SHIMADZU
Excellence in Science

Application News

Supercritical Fluid Chromatography Nexera™ UC
Open Access Software Open Solution

Measurement of Experimental Polar Surface Area Using Supercritical Fluid Chromatography

Shotaro Hirota, Rika Ogami, Yusuke Masuda

User Benefits

- Experimental polarity surface area (EPSA) can be measured using supercritical fluid chromatography (SFC).
- EPSA can be measured in open access environment provided by Open Solution software.

■ Introduction

In new drug development, polarity of a drug is an important factor affecting its permeability to target sites. Polar surface area (PSA) represents the total exposed areas of polar parts on a surface of a molecule. Experimental data have demonstrated a strong positive correlation between PSA and membrane permeability1), 2). Previous studies suggested that molecules with PSA more than 140 Å^2 tend to exhibit poor cell membrane permeability and oral bioavailability3). Molecules that can cross the blood-brain barrier are generally required to have PSA of approximately 60–70 Å^2 4). Furthermore, it has been verified that peptides with PSA more than 100 Å^2 do not show significant passive permeability5).

PSA calculated based on the bonding patterns of atoms within a molecule is called topological polarized surface area (TPSA), which is used for predicting the physical properties of candidate compounds in the early stages of drug discovery. However, TPSA is calculated without considering the three-dimensional structure of the molecule. Therefore, TPSA may not accurately evaluate the total exposed polar surface area of the molecule where its steric structure changes due to intramolecular hydrogen bonding like a peptide, a middle-size molecule. On the other hand, EPSA is a measured value based on SFC retention time (Fig. 1). Specifically, it is measured using a calibration curve created under analytical conditions where longer SFC retention times correlate with larger exposed polar area on the molecular surface. EPSA is considered effective even for molecular groups where accurate membrane permeability prediction is difficult using TPSA 2), 5). In this paper, we present a case study in which we measured the EPSA of peptides using the Nexera UC supercritical fluid chromatograph, a photodiode array (PDA) detector, and the LCMS-2050 single quadrupole mass spectrometer. Furthermore, to measure the EPSA of large numbers of compounds synthesized during the drug discovery phase with high throughput, it is necessary to maintain the instrument in a state ready for analysis at all times and to establish an environment where reliable data can be obtained regardless of how the instrument was used by the previous operator. In the latter half of this paper, we introduce a workflow for high-efficiency EPSA measurement and the implementation of open access to the instrument using the open-access software Open Solution.

■ Detector Selection and System Configuration

To measure the EPSA of a wide range of compounds, it is necessary to select an appropriate detector. While PDA detectors excel at the selective detection of compounds with UV absorption, they cannot detect compounds that do not exhibit UV absorption. On the other hand, MS detectors detect compounds based on ionization, so they may be able to detect compounds regardless of whether they exhibit UV absorption. However, since not all compounds ionize easily, compounds that are difficult to ionize may also be difficult to detect using MS. Therefore, by equipping a single system with both a PDA detector and an MS detector, it becomes possible to detect a wide range of compounds (Fig. 2). Furthermore, since EPSA is calculated from retention time, it can be determined not only from the retention times in the PDA chromatogram but also from those in the MS chromatogram. Consequently, by using both a PDA detector and an MS detector in combination, it is possible to calculate the EPSA for a greater number of compounds.

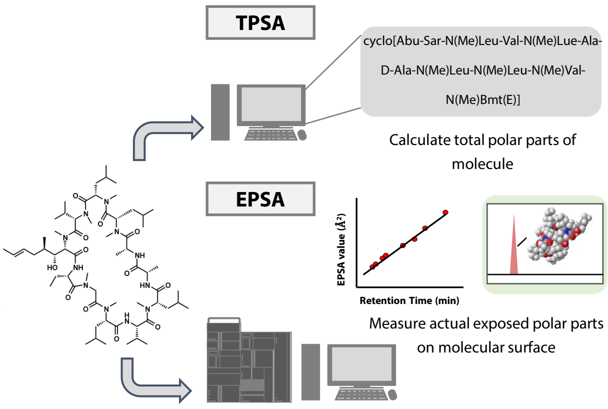


Fig.1 Two approaches to PSA evaluation based on calculation (TPSA) and measurement (EPSA)

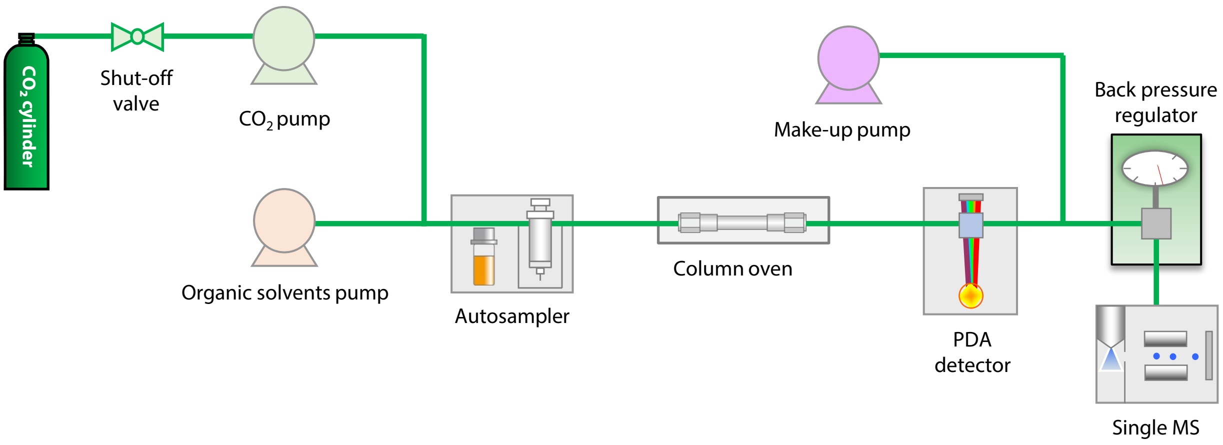


Fig. 2 System flow diagram （Nexera UC）

■ Analysis of Standard Solution and Creation of Calibration Curve

The nine compounds listed in Table 1 were dissolved in dimethyl sulfoxide (DMSO) to prepare standard solution for calibration curve (each compound concentration: 1.0 g/L). These were analyzed under the conditions shown in Table 2. Fig. 3 and Fig.4 show the chromatograms of standard solution obtained using PDA detector and single quadrupole mass spectrometer LCMS-2050.

Table 1 Standard compounds for creating calibration curve

	Name	Assigned EPSA Value (Å²) *1	Monoisotopic mass *2
(A)	Lidocaine	47	234.17
(B)	Antipyrine	61	188.09
(C)	Chlorpromazine	68	318.10
(D)	Desipramine	87	266.18
(E)	Pindolol	103	248.15
(F)	Diclofenac	135	295.02
(G)	*m*-Nitrobenzoic Acid	157	167.02
(H)	Bumetanide	185	364.11
(I)	Furosemide	230	330.01

*1 Values listed in Reference 2 are adopted.
*2 Rounded to the nearest unit mass

Table 2 Analysis conditions for Nexera and LCMS-2050

System	: Nexera UC
Column	: Phenomenex Chirex (*S*)-Val and (*R*)-NEA (50 mm × 4.6 mm I.D., 5.0 µm)
Column Temp.	: 40 °C
Injection volume	: 2 µL (1.0 g/L)
Sample solvent	: DMSO
Mobile phases	
Pump A	: CO_2
Pump B(Organic)	: 20mM Ammonium Formate in Methanol
Flow rate	: 4.0 mL/min
Time program(%B)	: 5%(0 min)→65%(2.75-4 min)→98%(4.01-5.5 min)→5%(5.51-6min)
Detection(PDA)	: 254 nm(SPD-M40, high-pressure flow sell)
BPR pressure	: 20.4 MPa
BPR Temp.	: 70 °C
System	: LCMS-2050
Ionization	: ESI/APCI(DUIS),positive and negative mode
Mode	: SCAN(*m/z* 100-2000)
Nebulizing gas	: 2.0 L/min(N_2)
Drying gas	: 5.0 L/min(N_2)
Heating gas	: 7.0 L/min(N_2)
DL Temp.	: 200 °C
Desolvation Temp.	: 450 °C
Interface voltage	: +3.0 kV/-2.0 kV
Make-up pump	
Mobile phase	: Methanol
Flow rate	: 0.5 mL/min

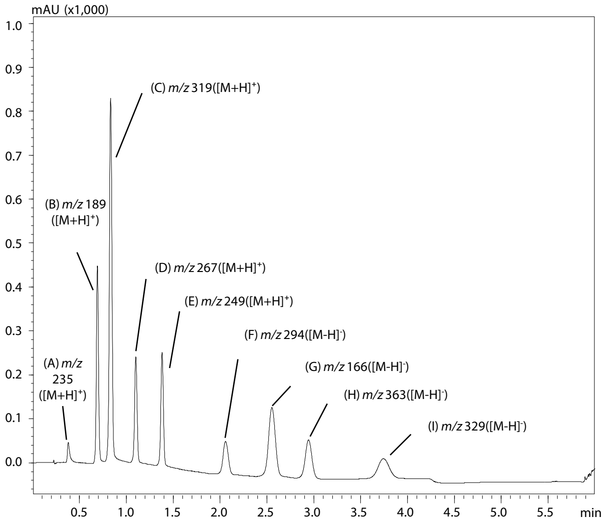


Fig. 3 Analytical results of standard solution (PDA chromatogram)

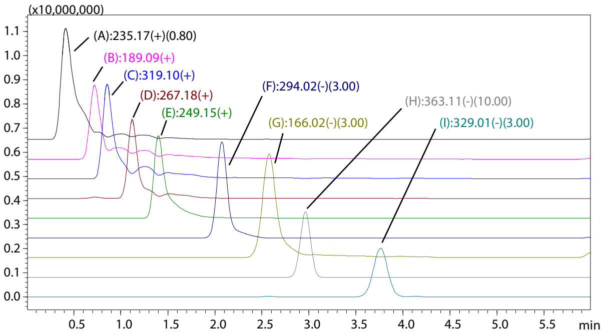


Fig. 4 Analytical results of standard solution (MS chromatogram)

The calibration curve was established using the retention times of respective compounds and the corresponding TPSA values listed in the references (Fig. 5), resulted in excellent linearity with a contribution factor of 0.99 or higher for EPSA values ranging from 47 to 230 Å².

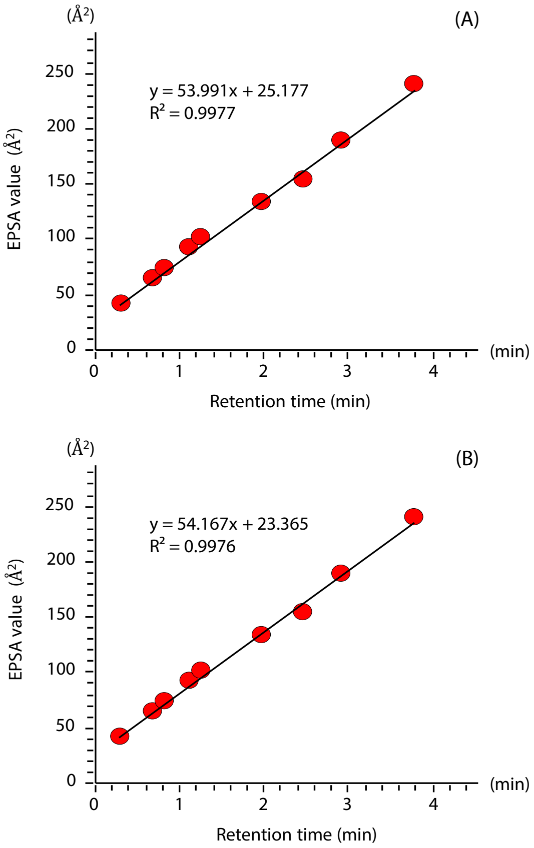


Fig. 5 Calibration curve created using retention times and corresponding TPSA of respective compounds in standard solution
(A): Using retention times from the PDA detector
(B): Using retention times from the MS detector

■ EPSA Measurements of Peptide Drugs

Cyclosporine A, a cyclic polypeptide antibiotic produced by fungi, and ritonavir, a peptide mimetic drug, were each dissolved in DMSO to make sample solutions (each compound concentration: 0.1 g/L). Respective sample solutions were analyzed under the conditions shown in Table 2. Fig. 6 shows the analysis results for Ritonavir.

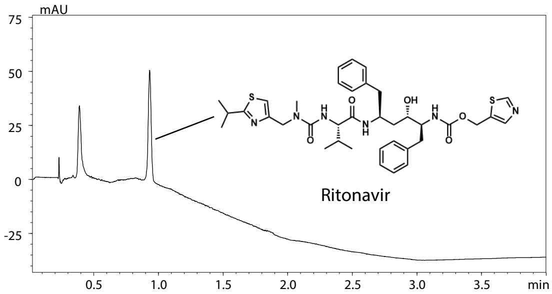


Fig. 6 Analytical result of Ritonavir (PDA chromatogram)

Fig. 7 and 8 show the analytical results for cyclosporin A. While cyclosporin A is difficult to detect using a PDA detector, it can be detected using an MS detector.

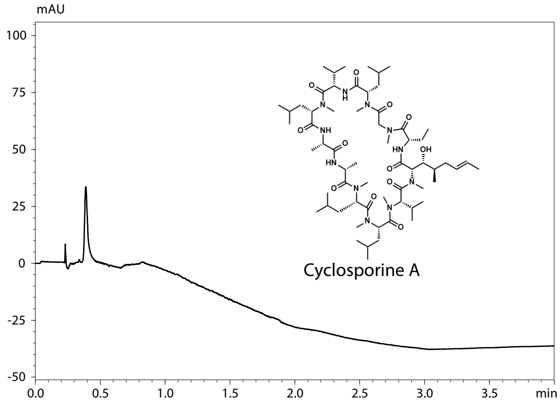


Fig. 7 Analysis result for cyclosporin A (PDA chromatogram)

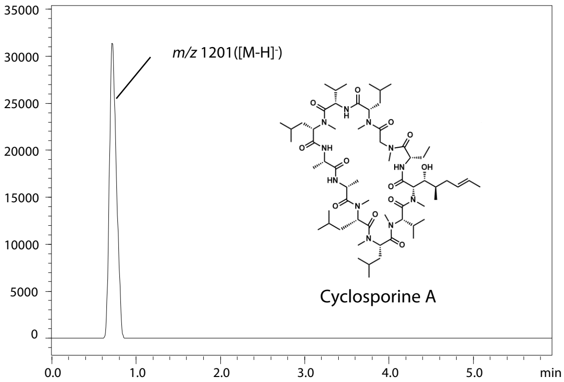


Fig. 8 Analysis result for cyclosporin A (MS chromatogram)

EPSA for cyclosporine A and ritonavir were measured using retention times and the calibration curve in Fig. 5. EPSA and TPSA for both compounds are shown in Table 3. TPSA, which is calculated based on the overall polarity of the molecule without considering its steric structure, resulted in high values for both compounds. In contrast, EPSA, which evaluates the actual exposed polar surface area of the molecule based on direct measurement, resulting in lower values compared to TPSA. Thus, even for medium size molecules like peptides, where TPSA may overestimate polarity, EPSA provides more appropriate measurement.

Table 3 TPSA and EPSA for cyclosporine A and ritonavir [Å^2]

	Cyclosporin A	Ritonavir
TPSA	278	145
EPSA	62	75

■ Setup of Open Access Environment with Open Solution

In a laboratory engaged in early-stage drug discovery, where a large number of compound samples are evaluated daily and specific instruments are shared among multiple researchers and staff, open access to instruments is a potential approach for simultaneous achievement of efficient instrument utilization and standardized analysis workflow. Open Solution provides the reduction of human error and the maximization of instrument operating rate by standardizing the workflow from setting sample to starting analysis.

The automatic conditioning function of Open Solution enables instrument operation tailored to user's workflow, such as starting analysis at fixed time or day. Features like automatic idling (automatic switching to low flow rate of mobile phase delivery when analysis is not executed for a certain period to save mobile phase consumption while keeping ready-to-analyze condition) and automatic shutdown (shutting down after long unoperated interval) help to set up open access environment where instruments are always ready for use from startup to shut down(Fig. 9).

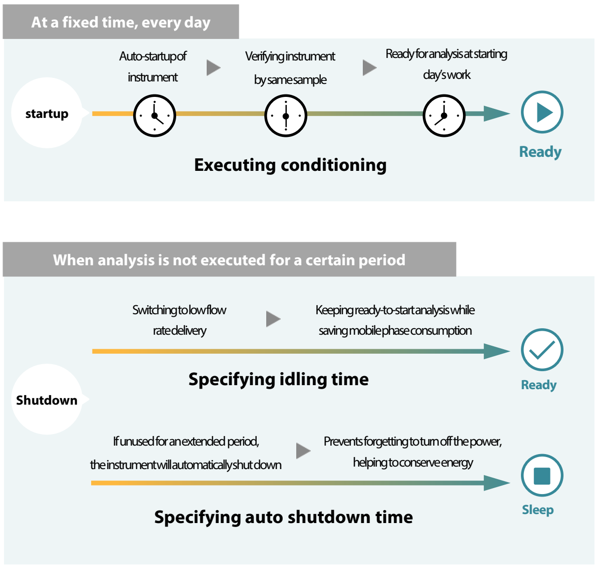


Fig. 9 Setup of open access environment using Open Solution

■ Verification of Instrument Condition Using System Suitability Test (SST)

SST is an effective way for ensuring the ongoing integrity of instruments and maintaining the reliability of analytical results. Regular execution of SST enables early detection of abnormalities often overlooked during routine operation, such as column degradation, tubing clogging, pump flow deviation, or detector sensitivity reduction, resulting in prevention of sample loss, analysis rework, and unplanned downtime. Since EPSA is calculated based on the retention time of target compounds, verifying instrument condition via SST is important for determining the reliability of obtained EPSA values. Using Open Solution provides automatic execution of SST under pre-set conditions and automatic determination of whether the instrument works properly. Furthermore, SST Viewer of Open Solution allows visual identification of abnormal values out of SST criteria. By comparing these values with previous SST results, the time of occurrence and trend of abnormalities can be confirmed at a glance.

An example is shown below. An SST based on retention time was performed using diclofenac (1.0 g/L) dissolved in DMSO. For one day, the salt concentration in the modifier was intentionally changed from 20 mmol/L to 10 mmol/L. Consequently, measurements were performed under conditions that did not meet the criteria(Fig. 10). The results clearly showed that the instrument condition was not suitable for correct calculation of EPSA. Fig. 11 shows the SST Viewer results screen. Thus, combination use of regular execution of SST and automatic judgement and record management functions provides rapid verification whether instrument condition affects results of retention time-dependent analyses or not, contributing to enhanced reliability and efficiency of daily data analysis.

Table 4 System Stability Test Settings
(Analysis conditions are listed in Table 2)

SST Criteria
Target Compound : Diclofenac (1.0 g/L)
SST Execution Parameters : Retention Time
lower limit : 2.100 min
upper limit : 2.200 min

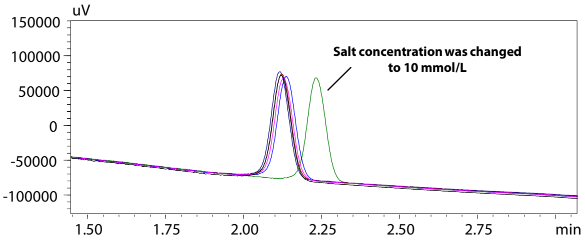
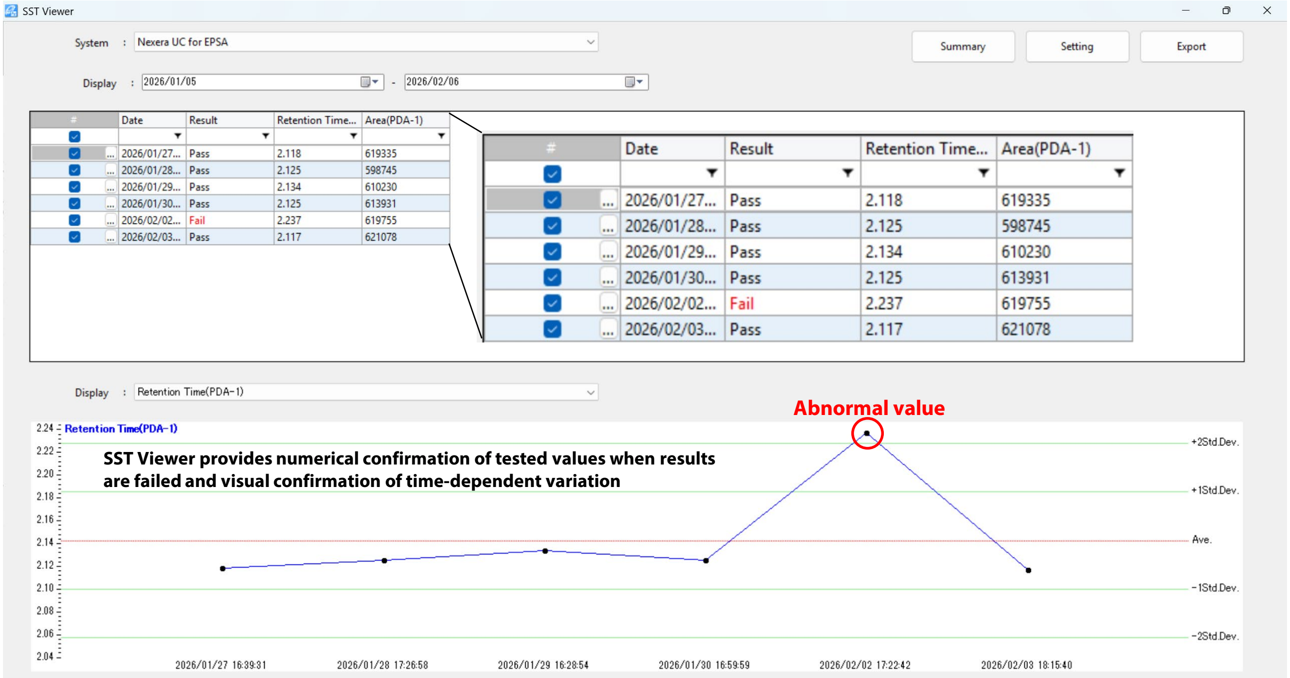


Fig. 10 Analytical result for diclofenac (PDA chromatogram)



System : Nexera UC for EPSA

Display : 2026/01/05 - 2026/02/06

#	Date	Result	Retention Time...	Area(PDA-1)
☑				
☑	2026/01/27...	Pass	2.118	619335
☑	2026/01/28...	Pass	2.125	598745
☑	2026/01/29...	Pass	2.134	610230
☑	2026/01/30...	Pass	2.125	613931
☑	2026/02/02...	Fail	2.237	619755
☑	2026/02/03...	Pass	2.117	621078

Display : Retention Time(PDA-1)

Abnormal value

SST Viewer provides numerical confirmation of tested values when results are failed and visual confirmation of time-dependent variation

Fig. 11 SST result confirmation screen by Open Solution SST Viewer

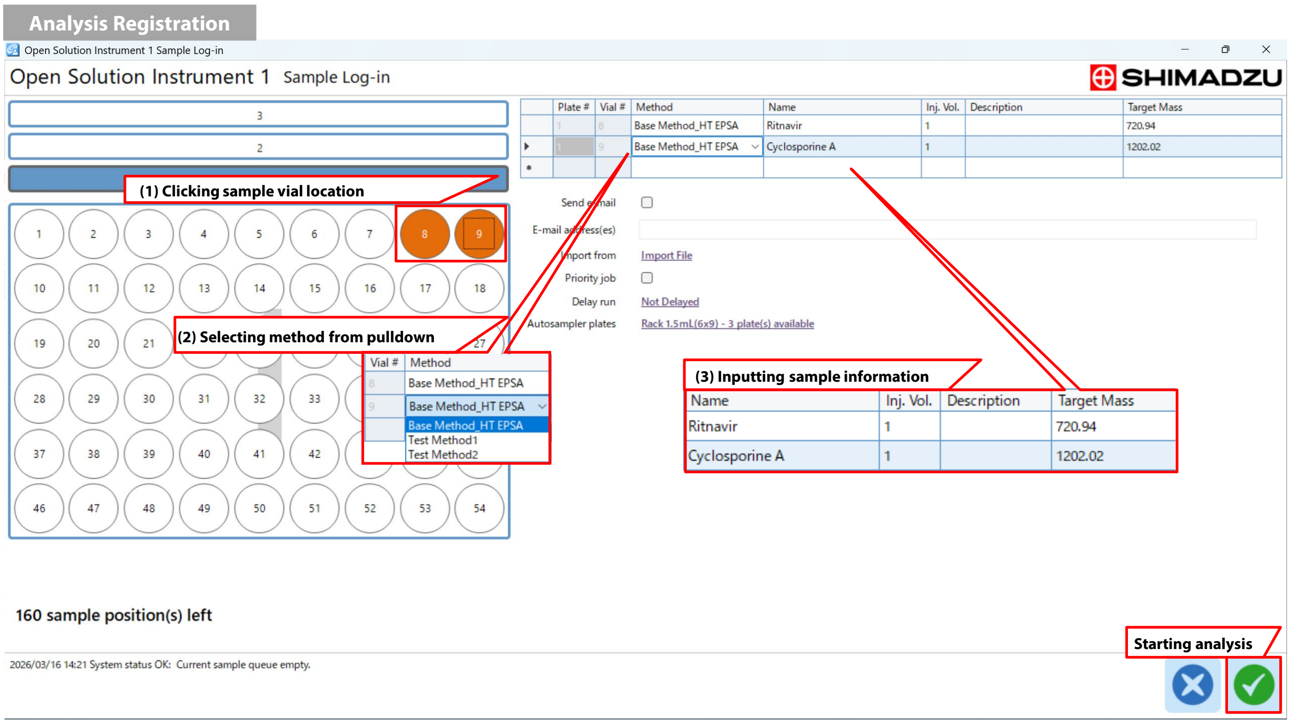
■ Analytical Workflow Using Open Solution

Samples in the early stages of drug discovery may contain various impurities in addition to the expected target compound. Combination use of PDA detector and single quadrupole mass spectrometer LCMS-2050 provides application of acquired spectral and mass information to identify known compounds and estimating unknown impurities as well as utilization of chromatographic retention times from PDA and MS detectors to calculate EPSA.

Furthermore, introducing Open Solution unifies the operational workflow from sample setting sample to starting analysis to reduce human error and to improve instrument operating rate simultaneously. EPSA calculation workflow utilizing Open Solution is as follows. Analysis registration in Open Solution is designed to be completed on a single screen.

Simply select a previously registered method file from the pull-down menu, enter the targeted mass number and sample information, then click the Start key to start analysis (Fig. 12). This screen is clearly organized for easy setting of required information to allow intuitive operation even for first-time users.

During data processing phase, Open Solution Results display screen of Open Solution can be flexibly rearranged to suit user’s requirement. Consequently, UV/MS chromatograms and spectra can be organized for easy viewing to allow quick confirmation of target peak retention time and spectral information. Additionally, automatic execution of column conditioning (pre-run/post-run) for each analysis can be scheduled, resulting in optimal starting condition regardless of previous users' condition. Therefore, the necessary reliability of data required for EPSA calculation can be guaranteed. Fig. 13 shows the Open Solution results analysis screen.



Open Solution Instrument 1 Sample Log-in

	Plate #	Vial #	Method	Name	Inj. Vol.	Description	Target Mass
	1	8	Base Method_HT EPSA	Ritnavir	1		720.94
▸	1	9	Base Method_HT EPSA	Cyclosporine A	1		1202.02
*							

Send e-mail ☐

E-mail address(es)

Import from Import File

Priority job ☐

Delay run Not Delayed

Autosampler plates Rack 1.5mL(6x9) - 3 plate(s) available

(1) Clicking sample vial location

(2) Selecting method from pulldown

Vial #	Method
8	Base Method_HT EPSA
9	Base Method_HT EPSA
	Base Method_HT EPSA Test Method1 Test Method2

(3) Inputting sample information

Name	Inj. Vol.	Description	Target Mass
Ritnavir	1		720.94
Cyclosporine A	1		1202.02

160 sample position(s) left

2026/03/16 14:21 System status OK: Current sample queue empty.

Starting analysis

Fig.12 Workflow from sample registration to starting analysis on Open Solution

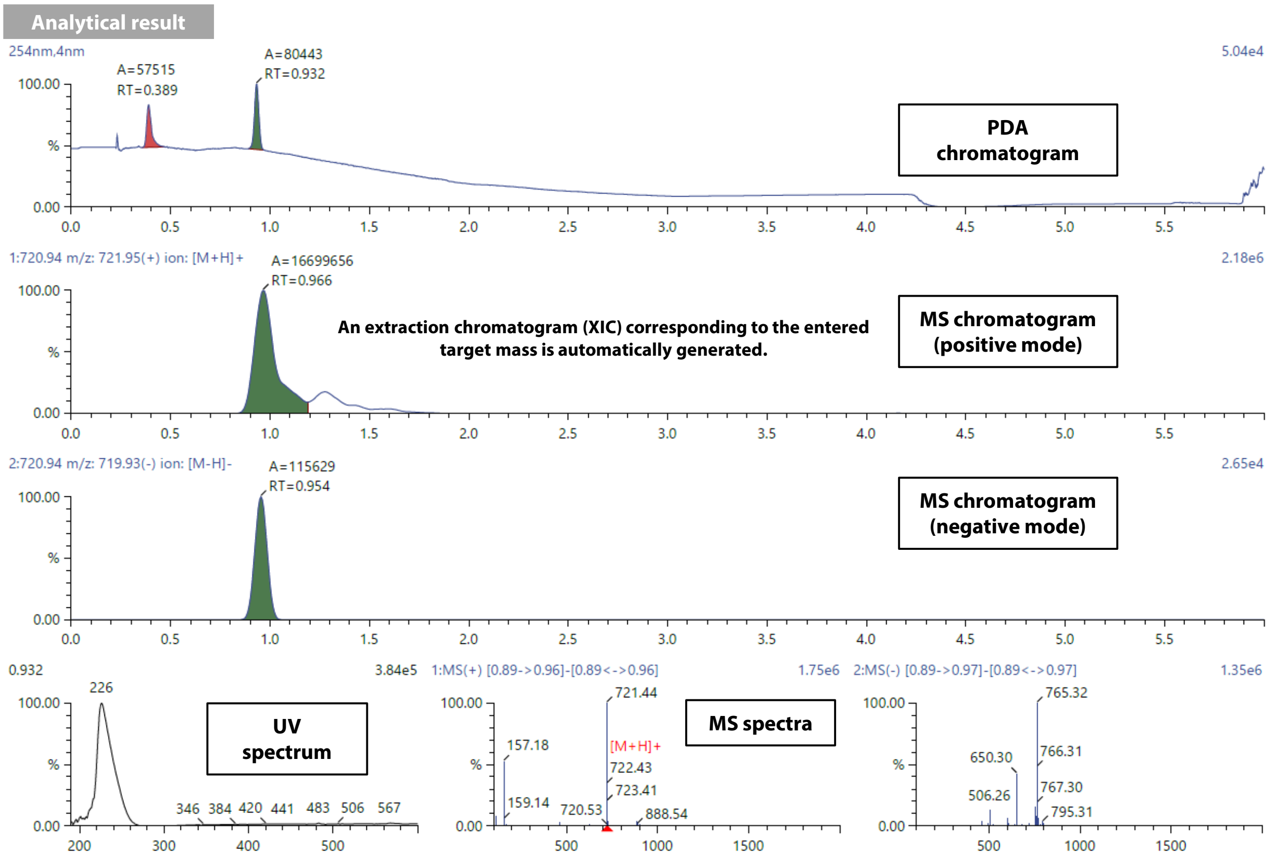


Fig. 13 Analytical result screen on Open Solution (indication result for ritonavir)

■ Conclusion

This article describes an experimental method for measuring EPSA by combination use of SFC and single quadrupole mass spectrometer LCMS-2050, and the setup of open access environment using Open Solution to support its practical operation. EPSA calculated based on retention time in SFC will be a useful indicator for evaluating permeability, particularly for cyclic peptides and middle molecules, as it can reflect effects such as steric conformation and intramolecular hydrogen bonding that TPSA cannot fully utilize.

On the other hand, in daily operations, a large number of small-volume samples can be processed at high throughput while keeping consistent data quality utilizing functions of setup of the open access environment, automatic conditioning, and monitoring instrument condition via SST, all provided by Open Solution. This is expected to accelerate the screening and candidate selection processes in drug discovery and medium-molecule research.

＜References＞

1) Palm, K., Stenberg, P., Luthman, K. et al. “ Polar Molecular Surface Properties Predict the Intestinal Absorption of Drugs in Humans.“ *Pharm Res* 1997, 14, 568–571

2) Yue-Ting Wang, Edward Price, et al. “High-Throughput SFC-MS/MS Method to Measure EPSA and Predict Human Permeability“ *J. Med. Chem.* 2024, 67, 16, 13765–13777

3) Daniel F. Veber, Stephen R. Johnson, et al. “Molecular Properties That Influence the Oral Bioavailability of Drug Candidates“ *J. Med. Chem.* 2002, 45, 12, 2615–2623.

4) Pajouhesh, H., Lenz, G.R. “ Medicinal chemical properties of successful central nervous system drugs ”.*Neurotherapeutics* 2005, 2, 541–553.

5) Goetz GH, Philippe L, Shapiro MJ. EPSA: A Novel Supercritical Fluid Chromatography Technique Enabling the Design of Permeable Cyclic Peptides. *ACS Med Chem Lett.* 2014 Aug 4;5(10):1167-72.

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

01-01161-EN First Edition: Apr.. 2026

Please fill out the survey

Related Products

Some products may be updated to newer models.

Nexera UC

Supercritical Fluid Extraction / Supercritical Flu…

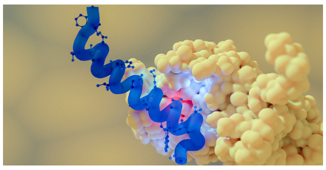
LCMS-2050

High-Performance Liquid Chromatograph Mass Spectro…

Open Solution

Support Software for Data Acquisition, Analysis, a…

Related Solutions



Peptide Therapeutics

Pharmaceutical and Biopharmaceutical

Small Molecule Pharmaceutical

Price Inquiry

Product Inquiry

Technical Service / Support Inquiry

Other Inquiry