*, 566–573.
- (16) McClain, R. T.; Dudkina, A.; Barrow, J.; Hartman, G.; Welch, C. J. Evaluation and Implementation of a Commercially Available Mass-Guided SFC Purification Platform in a High Throughput Purification Laboratory in Drug Discovery. *J. Liq. Chromatogr. Relat. Technol.* **2009**, *32*, 483–499.
- (17) Welch, C. J.; Henderson, D. W.; Pipik, B.; Shaimi, M.; Schenk, D.; Conlon, D. Shortcut to Selectivity: Make Them All and Let Preparative Chromatography Sort it Out. *Org. Process Res. Dev.* **2010**, *14*, 905–907.
- (18) De la Puente, M. L.; López Soto-Yarritu, P.; Burnett, J. Supercritical Fluid Chromatography in Research Laboratories: Design, Development and Implementation of an Efficient Generic Screening for Exploiting this Technique in the Achiral Environment. *J. Chromatogr. A* **2011**, *1218*, 8551–8560.
- (19) Hamman, C.; Schmidt, D. E., Jr.; Wong, M.; Hayes, M. The Use of Ammonium Hydroxide as an Additive in Supercritical Fluid Chromatography for Achiral and Chiral Separations and Purifications of Small, Basic Medicinal Molecules. *J. Chromatogr. A* **2011**, *1218*, 7886–7894.
- (20) Berger, T. A.; Berger, B. K. Minimizing UV Noise in Supercritical Fluid Chromatography. I. Improving Back Pressure Regulator Pressure Noise. *J. Chromatogr. A* **2011**, *1218*, 2320–2326.
- (21) Welch, C. J. The Use of Preparative Chiral Chromatography for Accessing Enantiopurity in Pharmaceutical Discovery and Development. In *Comprehensive Organic Synthesis*; Welch, C., Ed.; Newnes, 2013; Vol. 9, pp 143–159.
- (22) Nexera Prep SFC Preparative Supercritical Fluid Chromatography System. <https://www.ssi.shimadzu.com/products/liquid-chromatography/nexera/nexera-prep-sfc.html> (accessed on May 12, 2020).
- (23) Nexera UC Prep. [https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/Products/literature/hplc/lc190-e236-NexeraUC\\_Prep\\_SSI.pdf](https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/Products/literature/hplc/lc190-e236-NexeraUC_Prep_SSI.pdf) (accessed on May 12, 2020).



## Pharmaceutical

# Application of HPLC in Quality Analysis of Hydroxychloroquine Sulfate

Yang Le, Analytical Applications Center, Shimadzu (China) Co., LTD

**Abstract** In this study, methods were established for analysis of hydroxychloroquine sulfate and related substances following European Pharmacopoeia (EP10.0) and for determination of hydroxychloroquine sulfate content in tablet following US Pharmacopoeia (USP 43). Using Shimadzu UHPLC system, well separation was achieved between hydroxychloroquine sulfate and impurity C, as well as between impurity B and impurity C referring to the EP assay. Using Shimadzu HPLC system with reference to the USP assay, the content of hydroxychloroquine sulfate in tablet was determined with achieving the desired peak separation and reproducibility described in the USP.

**Keyword** *hydroxychloroquine sulfate, HPLC, European Pharmacopoeia (EP), US Pharmacopoeia (USP)*

## Introduction

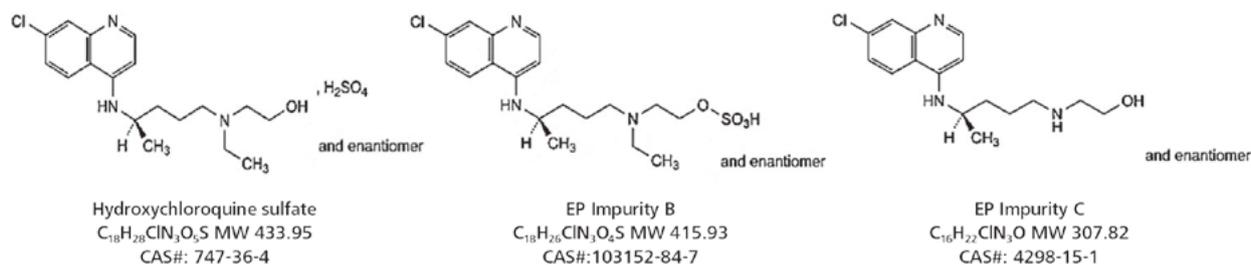
Hydroxychloroquine sulfate (CAS#: 747-36-4) was on the market first in 1955 in the USA. To date, it has been approved in more than 70 countries including Canada, France, German, Australia, China and Japan etc.. Hydroxychloroquine sulfate was used initially in the treatment of malaria. The drug was found to exhibit mild immunosuppression and immunomodulation effects in clinical use. Therefore, it has been used widely in the treatments of various diseases such as rheumatoid arthritis, systemic lupus erythematosus

(SLE), connective tissue disease (CTD) etc. Amid the pandemic of novel coronavirus disease (COVID-19) at present, some existing drugs come to public attention and hydroxychloroquine sulfate is one of such drugs.

Cell biology screening studies indicate that hydroxychloroquine sulfate exhibits significant suppression effect to coronavirus SARS/MERS. Molecular biochemistry evidences also provide primary support on its significant effect to the glycosylation level of receptor ACE2 of coronavirus. Recently, clinical trials on the evaluation of the safety and effectiveness for coronavirus disease with hydroxychloroquine sulfate were started urgently in many countries in the world.

The quality of a drug is a fundamental factor to guarantee the results of clinical trials. The EP and USP monographs have described the method and criteria for investigation of hydroxychloroquine sulfate and related substances, and for determination of hydroxychloroquine sulfate content in tablets.

In this work, hydroxychloroquine sulfate and related substances were analyzed following EP assay on Shimadzu UHPLC. The content of hydroxychloroquine sulfate in tablets was determined in refereeing to USP assay in order to provide comprehensive monitoring and evaluation on the quality of hydroxychloroquine sulfate tablet products.



## Experimental

### 1.1 Instrument

Shimadzu UHPLC system LC-30A and HPLC system LC-2040C 3D were used in this work.

The s/w used is LabSolutions™ DB ver 6.87, a chromatography workstation.

### 1.2 Analytical conditions

#### - EP assay (LC-30A UHPLC system)

Column : EP10.0 01/2017:2849, C18 50 X 2.1 mm I.D., 1.7 μm

Mobile phase: A - Methanol / Buffer (10:90 v:v); B Methanol / Buffer (85:15 v:v) \*

Flow rate : 0.7 mL/min

Injection volume : 4 μL

Column Temperature : 40°C

Autosampler temperature: 6°C

Elution mode: gradient solution, B initial conc. 0%, see time program in Table 1

\*Buffer is described in the monograph in the EP 10.0

Table 1. Gradient elution time program

Time (min)	Module	Command	Value (%)
1.00	Pumps	Pump B.Conc	0
11.00	Pumps	Pump B.Conc	100
11.10	Pumps	Pump B.Conc	0
12.50	Controller	Stop	

#### - USP assay (LC-2040C 3D HPLC system)

Column : Shim-pack™ GIST C18 250×4.6mmI.D., 5 μm (PN: 227-30017-08)

Mobile phase: Water/MeOH/CAN/phosphoric acid (800mL/100mL/100mL/2mL), containing 96 mg Sodium 1-pentanesulphonate

Flow rate : 1.0 mL/min

Injection volume : 20 μL

Column Temperature : 40°C

Autosampler temperature: 6°C

Elution mode : isocratic gradient

### 1.3 Sample preparation

As mentioned in EP 10.0 and USP 43.

## Results and Discussion

### 2.1 Determination of hydroxychloroquine sulfate related substances following EP method

In EP10.0 monograph, it requires peak resolution greater than 3.0 for Impurity C and hydroxychloroquine sulfate as well as for Impurity B and Impurity C.

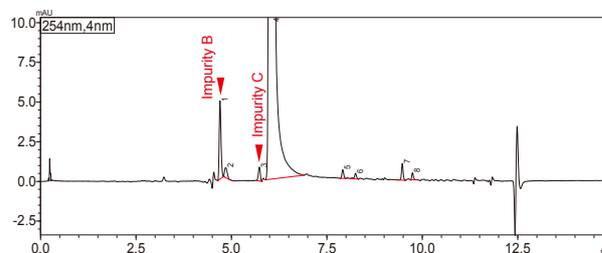


Fig. 1. Chromatogram of hydroxychloroquine sulfate related substances

Table 2. Chromatographic information of hydroxychloroquine sulfate (HCQ) and related substances

Peak#	Ret. Time	Area	Height	Area%	Resolution (EP)
<b>1</b> (Impurity B)	<b>4.701</b>	<b>16191</b>	<b>4885</b>	<b>0.251</b>	/
2	4.849	3007	642	0.047	1.337
<b>3</b> (Impurity C)	<b>5.732</b>	<b>3041</b>	<b>869</b>	<b>0.047</b>	<b>7.769</b>
<b>4</b> (HCQ)	<b>6.050</b>	<b>6421680</b>	<b>1570520</b>	<b>99.537</b>	<b>3.050</b>
5	7.917	1770	542	0.027	18.378
6	8.251	1377	355	0.021	3.624
7	9.475	3153	1040	0.049	13.984
8	9.742	1333	449	0.021	3.206
Total	8.242	6451552	1579303	100.000	3.889



The result indicates that the resolutions obtained are greater than 3.0 for hydroxychloroquine sulfate and Impurity C, as well as for Impurity B and Impurity C. This result meets the criteria stated in the EP monograph.

## 2.2 Determination of hydroxychloroquine sulfate content in tablet following USP assay

### 2.2.1 Result of resolution

In the USP method, it requires that the resolution must be not less than 1.8 for chloroquine phosphate (CQ) and hydroxychloroquine sulfate (HCQ). The resolution obtained is 3.728, which meets fully the criteria of R greater than 1.8.

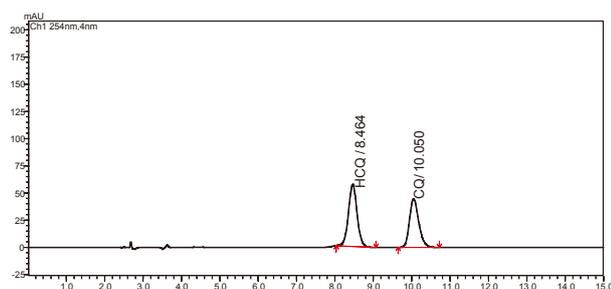


Fig. 2. Chromatogram of hydroxychloroquine sulfate (HCQ) and chloroquine phosphate (CQ)

Table 3. Result of resolution of hydroxychloroquine sulfate (HCQ) and chloroquine phosphate (CQ)

Peak#	Ret. Time	Area	Height	Theoretical Plate Number	Width (USP)/min	Resolution (USP)
HCQ	8.464	888,481	56,853	8107	0.410	/
CQ	10.050	751,613	44,490	8332	0.441	3.728 (>1.8)

### 2.2.2 Reproducibility

The USP assay requires that the peak area RSD% is lower than 1.5% (n=5). The standard solution of hydroxychloroquine sulfate (50 µg/mL) was injected for 5 times. The retention times and peak areas obtained were used to calculate RSD%. The results are shown in Table 4.

The results show that the peak area RSD% of hydroxychloroquine sulfate is 0.03% (n=5), which fully meets the USP criteria of less than 1.5%.

Table 4. Results of Reproducibility Tests (n=5)

Sample concentration	Retention Time (min)	Peak Area
hydroxychloroquine sulfate (50 µg/mL)	8.233	888,721
	8.234	888,407
	8.237	888,412
	8.240	888,213
	8.244	888,879
Average	8.238	888,526
SD	0.0047	268.15
RSD%	0.06	0.03

### 2.2.3 Analysis of actual sample

Twenty tablets of hydroxychloroquine sulfate (content 0.1 g per tablet) were obtained from a drugstore. Following USP procedure, the tablets were pre-treated and analysed. The results are shown in Table 5. The content is calculated using below equation:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$$r_U = \text{Peak area of sample} \quad r_S = \text{Peak area of standard}$$

$$C_S = \text{Conc. of standard (mg/mL)} \quad C_U = \text{Conc. of sample (mg/mL)}$$

The result shows that the content of hydroxychloroquine sulfate of the tablet is 99.99% as compared to the labelled content. This result meets the USP criteria (not lower than 93% and not higher than 107.0%).

Table 5. Result of HCQ content (%) as compared to the labelled content

Sample Name	Peak Area of Standard	Peak Area of Sample	Conc. of Std. (mg/mL)	Conc. of Sample (mg/mL)	Content (%)
hydroxychloroquine sulfate	888,526	888,481	0.05000	0.05002	99.95
		888,904		0.05000	100.04
		888,879		0.05003	99.98
Average		888,754		0.05002	99.99
SD		237.3715		0.000015	0.036864
RSD%		0.03		0.03	0.04

## Conclusion

Following EP10.0 assay, hydroxychloroquine sulfate and their related substances were analysed on Shimadzu UHPLC system. The results indicate that hydroxychloroquine sulfate and Impurity C,



Impurity B and Impurity C are well separated. The resolution of the separation measured meet the requirement described in the EP monograph. Using Shimadzu HPLC, the content of hydroxychloroquine sulfate in a tablet product was determined following USP 43 method. The resolution of the separation for hydroxychloroquine sulfate and chloroquine phosphate measured is 3.728 and the analysis reproducibility RSD% based on peak area is 0.03% (n=5). These results meet fully the criteria stated in the USP monograph for determination of hydroxychloroquine sulfate content in tablet.



**This article is published as  
Application News LC-199.**

<https://solutions.shimadzu.co.jp/an/n/en/hplc/hkl220025.pdf>



## Pharmaceutical

# Determination of genotoxic impurity NDMA in Ranitidine by LCMS-8050 and LCMS-9030

Li Qiang, Zheng Yunzhong, Li Changkun, Meng Haitao, Huang Taohong / Analytical Application Center, Shimadzu (China) Co. Ltd

## Introduction

Genotoxic Impurities (GTI) refer to compounds that damage cellular DNA directly or indirectly, produce gene mutations or in vivo mutagenesis, and have carcinogenic potential. N-dimethylnitrosamine (NDMA), also known as N-nitrosodimethylamine, is a yellow liquid that produced by the reaction of dimethylamine and nitrite under acidic condition. It is widely found in environment and has been identified as an animal carcinogen, with positive

results in a variety of short-term mutagenicity tests. In July 2018, nitroso compounds NDMA were successively detected in the sartan drugs, which caused widespread concern in the society and caused huge losses to related enterprises. In September 2019, FDA issued a statement reminding patients and medical personnel of find NDMA in ranitidine. In Dec 2019, the FDA and EMA issued a series of documents stating that NDMA was found in metformin, an oral diabetes medicine that helps control blood sugar levels.



Fig. 1. FDA documents about analytical methods of NDMA in Ranitidine

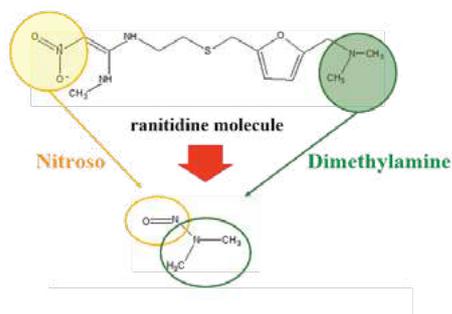


Fig. 2. Ranitidine and NDMA

The researcher found that ranitidine molecule contains both a nitrite and a dimethylamine (“DMA”) group which are well known to combine to form NDMA. Other experiment results showed that ranitidine can react with itself in normal analytical conditions (e.g. GC/MS oven temperature of 130°C) at high efficiency to produce NDMA at levels well in excess of the permissible daily intake limit for this probable carcinogen. Therefore, FDA has published LC-HRMS and LCMSMS methods for the determination of NDMA in ranitidine. Based on the methods conditions recorded in FDA documents, we developed a method for the analysis of NDMA in ranitidine drug substance and capsule preparations by Shimadzu LCMS-8050 Liquid Chromatography-Tandem Mass Spectrometry, and a method by LCMS-9030 Q-TOF Mass spectrometry for the reference of relevant testers.

## Experimental

### 1.1 Instrument

Shimadzu LCMS-8050 Liquid Chromatography-Tandem Mass Spectrometry. The specific configuration is as follows: pump LC-30AD×2, degassing unit DGU-20A5, autosampler SIL-30AC, column oven CTO-20AC, flow switching valve FCV-20AH2, system controller CBM-20A, UV detector SPD-20A, LCMS-8050 Triple Quadrupole Mass Spectrometer, LabSolutions Ver. 5.97 chromatography workstation. The LCMS-9030 Q-TOF system uses the same LC front-end configuration.



Fig. 3. LCMS-8050

### 1.2 Analytical conditions

LC conditions:

Column : ACE-C18-AR (4.6 mm I.D. × 150 mm L., 3 μm)

Mobile phase : A-0.1% formic acid in water; B-0.1% formic acid in methanol

Flow rate : 0.8 mL/min

Injection volume : 10 μL

Column temp. : 40°C

Elution method : Gradient elution, initial phase B concentration is 5%

Table 1. Time program of mobile phase for NDMA determination

Time(min)	Module	Command	Value
1	pump	B.Conc	5
2	pump	B.Conc	13
4.93	controller	Event	1*
6	pump	B.Conc	13
6.1	pump	B.Conc	100
10	pump	B.Conc	100
10.1	pump	B.Conc	5
14	controller	Stop	

Note: \* “1” means the flow path is switched to waste;

Table 2. MS conditions:

Interface	: APCI (+)
Interface temperature	: 300°C
Nebulizing flow	: 3 L/min
DL temperature	: 180°C
Interface voltage	: 3.5 kV
Drying gas flow	: 5.0 L/min
Heat block temperature	: 200°C
Acquisition mode	: MRM
MRM parameter	: refer to Table 3

Table 3. MRM parameters for NDMA

Name	Transitions	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
NDMA	75.15>43.05*	-11	-12	-17
	75.15>58.10	-13	-18	-17

Note: \*means quantitative ion;



### 1.3 Standard solution preparation

An 8-points calibration curve of NDMA was prepared at concentration of 1, 2, 5, 10, 20, 50, 100, 200 ng/mL from 100 mg/L stocked standard solution. The diluent was water.

### 1.4 Sample preparation

Drug substance: Accurately weigh 120 mg of drug substance into a 15 mL glass centrifuge tube. Add 4.0 mL of water into the tube and mix the solution using a vortex mixer until dissolved. Filter the supernatant with a 0.22  $\mu$ m filter.

Drug product: Accurately weigh the appropriate contents and dissolve it with water to reach the target concentration of 30 mg/mL API. Vortex the mixture for 40 min and filter with a 0.22  $\mu$ m filter.

## Results and discussion

### 2.1 Chromatographic separation of NDMA and API

10 ng/mL NDMA standard solution and the ranitidine drug substance was prepared as described above (c.f. section 1.3 and 1.4). The chromatogram was monitored by MRM and UV detector for NDMA and ranitidine respectively. The results showed good chromatographic separation of the two compounds.

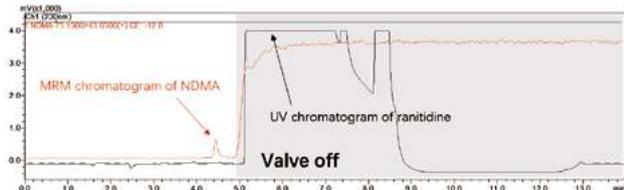


Fig. 4. NDMA standard (10 ng/mL) MRM chromatogram and UV chart of drug substance

### 2.2 Selectivity and Sensitivity

Selectivity is an important indicator that directly affects the credibility of the established method and is a prerequisite for accurate quantitative results later. For NDMA, two transitions were selected through optimization, one for quantitative and the other for reference. The transition ion channel of "75>43" has higher baseline noise than that of "75>58", but its signal intensity is significantly higher than the latter. Therefore, this channel is selected as a quantitative ion channel whereas the other is used as reference ion. The following figure showed sensitivity result of NDMA at 1.0 ng/mL, which showed the s/n ratio above 13.

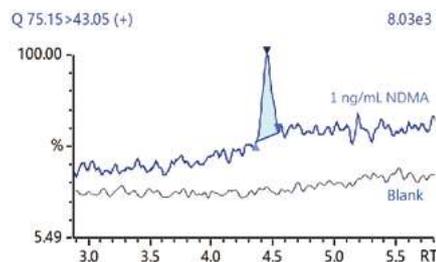


Fig. 5. Selectivity and sensitivity test of NDMA by LCMS-8050

According to the documents issued by FDA, NDMA impurity was separated from ranitidine by HPLC and was detected by a high-resolution and high-mass accuracy (HRAM) mass spectrometer. High sensitivity detection is achieved by monitoring the accurate  $m/z$  value of the protonated impurity ion. LCMS-9030 is a new released high-resolution Q-TOF MS, which has multiple patented hardware designs and accurate and stable mass accuracy. NDMA peak areas from the extracted ion chromatograms (EIC) with a  $m/z$  tolerance of 15 ppm are used for quantitation. The NDMA  $m/z$  value to be extracted is 75.0553. The chromatogram of NDMA with the concentration of 1 ng/mL was showed as below. It can be found that the extract ion chromatogram obtained by high resolution mass spectrometry has better selectivity and sensitivity.

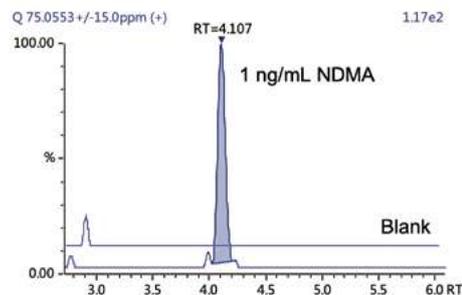


Fig. 6. Selectivity and sensitivity test of NDMA by LCMS-9030



Fig. 7. LCMS-9030

### 2.3 Linearity

The standard solution was prepared according to section 1.3. A good linearity was achieved with a linear correlation coefficient



greater than 0.999 and an accuracy ranges 93.2%–110.6%. The curve results are shown in Fig. 8.

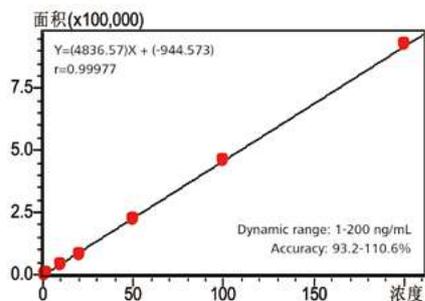


Fig. 8. Calibration curve of NDMA by LCMS-8050

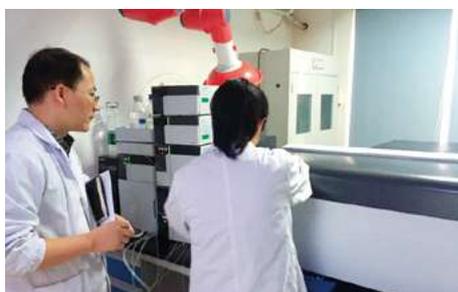


Photo: Customers of Institute for Food and Drugs Control use LCMS-8050 to detect NDMA in ranitidine

For LCMS-9030, standard solutions of NDMA at eight different concentrations (1.14, 2.28, 4.56, 9.12, 18.2, 36.5, 73 and 146 ng/mL) were prepared in methanol. Each of these solutions was injected and the extraction ion chromatogram of  $m/z$  75.0553 with 15 ppm tolerance was processed. A graph was plotted of NDMA peak area against concentration. The plot was linear in the range 1.14 ng/mL to 146 ng/mL for NDMA with a correlation coefficient 0.9999. Accuracy of each standard solutions was 86.95–112.32%.

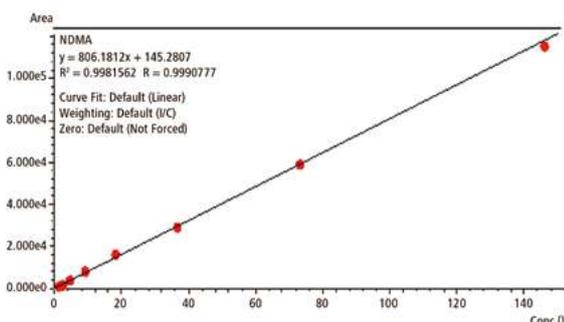


Fig. 9. Calibration curve of NDMA by LCMS-9030

## 2.4 Repeatability

Prepare standard solutions of three concentrations of low, medium, and high then conduct 6 injections successively to examine the repeatability of retention time and peak area. The relative standard

deviations (RSD%) of the retention times and peak areas of NDMA at three levels were between 0.06–0.13% and 1.68–2.96%, respectively, which met the FDA method requirements (retention time RSD% <2%, peak area RSD% <10%).

## 2.5 Real world sample test

Prepare the drug substance and capsule according to 1.4 and each sample is measured duplicate. The chromatograms are shown in Fig. 10. The test results are shown in Table 4.

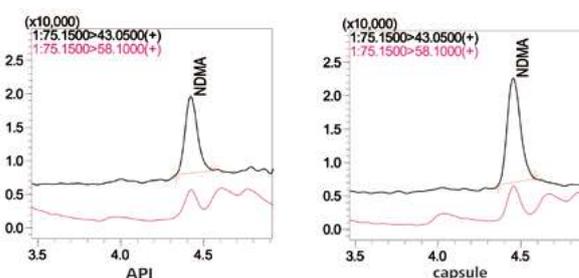


Fig. 10. Chromatogram of real sample test

Table 4. Test results of NDMA in ranitidine drug substance and capsule by LCMS-8050

Name	Test concentration (ng/mL)	Impurity content (ppm)
API	3.89	0.13
capsule	5.25	0.17

The developed LC-QTOF-MS method was used for determination of NDMA impurities in ranitidine drug substance. Six lots of samples were prepared and tested. From the calibration curve, the amount of NDMA in dry powder of ranitidine substance was calculated. The retention time difference of NDMA between in sample solution and in the standard solution was found to be less than 0.04 min, which was acceptable as it is less than 2%. The assay value of NDMA was found to be less than 0.33 ppm, the acceptable limit what the FDA considers safe for these medicines.

Table 5. Test results of NDMA in ranitidine drug substance by LCMS-9030

No.	Impurity content (ppm)
1	0.13
2	0.11
3	0.03
4	0.04
5	ND
6	ND



## 2.6 Spiked recovery

The ranitidine drug substance was prepared according to section 1.4. The standard was spiked at 80%, 100%, and 120% of the concentration in the drug substance, and measured triplicates. The recoveries of the three concentrations are between 90.3% and 100.9%, and the relative standard deviations (RSD%) of three parallel samples are between 1.77 and 4.93%, indicating that the method is accurate and reliable.

Table 6. Recovery of NDMA spiked to API at 80,100 and 120% level. Each level has triplicated samples.

Name	Spiked concentration (ng/mL)	Test concentration (ng/mL)	Recovery	RSD/%
API	—	7.59	—	—
80%	5.6	12.75	92.1	1.77
100%	7.0	14.65	100.9	4.93
120%	8.4	15.18	90.3	4.85

## Conclusion

For NDMA in different drugs, several instrumental methods were developed as mentioned above. It is noted that method selection is based on the chemical properties of the drug, GTI and limit of quantification regulated by the authorities. In this study, the triple quadrupole mass spectrometer LCMS-8050 and Q-TOF MS LCMS-9030 were used to establish methods for determining NDMA in ranitidine drug substance and capsule. The results for real samples showed that methods could be used for quality control and commodity inspection for pharmaceutical companies or Drug Inspection Agency. The method optimized by adjusting LC parameters based on the original method can be used for the detection of nizatidine and Bismuth ranitidine citrate. The LCMS method for metformin is also established in the same way. It is important to note that the retention time of metformin is weaker than that of NDMA, and thus better separation is required to obtain accurate results. Alternatively, GCMSMS or GCMS is also a choice for NDMA analysis. It is applicable when the compound is thermo stable.

## Reference

1. Zantac (ranitidine): Safety Information—NDMA Found in Samples of Some Ranitidine Medicines. <https://www.fda.gov>
2. Formation of NDMA from ranitidine and sumatriptan: The role of pH, Ruqiao Shen, Susan A Andrews, Water Research, 2013(47) 802-810.
3. Structural alerts of genotoxic impurities, MA Lei et al, Chinese Journal of New Drugs 2014, 23(18)
4. FY19-177-DPA-S. Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Ranitidine Drug Substance and Drug Product, FDA.
5. FY19-059-DPQR-S, Review of Potential NDMA Formation from Ranitidine HCl (July 5, 2019), FDA.
6. Statement on new testing results, including low levels of impurities in ranitidine drugs, <https://www.fda.gov>
7. Questions and Answers: NDMA impurities in ranitidine (commonly known as Zantac) <https://www.fda.gov>
8. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for the Determination of NDMA in Ranitidine Drug Substance and Solid Dosage Drug Product, <https://www.fda.gov>
9. Determination of N-nitrosodimethylamine (NDMA) in metformin products by HRAM-GCMS. Pharmaceutical Laboratory Applied Sciences Group, Health Sciences Authority, Singapore.
10. NDMA impurity in valsartan and other pharmaceutical products: Analytical methods for the determination of N-nitrosamines, Maria Kristina Parr, Jan Felix Joseph, Journal of Pharmaceutical and Biomedical Analysis, 2019 (164) 536-549



## Pharmaceutical

# Determination of genotoxic impurity NDMA in Metformin by LCMS-8050 and GCMS-TQ8050 NX

Zheng Jia, Huang Gang, Gao Jie, Shen Lingling, Fan Jun, Huang Taohong / Analytical Application Center, Shimadzu (China) Co. Ltd

### Introduction

Genotoxic Impurities (GTI) refer to one group of potential carcinogenic compounds which damage cellular DNA directly or indirectly and induce gene mutations or in vivo mutagenesis. N-dimethylnitrosamine (NDMA) is such a type of genotoxic impurity and classified as Group 2A carcinogen by IARC; however it has been found in some common drugs. In July 2018, the EMA announced that NDMA was detected in the anti-stress drug, valsartan. After more than one year, it was detected again in ranitidine, a drug for treating high gastric acid secretion diseases, according to report from FDA and EMA in September 2019. Thus, we established the complete solution for the detection of NDMA in the drugs of valsartan and ranitidine. However, in December 2019, the FDA and EMA reported in an official announcement that NDMA was found in metformin, an oral diabetes medicine that helps control blood sugar levels in official documents. The pharmaceutical matrix of metformin is different from that of valsartan and ranitidine. Therefore, a new method of extracting and detecting the NDMA in metformin is extremely required.

According to FDA, the upper limit of NDMA in metformin is 96 ng/day with the assumption of the maximum daily dosage of metformin as 2 g, so the maximum content of NDMA in metformin is 48 ng/g. To meet or even superior to that detection requirement from different customers, we developed three methods to detect NDMA in metformin based on the recipe published by National Institutes for Food and Drug Control and Health Sciences Authority of Singapore. The first is to use the LCMS-8050 with a LC-MS/MS protocol for the analysis of NDMA in metformin hydrochloride tablets and active pharmaceutical ingredient (API) besides sustained release tablets. Meanwhile, due to NDMA is a volatile small molecule, we also built a GC-MS/MS method by GCMS-TQ8050 NX with higher sensitivity and better anti-interference capacity for the detection of NDMA with lower concentration in hydrochloride metformin tablets and API. Additionally, given that the detection cost, we also built an inexpensive method using GCMS named GCMS-QP2020 NX to determine the concentration of NDMA in metformin according to the method published by Health Sciences Authority of Singapore.



## 1. Determination of NDMA in metformin by LC-MS/MS Experimental

### 1.1 Instrument (LC-MS/MS)

Liquid Chromatography-Tandem Mass Spectrometry: LCMS-8050, Shimadzu Corporation.

The specific configuration is as follows: pump LC-30AD×2, degassing unit DGU-20A, autosampler SIL-30AC, column oven CTO-30A, flow switching valve FCV-20AH<sub>2</sub>, system controller CBM-20A, PDA detector SPD-M20A, LCMS-8050 Triple Quadrupole Mass Spectrometer and LabSolutions Ver. 5.89 chromatography workstation.



Fig. 1. LCMS-8050

### 1.2 Analytical conditions

LC condition:

Column : ACE-C18-AR (4.6 mm I.D. × 150 mm L., 3 μm)

Mobile phase : A-0.1% formic acid in water;  
B-0.1% formic acid in methanol

Flow rate : 0.8 mL/min

Injection volume: 5 μL

Column temp : 40°C

Elution method : Gradient elution, initial phase B concentration is 5%

Table 1. Time program of mobile phase for NDMA determination

Time(min)	Module	Command	Value
1	pump	B.Conc	5
2	pump	B.Conc	13
3.2	controller	Event	1*
5.2	controller	Event	0*
6.0	pump	B.Conc	13
6.1	pump	B.Conc	100
10.0	pump	B.Conc	100
10.1	pump	B.Conc	5
14	controller	Stop	

Note: \* "0" means the flow path is switched to waste;  
\* "1" means the flow path is switched to Mass Spectrometer.

Table 2. MS condition

Interface	APCI (+)
Interface temperature	300°C
Nebulizing flow	3 L/min
DL temperature	180°C
Interface voltage	4.5 kV
Drying gas flow	5.0 L/min
Heat block temperature	200°C
Acquisition mode	MRM
MRM parameter	refer to Table 3

Table 3. MRM parameters for NDMA

Name	Transitions	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
NDMA	74.95>43.05*	-31	-16	-16
	74.95>58.10	-14	-16	-36

Note: \* means quantitative ion;

### 1.3 Calibration

A 6-points calibration curve of NDMA was prepared at concentration of 1, 2, 5, 10, 50, 100 ng/mL from 100 mg/L stock standard solution. The diluent was water.

### 1.4 Sample preparation

Accurately weigh 300 mg of sample into a 10 mL volumetric flask. Add water to dissolve and fix the volume to 10 mL. Filter the supernatant with a 0.22 μm filter.

## Results and discussion

### 1.5 Chromatographic separation of NDMA and API

100 ng/mL NDMA standard solution and the metformin drug substance was prepared as section 1.3 and 1.4. The chromatogram was monitored by MRM and UV detector for NDMA and metformin, respectively. The results showed good chromatographic separation of two compounds. We can transfer the flow path to mass spectrometry from 3.2 min to 5.2 min to avoid contamination.

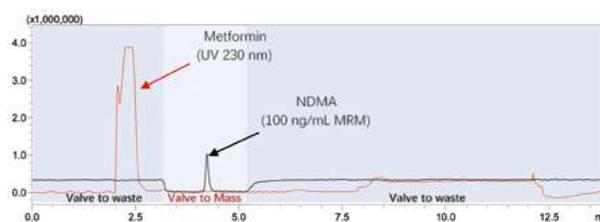


Fig. 2. NDMA standard (100 ng/mL) MRM chromatogram and UV chart of drug substance



### 1.6 Linearity and Sensitivity

The standard solution was prepared according to section 1.3. A good linearity was achieved with a linear correlation coefficient greater than 0.999 and an accuracy ranges 93.1%–110.9%. The curve is shown in Fig. 3. Fig. 4 shows sensitivity result of NDMA at 1.0 ng/mL, displaying a S/N above 13, which meets the requirements of the National Institutes for Food and Drug Control method ( $S/N > 10$ , 1.0 ng/mL).

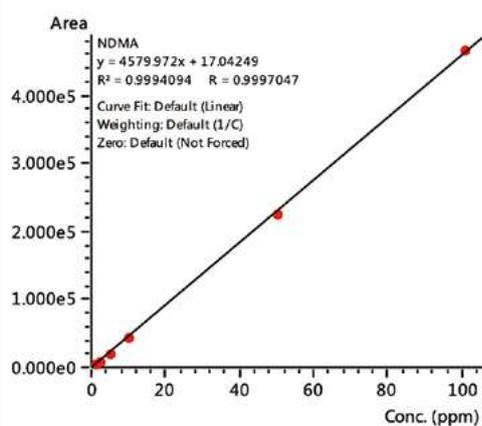


Fig. 3. Calibration curve of NDMA (1–100 ng/mL)

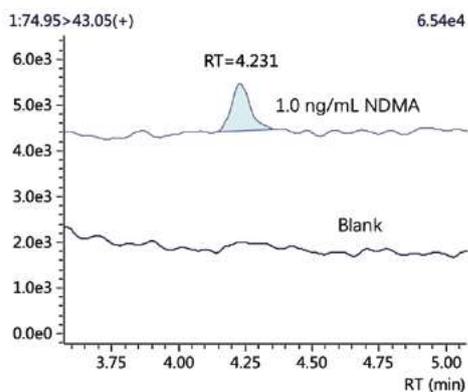


Fig. 4. Sensitivity test of NDMA by LCMS-8050

### 1.7 Repeatability

Standard solutions of 2 ng/mL was prepared according to section 1.3, and then 6 injections were conducted successively to examine the repeatability of retention time and peak area. The Relative standard deviations (RSD%) of retention time and peak area of NDMA were obtained as 0.08% and 7.81%, respectively.

### 1.8 Actual sample test

The metformin hydrochloride tablets were prepared according to 1.4. The chromatogram is shown in Fig. 5, and test result was shown in Table 4.

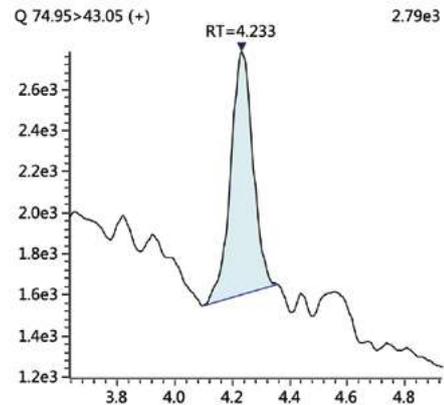


Fig. 5. Chromatogram of actual sample

Table 4. Test results of NDMA in metformin hydrochloride tablets

Name	Test concentration (ng/mL)	Impurity content (ng/g)
tablets	1.35	27.0

### 1.9 Spiked recovery

The metformin hydrochloride tablet was prepared according to section 1.4. The standard solution was spiked at 1, 2 and 5 ng/mL in the tablets, and measured in triplicates. Recoveries of three concentrations were between 86.0% and 100.0%, and RSD% of three parallel samples were between 1.16 and 3.58%, indicating the method is accurate and reliable.

Table 5. Recovery of NDMA spiked to metformin tablets (n=3)

Name	Spiked concentration (ng/mL)	Test concentration (ng/mL)	Recovery	RSD/%
Tablets	—	ND	—	—
Spiked 1	1	0.86	86.0	1.16
Spiked 2	2	2.00	100.0	1.26
Spiked 3	5	4.70	94.0	3.58



Photo: Customer of Institute for Food and Drugs Control use LCMS-8050 to detect NDMA in metformin



## 2. Determination of NDMA in metformin by GC-MS/MS Experimental

### 2.1 Instrument (GC-MS/MS)

Gas chromatography-tandem mass spectrometry: GCMS-TQ8050 NX, Shimadzu Corporation.



Fig. 6. GCMS-TQ8050 NX

### 2.2 Analytical conditions

Column : SH-Rtx-Wax, 30 m×0.25 mmID×0.5 μm  
 Oven program : 60°C (0.5min)\_15°C/min\_ 150°C \_20°C/min\_  
 240°C (2min)  
 Linear velocity : 51.6 cm/sec  
 Injection mode : Splitless  
 Injection volume : 2 μL  
 Ionization mode : EI  
 Ion source Temp : 230°C  
 Interface Temp : 240°C  
 Acquisition mode: MRM (See Table 6)  
 Detector Voltage : Relative to tuning results+0.8kV

Table 6. Information of NDMA

Compound	CAS No	Retention time (min)	Transition 1	CE (V)	Transition 2	CE (V)
NDMA	62-75-9	4.988	74.00>44.10	6	74.00>42.10	24

### 2.3 Standard solution preparation

An 8-points calibration curve of NDMA was prepared at concentration of 0.25, 0.5, 1, 5, 10, 20, 50 and 100 ng/mL. The diluent was dichloromethane.

### 2.4 Sample preparation

The drug was crushed evenly before the sample containing 0.5 g of metformin effective ingredient was weight. And then, the sample was added with 10 mL 1N hydrochloric acid solution, stirred and shaken, added 10 mL dichloromethane, stirred and shaken for 10

min, centrifuged for 10 min at 4000 rpm successively. After all the steps before, the organic phase was taken to filter through 0.22 μm filter membrane.

## Results and discussion

### 2.5 Linearity and Sensitivity

The standard solution was prepared according to section 2.3. A good linearity was achieved with a linear correlation coefficient 0.999 as shown in Fig. 7. Fig. 8 shows the MRM chromatogram of NDMA (0.25 ng/mL), and the S/N of NDMA (0.25 ng/mL) is 14.5. The limit of detection (0.25 ng/mL) convert to a concentration 5 ng/g in real sample far less than 48 ng/g, the upper limit of NDMA in metformin. GCMS-TQ8040 NX can also reach the limit of detection 5 ng/g through the test in customer's laboratory.

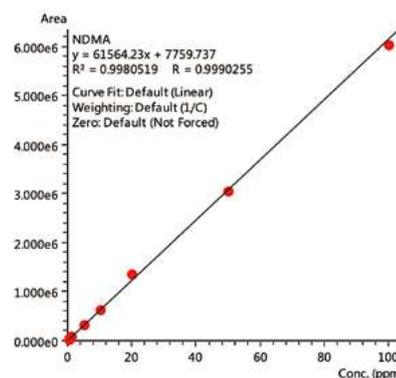


Fig. 7. Calibration curve of NDMA (0.25–100 ng/mL)

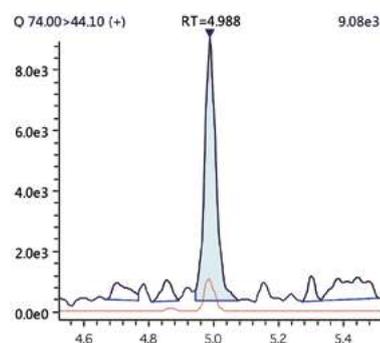


Fig. 8. MRM chromatogram of NDMA (0.25 ng/mL)

### 2.6 Repeatability

Standard solutions of 0.5, 1 and 5 ng/mL were taken successively to examine the repeatability of peak area with 6 injections for each concentration. The relative standard deviations (RSD%) of peak areas at three levels were between 2.76–4.03%.



## 2.7 Actual sample test

The metformin hydrochloride tablet was prepared according to section 2.4. The MRM Chromatogram of NDMA in metformin hydrochloride tablet is shown in Figure 9, and the concentration is 13.6 ng/g.

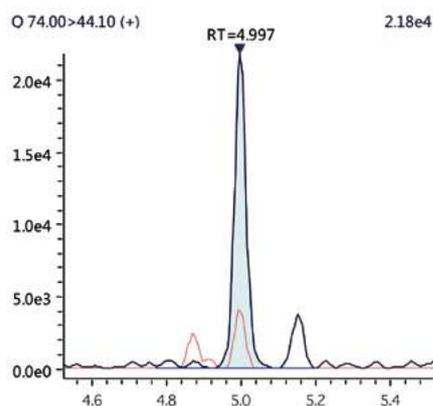


Fig. 9. Chromatogram of NDMA in metformin hydrochloride tablet

## 2.8 Spiked recovery

The metformin hydrochloride tablet was prepared according to section 2.4. The spiked concentration is 20, 40 and 100 ng/g respectively, and each level was tested for 6 times. Spiked recoveries of the actual sample were about 85%, and RSD were lower than 5%.

## 3. Determination of NDMA in metformin by GC-MS

Except for the two methods shown above, we also established an economical GC-MS method by GCMS-QP2020 NX in SIM mode to detect NDMA in metformin. The results showed that the linear correlation coefficient ( $r$ ) of NDMA was 0.999 in the concentration range of 1–100 ng/mL, indicating good linearity. Figure 11 shows the SIM chromatogram of NDMA (1 ng/mL), and the S/N of NDMA (1 ng/mL) is calculated as 16.2. However, it should be noted that one of the impurities in dichloromethane, DMF, may interfere with the peak of NDMA, and they can be separated completely by adjusting the program of column oven temperature. The repeatability was evaluated by six injections of NDMA (2 ng/mL), and the relative standard deviation (RSD%) of the peak area ratio was 3.99%. We also detected the concentration of NDMA in an actual metformin hydrochloride sustained release tablets by GC-MS. The NDMA in sustained release tablets was extracted with dichloromethane directly without dissolving by HCl solution in advance, which is somewhat different from the pretreatment

method of metformin hydrochloride tablets. The test result of actual sample was 68 ng/g. The average recovery was 93.0% at spiked level of 48 ng/g for the actual sample.

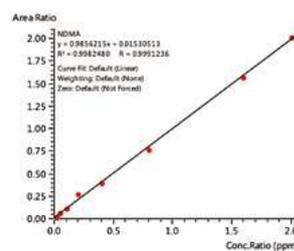


Fig. 10. Calibration curve of NDMA (1–100 ng/mL)

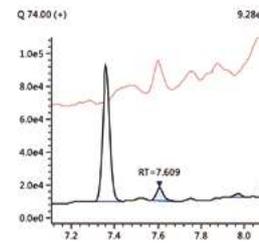


Fig. 11. SIM chromatogram of NDMA (1 ng/mL)

## Conclusion

We developed three methods to detect NDMA in metformin, including LC-MS/MS method, GC-MS/MS method and GC-MS method. The GC-MS/MS method by GCMS-TQ8050 NX is the most sensitive method with the detection limit of 5 ng/g, and GCMS-TQ8040 NX can also reach such a detection concentration as low as 5 ng/g. The cost-effective GC-MS method is also used to detect NDMA especially with high concentration in metformin hydrochloride sustained release tablets. In the end, the laboratories with existing LC-MS/MS instruments can directly use it to detect NDMA in metformin, and the results will completely meet to the detection requirements of the National Institutes for Food and Drug Control and the FDA.

## Reference

1. Determination of NDMA in metformin hydrochloride capsules and enteric coated tablets by LC-MS/MS, National Institutes for Food and Drug Control.
2. Determination of NDMA in metformin hydrochloride API and formulations, National Institutes for Food and Drug Control.
3. Determination of N-nitrosodimethylamine (NDMA) in metformin products by HRAM-GCMS. Pharmaceutical Laboratory Applied Sciences Group, Health Sciences Authority, Singapore.
4. Combined Direct Injection N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), and N-Nitrosodibutylamine (NDBA) Impurity Assay by GC-MS/MS, FDA
5. NDMA impurity in valsartan and other pharmaceutical products: Analytical methods for the determination of N-nitrosamines, Maria Kristina Parr, Jan Felix Joseph, Journal of Pharmaceutical and Biomedical Analysis, 2019 (164) 536-549



## Pharmaceutical

# The Necessity of Analyzing Elemental Impurities in Drug products, and Approaches to Their Measurement

Tadashi Taniguchi, Shimadzu Corporation

Elemental impurities in drug products formulations consist of the residue of catalytic components added when synthesizing the pharmaceuticals, as well as chemicals originating through unexpected contamination from manufacturing instruments, and chemicals included in the structural components of the formulations. Elemental impurities impede the active ingredients in Drug products, and can have toxic effects on patients, so keeping the amount of elemental impurities in Drug products within permitted limits is viewed as very important. Accordingly, evaluations have long been performed using the heavy metals test method. However, this test method stains metallic contaminants using a reagent, and is limited to expressing the results in terms of the amount of lead. It is not specifically intended for elements, is lacking in quantitative ability, and the standard values are not necessarily based on toxicity. Other issues have also been pointed out, including the inability to evaluate Pd and Pt and other metallic catalysts utilized for synthesis.

Accordingly, at the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the toxicity of elemental impurities was considered in Guidelines for Elemental Impurities (Q3D), and permitted daily exposure levels for 24 elements were configured as guideline levels. These

include the so-called big four: lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), as well as residual metal catalysts added intentionally in the synthesis of the drug substances. In Europe and the USA, the permitted daily exposure (PDE) for 24 elements, as well as their measurement methods, are specified in the respective Pharmacopoeia, in accordance with the ICH Q3D. These apply to all commercially available formulations as of January 2018.

USP: USP <232> Elemental Impurities-Limits

USP <233> Elemental Impurities-Procedures

EP : Ph,Eur, Chapter 5.20: Elemental Impurities in Pharmaceutical-Limits

Ph Eur, Chapter 2.4.20: Determination of elemental impurities

Methods for measuring elemental impurities are indicated in the USP <233>, and the Ph. Eur., Chapter 2.4.20, and inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) are noted as the measurement instruments. For both instruments, multi-element analysis can be performed by dissolving the samples using devices such as a microwave sample decomposition unit. These instruments can quickly perform analysis of the 24 elements indicated in the ICH Q3D guidelines in a short time (several seconds per sample). Further, ICP-MS is capable of high sensitivity analysis of the concentrations in measurement sample solutions at the ppt



(pg/mL) level, thereby supporting the analysis of even low concentration. This section will introduce examples of the analysis of drug products utilizing these instruments.



#### **Analysis of ICH Q3D Guideline for Elemental Impurities in Drug Products Using ICPMS-2030**

<https://solutions.shimadzu.co.jp/an/s/en/icp/jpb416006.pdf>



#### **Analysis by ICP Atomic Emission Spectrometry in Accordance with the ICH Q3D Guideline for Elemental Impurities Using ICPE-9820**

<https://www.shimadzu.com/an/literature/icp/jpb414004.html>



#### **Analysis of Elemental Impurities in Pharmaceutical Products Following USP <232>/<233> on ICPMS-2030**

<https://www.shimadzu.com/an/literature/icp/apb419001.html>

With the ICP-OES or ICP-MS, if the sample is a solid, pretreatment is required to create a sample solution. Conversely, with the

energy dispersive X-ray (EDX) method, solids, liquids, and powders can all be measured without pretreatment, which means that this measurement method can improve productivity. This method is adopted as a general analysis method in the USP <735> and the European Pharmacopoeia Ph. Eur., Chapter 2.2.37. At the present stage, there are sensitivity issues for the measured elements. However, with the specified elements, it is of interest as a measurement method that can perform measurements without pretreatment and in a short time.



#### **Pharmaceutical Elemental Impurities Analysis System**

##### **Energy Dispersive X-ray Fluorescence Spectrometer**

[https://www.shimadzu.com/an/elemental/edxrf/pharma\\_impurities/index.html](https://www.shimadzu.com/an/elemental/edxrf/pharma_impurities/index.html)



#### **ICH Q3D Elemental Impurities Analysis of Drug Substances by EDX**

<https://solutions.shimadzu.co.jp/an/n/en/edxrf/jpq219010.pdf>

# Shimadzu Selection

These articles were selected by Shimadzu. Relating pharmaceutical analysis and development, they are from posters presented at ASMS 2019 and from application notes. They feature a variety of instruments we produce and include cutting-edge technologies. Please obtain the articles of your interest through the links on the titles.



## Microflow LC-MS/MS Analysis of Monoclonal Antibody in Human Plasma at ng/mL Level with nSMOL Proteolysis

A highly sensitive quantitation of the therapeutic monoclonal antibody Trastuzumab using a combination of nSMOL proteolysis and the Nexera Mikros system is introduced. Using this microflow system, the lower limit of quantitation (LLOQ) was 0.00763  $\mu\text{g/mL}$  which is higher than the LLOQ of 0.060  $\mu\text{g/mL}$  when using a conventional semi-microflow LC/MS system (cited from Application News No. C145A), indicating a sensitivity 7.9 times higher.



## Analysis of Modification Site of Chemically Modified Antibody Using MALDImini-1 Compact MALDI Digital Ion Trap Mass Spectrometer

In evaluations of antibody drug conjugates (ADC), a new class of anti-cancer drugs in which an antibody is bound to a cytotoxic drug, the compound binding site on the protein is one of the critical quality properties. This article reports an example in which a pseudo ADC was created by artificially binding a low-molecular compound to a standard research antibody, and the chemical modification site was analyzed by using a compact MALDI digital ion trap (DIT) mass spectrometer.



## Simplified Mass Measurement of Chemically-Modified Antibodies: Determination of the Presence of the Number of Modifications Using a Linear Benchtop MALDI-TOF MS

Antibody drug conjugates (ADC) appeared in the 2000s with the expectation they would serve as more effective anti-cancer drugs than previous small-molecule pharmaceuticals through the combination of the antibody's high selectivity and the availability of a small-molecule drug. This article introduces an example of analyzing the pseudo ADC, which was created by artificially binding low-molecular compounds to a standard research antibody, using a benchtop MALDI-TOF MS.



## Characterization of Insoluble Subvisible Particles in Biopharmaceuticals by Flow Imaging Method

In characterization of insoluble subvisible particulate matter in biopharmaceuticals, particles with sizes of 10  $\mu\text{m}$  and larger are characterized by the light obscuration (LO) method as a test method in the Japanese and United States Pharmacopoeias. However, in recent years it has been reported that the LO method has poor sensitivity for highly transparent particles (e.g. aggregates of proteins) in comparison with the flow imaging (FI) method. As an additional limitation of LO, it is also impossible to predict the type of particle from the particle image in some cases.



## Fast Analysis of Glycoprotein-Derived Glycans Using the MultiNA Automated Microchip Electrophoresis System

High-throughput quantitative and qualitative glycoanalytical procedures are required for the glycoscientific research field (e.g. metabolism of glycans, glyco-biomarker discovery, and quality control of glycoprotein pharmaceuticals). Among currently available analytical techniques, microchip electrophoresis is one candidate for high-throughput quantitative and qualitative glycoanalysis. In this article, we describe a strategy for high-throughput glycoanalytical procedures for the relative quantitation of N-linked glycans using the MultiNA microchip electrophoresis system, which is specialized for DNA/RNA analysis.



## Peptide Mapping of Monoclonal Antibody (mAb) Using Nexera Bio UHPLC and Q-TOF Mass Spectrometer with a Shim-pack GISS-HP Column

Monoclonal antibody (mAb)-based biotherapeutics are emerging as one of the fastest-growing categories of biologic drugs being developed today. Peptide mapping is a key analytical method for quality assurance of mAb products. In the previous application news AD-0176, we had developed a mAb peptide mapping workflow by using both Nexera Bio UHPLC and LCMS-9030 (Q-TOF) systems, yet the method is time consuming, taking 2 hours for each analysis. In this report, we aim to optimize the workflow to reduce the running time and maintain the 100% peptide sequence coverage on Shimadzu LCMS-9030 (Q-TOF) mass spectrometer.



## Disulfide Bond Characterization of Monoclonal Antibody (mAb) Using Q-TOF Mass Spectrometer

Monoclonal antibody (mAb) is emerging as the fastest-growing category of biotherapeutics with a wide range of therapeutic and diagnostic applications. Higher-order structure of mAb plays a critical role in the efficacy and safety. Herein, we report a LCMS-based method to precisely characterize disulfide bonds in mAb biosimilar by a comparative analysis of non-reduced and reduced conditions. The peptides were gradient-eluted and analyzed using a Shimadzu LCMS-9030 Q-TOF mass spectrometer for MS scan and MS/MS analysis.



## In-depth Peptide Mapping of Monoclonal Antibody by A de novo Peptide Sequencing Method on Q-TOF Mass Spectrometer with Data-independent Acquisition

Peptide mapping is an important and efficient technique for the primary structure characterization of monoclonal antibodies (mAbs). It involves enzymatic digestion (e.g., trypsin) and enables the direct detection of single amino acid changes. Also, it can provide additional information of post-translational modifications (PTMs). However, we have to note that peptide mapping is usually performed in a comparative manner, in which the peptide map of a mAb product is compared to that of a reference in a side-by-side experiment, which is highly dependent on the reproducible sample preparation and extremely time consuming.



### Monoclonal Antibody Workflows on the Shimadzu Q-TOF LCMS-9030 Using the Protein Metrics Software Suite

Accurate characterization of monoclonal antibodies is essential to development of biotherapeutics. Thorough understanding of biotherapeutic properties aids in the optimization of bioprocess production, product formulation, and product dosage. In this application note, we use the new Shimadzu Q-TOF LCMS-9030 to characterize the recombinant human IgG1 NIST mAb reference standard as a model of biotherapeutic monoclonal antibodies.



### Novel Platform for Online Sample Preparation and LC-MS/MS Analysis of Drugs in Biological Matrices

Currently, sample preparation in forensic laboratories involves using time-consuming SPE or LLE. The multi-step sample preparation can lead to more human error, irreproducible results, and endanger the analyst by exposing them to biohazardous materials. The Clinical Laboratory Automation Module (CLAM) was developed to meet these needs and offer a safer, hands-free approach to postmortem sample analysis.



### High-Sensitivity Analysis of drugs in ultra-small volumes plasma samples using Micro-Flow LC-MS/MS

Drugs and other xenobiotics entering the body are generally subject to metabolism that facilitates their detoxification and elimination. The ability to predict the metabolic fate of compounds before the first doses are given to humans is highly desirable for reasons of both efficacy and safety. However, the size of the animal models usually restricts the sample volume that can be safely taken during a pharmacokinetic time study for the drug and its metabolites. This quickly results in the need for many animals and a significant amount of drug material.



### A Comprehensive N-Glycan Profiling Analysis of Bevacizumab Biosimilar by UHPLC with Fluorescence Detection and Q-TOF Mass Spectrometry

Global biopharmaceutical market is entering a new era of biosimilars - generic copies of commercialized monoclonal antibodies (mAbs), with the aim of providing less-expensive medication options. In this work, we established a robust, sensitive and reproducible analytical system on the basis of a Nexera Bio UHPLC coupled with Fluorescence detection and Q-TOF Mass Spectrometry for N-glycan profiling analysis of a bevacizumab biosimilar sample.



### High Sensitivity Identification of Drug Metabolites with Increasing Ionization Efficiency using A Novel LC-ESI Interface and Q-TOF

Conventional LC flow rate instruments may be unable to detect minor metabolites due to the lack of sensitivity. Although nanoflow ESI helps to enhance the signal, the analytical time becomes longer and robustness is challenging. In this work, we demonstrate the ability of a micro-flow LC-ESI system with a high performance Q-TOF to detect low levels of drug metabolites.



### Qualitative Characterization and Quantitative Assessment of Monoclonal Antibodies Using Protein Metrics and nSMOL coupled with the Shimadzu LCMS-9030 QTOF

In this poster, we use the new LCMS-9030Q-ToF to qualitatively characterize NIST mAb reference standard as a model of biotherapeutic monoclonal antibodies. Assessments of intact profile, protein subunits (heavy chain, and light chain), and peptide fragments were included in experimental design. To aid in the quantitative assessment of Bevacizumab in biological matrix, the Shimadzu nSMOLTM technology was utilized for increased sensitivity by selectively digesting the antibody's FAB region and reducing matrix interference.



### Latest topics 1

# Global Pharma Summit 2019 Report

On November 14th, 2019, Shimadzu (Asia Pacific) hosted The Global Pharma Summit in Singapore. This was seventh chapter of the event, with theme of “Transforming the Future of Pharma”. This technology forum reaffirmed Shimadzu’s commitment to contribute towards growth and development of Pharma business in Asia. The event brought 103 pharmaceutical industry leaders, health-care providers and researchers to Singapore from twenty countries around the world.

In his opening remarks, Shimadzu CEO Dr. Teruhisa Ueda very eloquently framed the event and set the expectations right, stating that “most things in the world today are powered by AI. This summit is also harnessing what Shimadzu calls ‘Analytical Intelligence’. This only-of-its-kind innovation in the world will be the backbone of all future labs [...] and you will experience this disruptive technology first-hand at this summit.”

## Global Trends in the Pharmaceutical Industry

In recent years, the pharmaceutical industry has started integrating a new intelligence, which has given rise to new demands in both the research process and the laboratory itself. Adopting AI (artificial intelligence) to improve efficiency for various tasks, integrating a multitude of systems, improving productivity and reducing human error through automation – all of these trends have created a climate in which reliable, more efficient analysis in research and development is expected.

## Increasing Laboratory Efficiency: Powered by AI

Similar to other industries, pharmaceutical industry has experienced improvements in its processes, specifically by improving efficiency and decreasing research time due to AI. At the conference, Dr. Jimmy Yen-Chu Lin from Insilico Medicine outlined the company’s AI platform for drug discovery. To hear Dr. Lin say it, “our goal in using AI is to decrease the research, discovery and preclinical test time from 3–4 years to a few months. By focusing on the precious time in the developmental stage as well as through diminishing costs, we can aim to get more drugs approved.” During his presentation he introduced real and practical application of AI to their research. Dr. Jimmy demonstrated utilization of AI for screening antibody drug candidates in cancer treatment or the search process for low molecular lead compounds, which has been called “finding a needle in a haystack”.



Dr. Jimmy Yen-Chu Lin

## Medical Cannabis

Anticipating the spread of medical marijuana in Thailand, Chao Phya Abhaibhubejhr Hospital is looking at developing the cultivation, manufacturing and quality control of cannabis, in addition to patient monitoring and establishing an educational system with regards to the drug. Though research is still being performed regarding the safety and efficacy of cannabis for medical use, and while it remains to be approved by the country's regulatory institution, one of the hospital's researchers, Dr. Pekakrong Kwankhao, described the situation in Thailand: "In February of 2019, usage of medical marijuana was legalized, and currently we are at the stage where only hospitals and accredited agencies may treat with the drug. In our hospital, we are exploring cannabis' effect on quality of life among cancer patients and developing medical products to that end."

The CEO of Front Range Biosciences Inc. USA, Dr. Jonathan Vaught, introduced the chemical diversity and potential of the medical effects of cannabis through outlining two examples of recent research, one concerning the effects of THC on cancer cell biology and another on applied research together with autistics. He stated that "cannabis has wide applications, both for medical as well as commercial use as wellness products. The secret to unlocking its potential lies in understanding its make-up and chemical diversity."



Dr. Jonathan Vaught

## Shimadzu Analytical Intelligence and Workflow Solutions for The Pharma Industry

By listening to the needs of researchers and other users of our products, Shimadzu has proposed its new concept of "Analytical Intelligence" to usher in a new intelligence for analytical instruments.

Analytical Intelligence describes automated support functions utilizing digital technology, such as M2M, IoT, and Artificial

Intelligence (AI), that enable higher productivity and maximum reliability. It consists of systems and software that simulate expert operators automatically, determining whether conditions and results are good or not, providing feedback to users, and solving common problems. It increases data reliability by compensating for any differences between users in their instrument knowledge or experience.



Including the new Nexera UHPLC series which was released in March 2019, Shimadzu has already integrated artificial intelligence as Analytical Intelligence for a number of instruments.



Learn more about Analytical Intelligence:

[https://www.shimadzu.com/an/analytical\\_intelligence.html](https://www.shimadzu.com/an/analytical_intelligence.html)

At the end of this year's summit, key panel discussed "Transforming The Future of Pharma". Topics of discussion were wide, and perspectives on the theme were shared from viewpoints of manufacturing, CROs, clinical research as well as data science and technology of tools. Irrespective of the business or workflow, panel agreed that major trends of the pharmaceutical industry are improving efficiency, increasing productivity, and integrating systems all by digital transformation.

In addition, the development of drugs to treat incurable diseases, as well as developing human resources that have expertise in data analysis (made necessary by the stark increase in data overall), were also cited as important questions in the industry.

## Take Home Message — for Present and Future

Shimadzu's Shuzo Maruyama summed up the summit as follows: "This year's summit was a chance to truly feel the various changes happening in the pharmaceutical industry stemming from new technology aiming to improve productivity and increase efficiency. As a provider of indispensable analytical technology, we aim to provide solutions, supporting researchers, reducing downtime in the lab and reducing human error through Analytical Intelligence. This is how we will provide new value for the industry as a whole."

The pharmaceutical industry's aim is to support our health. With regards to that, Shimadzu aims to not only continue to provide new products but is looking towards being a comprehensive healthcare solution provider for its global partners through its paradigm of Analytical Intelligence as AI technologies transform industries, including pharma. These activities are done with the grand goal of realizing a healthier, better lifestyle for everybody, and Shimadzu looks forward to addressing industry challenges on a global stage.



## Latest topics 2

# Shimadzu Scientific Instruments Partners with Northern Michigan University to Support Medicinal Plant Chemistry Program

Shimadzu Scientific Instruments (SSI) and Northern Michigan University (NMU) have partnered to establish the Shimadzu Analytical Core Laboratory for Medicinal Plant Sciences. Through this collaboration, SSI has donated instrumentation for the laboratory to support NMU's first-in-the-nation medicinal plant chemistry program. This rigorous chemistry program gives students and faculty access to cutting-edge equipment and technology to conduct medicinal plant research.

As part of the Shimadzu Partnership for Academics, Research and Quality of Life (SPARQ) program, SSI provided an in-kind donation of instrumentation for the laboratory valued at nearly \$851,000, along with a framework for research collaborations and internships. The Shimadzu instruments include multiple mass spectrometers: gas and liquid chromatography (GC-MS and LC-MS/MS), and inductively coupled plasma (ICP-MS), all of which serve a vital role in ensuring product quality and safety.

Shimadzu's cannabis and hemp testing solutions cover a broad range of applications, from accurate potency testing and terpene profiling to contaminate testing for pesticides, residual solvents, heavy metals and mycotoxins/afatoxins.

"The serious education and research on medicinal plants undertaken in this unique undergraduate program will help to overturn the misinformation and stigma associated with hemp and cannabis as it applies to human and environmental health," said Scott Kuzdzal, vice president of marketing for Shimadzu Scientific

Instruments. "NMU students in the medicinal plant chemistry program now have access to the same level and quality of analytical instrumentation that they will encounter after graduation in commercial, academic and medical research labs."

NMU President Fritz Erickson added, "Having access to the same top-caliber equipment they will work on in the industry gives our students a real advantage heading into their careers. We thank Shimadzu for recognizing Northern's innovative culture, our boldness in launching the medicinal plant chemistry program and the high quality of our faculty and students."

Top image: At the laboratory dedication (from left): Scott Kuzdzal of Shimadzu; NMU student Josie Molohan; NMU President Fritz Erickson; and Phil Martin of Shimadzu.



**For more information, visit SSI's special site for cannabis and hemp testing solutions:**

<https://www.shimadzu.com/an/industry/cannabis/index.html>



**Note: The above content may not be available in all countries or regions. If you are not allowed to view, please visit the following page.**

<https://www.ssi.shimadzu.com/industry/cannabis-testing-solutions.html>



**For more on NMU's medicinal plant chemistry program**

<https://www.nmu.edu/chemistry/medicinal-plant-chemistry>



Providence Portland Medical Center

Latest topics 3

# Shimadzu and Providence Cancer Institute Partner to Advance Cancer Immunotherapy Research

Shimadzu Corporation (Shimadzu) and the Earle A. Chiles Research Institute, a division of Providence Cancer Institute (Providence) in Portland, Oregon, have entered into a joint research agreement to apply mass spectrometry technology to develop tools for personalized cancer immunotherapeutics.

Shimadzu and Providence will develop technologies to reliably identify cancer antigens recognized by an individual's immune system, and to analyze the pharmacokinetics of immunotherapy drugs, with the goal of bringing these novel diagnostics to early clinical application.

"Immunological therapy has emerged as an effective cancer treatment. An important part of this research is to identify clinical signatures that can predict the efficacy of immunological therapies," said Masayuki Nishimura, Ph.D., Director – Innovation Center, Shimadzu Scientific Instruments (SSI). "This partnership will integrate Providence's leading cancer researchers and clinical resources with Shimadzu's analytical technologies to contribute to the development of these new therapies. As a global leader in immunotherapy for cancer, Providence is an ideal partner to advance the application of mass spectrometry in immune-based cancer clinical research."

"In collaboration with Shimadzu, we want to build better diagnostics based on each patient's unique tumor microenvironment. This will help inform clinical care with current immunotherapies, and pave the way for the next generation of personalized immunotherapeutics," said Brian Piening, Ph.D., Technical Director,

Clinical Genomics and Assistant Member, Immune Omics Laboratory at Providence.

SSI will direct this collaboration through its Innovation Center in Columbia, Maryland, and a new bioscience laboratory in Canyon Park, Bothell, Washington.



The members involved in the project Dr. Hong-Ming-Hu, Dr. Bernard Fox, (Dr. Takashi Shimada, Shimadzu), Mr. John Cha, Dr. Yoshinobu Koguchi, Dr. Brian Piening, and (Dr. Noriko Iwamoto, Shimadzu) (left to right).

## About Providence Cancer Institute

Providence Cancer Institute, a part of Providence St. Joseph Health, offers the latest in cancer services, including diagnostic, treatment, prevention, education, support and internationally-renowned research. Providence Cancer Institute is home to the Earle A. Chiles Research Institute, a world-class research facility located within the Robert W. Franz Cancer Center in Portland, Oregon, and is a recognized leader in the field of cancer immunotherapy since 1993.



Visit [providenceoregon.org/cancer](https://providenceoregon.org/cancer) to learn more.  
<https://oregon.providence.org/>



#### Latest topics 4

# Celebrating 50 Years of Mass Spectrometry Innovation

The year 2020 marks 50 years since the release of Shimadzu's first mass spectrometry device, the LKB-9000. To celebrate this occasion, Shimadzu is planning a number of activities throughout the year.



**Dedicated 50th anniversary webpage:**

[https://www.shimadzu.com/an/news-events/celebrating\\_50\\_years/index.html](https://www.shimadzu.com/an/news-events/celebrating_50_years/index.html)



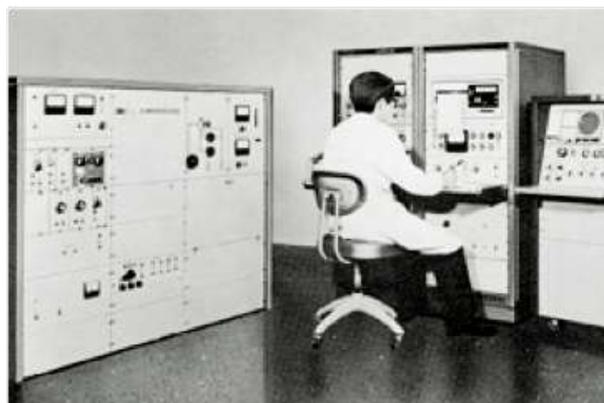
## A history of cutting-edge innovation

The LKB-9000 was released in 1970. By this time, Shimadzu had nearly 100 years of experience in manufacturing physics and chemistry equipment, and was well-positioned to bring Japan's first commercial GCMS device to market. The LKB-9000 was produced in cooperation with the Swedish firm LKB, but it wasn't long before Shimadzu engineers were improving on the base technology to develop various new GC/MS instruments such as the GCMS-6020 and GCMS-90 in the 1970s.

The world's first MALDI device, the LAMS-50K, came in 1987. The development of technology underlying this new ionization technique netted Shimadzu's Koichi Tanaka a share in the 2002 Nobel Prize for Chemistry.

In 2004 Shimadzu released the LCMS-IT-TOF, a world-first hybrid LC/MS model fusing IT and TOF for structural analysis etc.

Throughout the 2010s, a number of world-class triple quadrupole instruments were produced, for example the GCMSTQ8050 and LCMS-8060, which make full use of Shimadzu's unique UFMS (Ultra-Fast Mass Spectroscopy) technology.



The LKB-9000 in use

## User involvement

As part of our 50th anniversary activities, we are inviting researchers to test their knowledge of MS with a trivia quiz on a variety of topics. Shimadzu is also holding a “Memorable MS Moments” campaign where we ask customers to share breakthroughs or important results achieved with our instruments.

## Looking forward to the future of MS

To discover more about trends in MS instrumentation and the expectations of MS users, we are interviewing a number of experts worldwide who are utilizing Shimadzu MS technology in various research fields. In January’s interviews, Dr. Stanley Hazen from the US described the use of triple quad MS for probing the biological mechanisms of heart disease, and Prof. Wang Guanghui from China indicated a need for low-cost electrostatic ion trap devices.

Other user interviews will be published regularly on the anniversary webpage.

In addition, we talked to young Shimadzu engineers to hear their ideas about developments in store for mass spectrometry within the next 30 years. These discussions yielded a wide range of suggestions, from pocket-sized or wearable MS devices with high sensitivity to the idea of an “MS licence” to certify a user’s knowledge once MS becomes a more accessible technology.

In the future, Shimadzu hopes to build on our extensive experience in the field to continue expanding the limits of what’s possible with mass spectrometry. As we forge new research collaborations and work ever more closely with our customers, we look forward to many more years of innovation as a truly global vendor of MS instruments.



The future of MS: Technical discussions

# New Products

## LCMS-8060NX

(Liquid Chromatograph Mass Spectrometer)



The LCMS-8060NX is the culmination of Shimadzu's expertise in triple quadrupole MS, inheriting its excellent speed and world-class sensitivity from the LCMS-8060 while displaying improvements in robustness and ease-of-use. With robust parts that extend equipment lifetime and minimize downtime, as well as multiple Analytical Intelligence features to automate workflow steps from start-up to shutdown, the LCMS-8060NX is an indispensable tool to maximize your lab's productivity.

### Features

- World-class sensitivity and speed
- High robustness minimizes downtime
- Excellent ease-of-use for greater workflow efficiency

[Click here>>](#)

## UV-i Selection

(UV-VIS Spectrophotometers)



The UV-i Selection brand models are equipped standard with LabSolutions UV-Vis analytical data system. By including highly user-friendly operability, the ability to automatically analyze multiple samples continuously, and other features for satisfying a wide variety of customer needs in a single highly expandable design, the systems are well-suited for use in a wide range of fields, including academia, pharmaceuticals, and chemicals.

### Features

- Automatic pass/fail determination for improved efficiency
- Automatic measurement for improved operating efficiency
- Improved data analysis efficiency and stronger data management

[Click here>>](#)

## Shim-pack Bio HIC

(Hydrophobic Interaction Chromatography)



Shim-pack Bio Series

Shim-pack Bio HIC Column is a hydrophobic interaction chromatography (HIC) column packed with butyl bonded hydrophilic nonporous polymer particles (4  $\mu\text{m}$ ). HIC is suitable for the separation of analytes with slightly different hydrophobicity, such as antibody-drug conjugate (ADC) with different drug-antibody ratios (DAR). Shim-pack Bio HIC can be used for the analysis of DAR of ADC with relatively low pressure and high resolution.

### Features

- High separation capacity equivalent to 2.5  $\mu\text{m}$  is achieved with a low back pressure
- Enables high-speed analysis at a high flowrate
- Good Peak Shapes Even with High Load

[Click here>>](#)

Find us on 



Linked 



Shimadzu Corporation  
[www.shimadzu.com/an/](http://www.shimadzu.com/an/)

For Research Use Only. Not for use in diagnostic procedures.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2020  
Printed in Japan, July, 2020