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LC World Talk

SHIMADZU'S NEWSLETTER FOR THE HPLC GLOBAL COMMUNITY

Prominence UFLC
Achieving Ultra Fast Liquid Chromatography

LCMS-IT-TOF
Formula Predictor Software

Automated On-line Pretreatment LCMS System
for the Determination of Bisphenol A and 4-Octylphenol in Serum

PROMINENCE UFLC WITH XR-ODS COLUMNS enables users to shorten analysis time drastically and easily without extremely high pressure, while maintaining the high separation efficiency of conventional columns and system performance features such as reproducibility, carryover and durability.

PROMINENCE UFLC
ACHIEVING ULTRA FAST LIQUID CHROMATOGRAPHY

Prominence UFLC has been designed to achieve Ultra-Fast HPLC by utilizing the Prominence hardware series. In this respect, we are successful in demonstrating high speed and separation performance without resorting to high pressure, while providing high reliability and expandability not available with other fast LC systems.

Smaller particle columns such as sub-2µm columns generate higher pressures but also produce high separation efficiency. Sub-2µm columns often require specialized instruments that can operate under high pressure to demonstrate high separation efficiency. Our Shim-pack XR-ODS column was developed with the intention of creating a new high-speed analysis column at much lower pressure than experienced with sub-2µm columns. The Prominence UFLC has been optimized to enable high-speed analysis using the Shim-pack XR-ODS.

Shim-pack XR-ODS for High Speed and High Separation Efficiency

The Shim-pack XR-ODS, which is packed for use in reverse-phase chromatography, has 2.2µm diameter particles of totally porous high-purity silica gel with chemically bonded octadecylsilane (ODS) as the base material. Generally, columns using smaller particles provide good separation when using shorter length columns and help to minimize the reduction in column efficiency even if the mobile phase flow rate is high. This makes them well suited to high-speed analysis. However, since column pressure increases inversely proportional to the square of the diameter of the packing material particle, special HPLC instruments that have resistance to high pressure are required.

The specialized instruments that allow use of sub-2µm columns often sacrifice operability, reliability and application flexibility. The design of the new Shim-pack XR-ODS is based on an in-depth evaluation of a variety of factors such as separation performance, durability and column pressure. Use of 2.2 µm silica gel-based material reduces column flow resistance, allowing high-speed analysis, even with systems comprised of conventional HPLC hardware. Since the XR-ODS achieves a good balance between separation efficiency and pressure, resolution performance is maintained as in a general-purpose column (4.6mmi.d. x 150mm, 5µm) while greatly shortening analysis time (Figure 1). Moreover, column pressure is maintained at 30 MPa or less in most analytical conditions without using a specialized system for extremely high pressure. We reported the

development concept and some features and applications of the Shim-pack XR-ODS columns in our 2006 spring issue of *LC WorldTalk*. We hope this article will aid in the understanding of Shim-pack XR-ODS columns.

*See Figure 1.*

Prominence UFLC

Prominence UFLC responds to increased customer requests for ultra-fast analysis. Based on the existing Prominence components, improvements in flow path pressure (autosampler), high-speed gradient (controller and solvent delivery units), and reduction of peak broadening in flow paths (autosampler and detector cell) have been implemented. The maximum pressure of the SIL-20AHT/20ACHT autosampler has been raised to 35 MPa to correspond with Shim-pack XR-ODS pressure

tolerances. Basic specifications, including injection volume accuracy, precision, carryover and durability, are the same as those of the SIL-20A/20AC.

Ultra Fast

High-throughput analysis requires shortening of the total cycle time, which is defined as the separation, the injection interval and the column equilibration. The SIL-20AHT /20ACHT autosamplers boast the world's fastest injection (10 sec, 10 µL injection time), as well as suppressed carryover achieved by special surface coating of the needle and the improved needle seal. Figure 2 shows the analysis of 7 alkyl phenones, demonstrating ultra-fast gradient analysis with a 32-second cycle time. A fast autosampler and standard features such as automatic purging allow

Figure 1: Comparison of Shim-pack XR-ODS with conventional 5µm particle column.
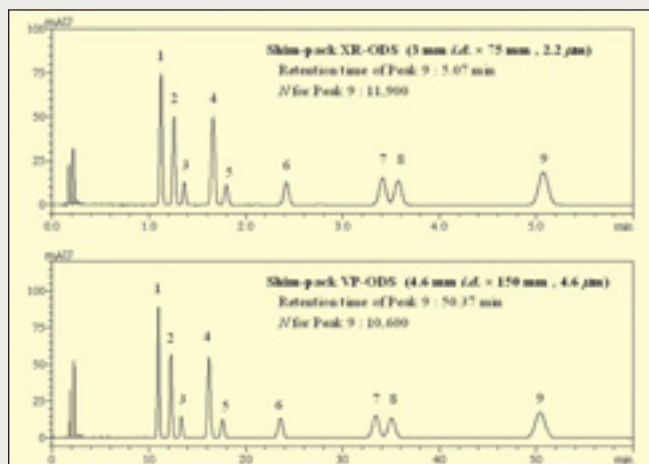
Chromatographic Conditions

Mobile Phase:	Water/Acetonitrile (40/60, v/v)
Flow Rate:	2.0 mL/min (XR-ODS)
	1.0 mL/min (VP-ODS)
Temperature:	60°C (XR-ODS)
	50°C (VP-ODS)
Detection:	Absorbance at 210 nm.
Peaks:	1: Fluorene
	2: Phenanthrene
	3: Anthracene
	4: Fluoranthene
	5: Pyrene
	6: Chrysene
	7: Benzo(b)fluoranthene
	8: Benzo(k)fluoranthene
	9: Benzo(g,h,i)perylene

Figure 2: Ultra-fast analysis using Prominence UFLC.
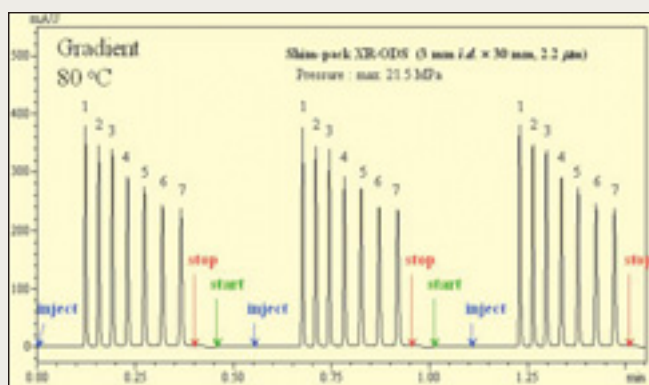
Chromatographic Conditions

Column:	Shim-pack XR-ODS (3 mm i.d. × 30 mm, 2.2 µm)
Mobile Phase:	Water/Acetonitrile (40/60 to 20/80 in 0.4 min, convex gradient)
Flow Rate:	3 mL/min
Temperature:	80°C
Detection:	Absorbance at 245 nm
Injection Volume:	4 µL (each 800 nmol), 0.1 min delayed injection
Peaks:	1: Actophenone
	2: Propiophenone
	3: Butyrophenone
	4: Valerophenone
	5: Hexanophenone
	6: Heptanophenone
	7: Octanophenone

users to shorten the overall analysis time and experience truly fast LC analysis. *See Figure 2.*

Maximizing Reliability

The UFLC modules have many design improvements to consumable parts such as check valves, pistons, and the autosampler rotor seal. These improvements allow UFLC components to operate reliably as a conventional or UFLC system, whereas with other systems currently on the market, the extremely high backpressure may contribute to more frequent replacement of consumable parts.

Steep gradients for performing short cycle time analyses and heat generated by high pressure in the column may produce poor reproducibility of retention time and peak area. Responsive tracking of a quickly changing flow rate is essential in high-speed gradient analysis. The Prominence UFLC features excellent solvent delivery flow performance with its pumping resolution of 3.7 nL using micro stroke technology (10 µL/stroke) and superior solvent delivery flow control (response of 0.1 second) to provide excellent retention time repeatability. Moreover, at higher flow rates, columns such as the XR-ODS, with an internal diameter of 2 mm to 3 mm, are most appropriate as they can better handle the increased mobile phase

Prominence *UFLC*
ULTRA FAST LIQUID CHROMATOGRAPH

Q&A: Fast HPLC

Q: Is high pressure required for performing fast HPLC?

A: The short answer is no. High pressure is not the goal; it is simply a consequence of using small particle (sub-2µm) HPLC columns. The use of these types of columns subjects hardware to greater stress due to increased resistance to flow and, subsequently, dramatically increases the back pressure, potentially sacrificing basic performance due to design constraints brought on by the need for a high pressure tolerance.

Q: Does high pressure influence consumable parts and maintenance?

A: Pushing any system to the extremes of its performance envelope, but particularly specialized systems running at higher pressures, increases stress on instrument components such as pump pistons and seals and the autosampler rotor and stator. This results in shorter consumable life and less durability even when using such specialized (more expensive) components.

Q: How does high pressure influence peak shape?

A: Under high-pressure conditions, frictional heating occurs in the column, causing a temperature gradient within the column's cross section. This means the mass transfer between the mobile phase and the packing material will be different across that area, possibly resulting in peak distortion. In such situations, any benefits to using high pressure are lost.

Q: What is the goal?

A: The goal of Fast LC is increased sample throughput while maintaining data quality, meaning how many samples can be analyzed per day, per hour, or even per minute. In order to achieve such high throughput, not only

must the run time of a single analysis be shortened, but the total cycle time of the injection sequence and run time needs to be optimized. Specialized inject-ahead routines or overlapping injections can shorten cycle times but often compromise an autosampler's carryover performance and should be avoided. Is waiting 45 seconds for the next injection of your 30-second analysis high throughput?

Q: What is the solution?

A: The ultimate answer lies in understanding what constitutes the chromatographic separation and optimizing those parameters while maintaining the reliability and precision demanded in the laboratory. Ideally, it would include HPLC hardware that, when coupled with an appropriate column packing, can perform precisely and reliably under a variety of conditions, like the Prominence Series.

Q: Why is Prominence the solution?

A: Simply stated—Excellent design, engineering and quality. The Prominence Series includes the proven micro-stroke solvent delivery mechanism for rapid, precise and reproducible gradient formation. The Prominence autosampler is capable of short injection cycle times and excellent carryover performance without sacrificing precision. Stable and accurate temperature control is critical to produce good data under UFLC conditions and can be found in the Prominence forced-air ovens, large enough to allow for flexible column configurations like column switching. Fast response time and high sensitivity coupled with a temperature-controlled, low delay volume flow cell are incorporated in the Prominence UV and PDA detectors. In other words, a good HPLC system should be engineered and capable of Fast HPLC from the start; a specialty system with extreme pressure tolerances, and the design sacrifices incorporated because of it, should not be required. Regardless, there should be no compromises in the quality of the data—namely reproducibility, peak shape, and quantitative accuracy.

Figure 3: Reproducibility of retention times and areas of 7 alkyl phenones.
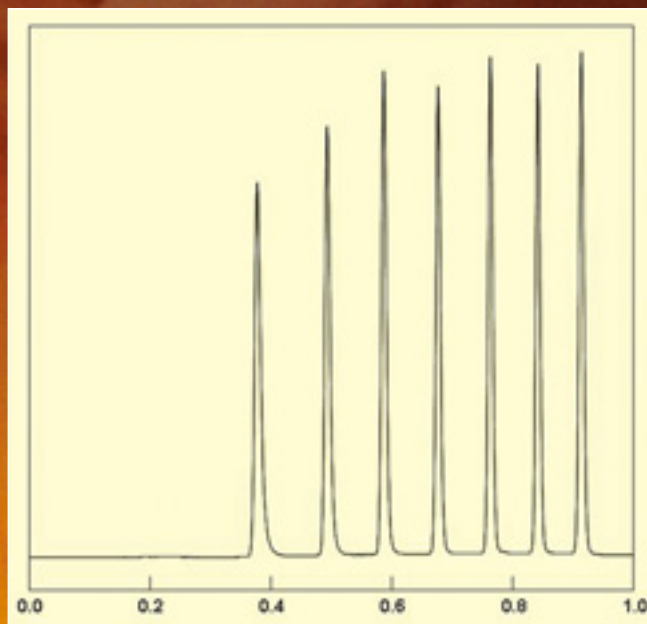
Chromatographic Conditions

Column:	Shim-pack XR-ODS (3 mm i.d. × 50 mm, 2.2 µm)
Mobile Phase:	A: Water, B: Acetonitrile, 0min (50%B) → 0.55min (95%B) → 0.70min (95%B)
Flow Rate:	1.5 mL/min
Temperature:	40°C
Detection:	Absorbance at 245 nm, 2µL injection, n=6

Compounds	Retention Time		Peak Area	
	Average	%RSD	Average	%RSD
Acetophenone	0.377	0.076	49018	0.193
Propiophenone	0.493	0.072	47260	0.167
Butyrophenone	0.587	0.070	49199	0.055
Valerophenone	0.677	0.065	45277	0.138
Hexanophenone	0.763	0.082	46613	0.138
Heptanophenone	0.842	0.087	43975	0.205
Octanophenone	0.914	0.080	48978	0.200

flow rate and minimize wear on instrument components, such as pump pistons and seals, autosampler rotor, etc.

As the column's internal diameter becomes smaller, it is necessary to decrease the sample injection volume in proportion to the column cross-sectional area (about 2 µL to 5 µL injection volume with 3 mm internal diameter column). With the Prominence UFLC, the SIL-20AHT/20ACHT uses a high-performance measuring pump and enhanced air tightness of flow lines to achieve peak area repeatability (RSD) of 0.3% or less, even with injections of 2 µL to 5 µL.

Carryover has gained a great deal of attention in recent years, especially with today's sensitive triple quad mass spectrometers. For ultra-fast LC, reduced carryover is a very important factor, but combating sample carryover may increase analysis cycle time if additional autosampler needle rinsing is required. The Prominence UFLC SIL-20AHT/20ACHT autosamplers successfully reduce carryover using the same proven techniques of the SIL-20A/AC models. These include inhibiting the adsorption of samples to needle and seal surfaces, and an option for two different rinse solvents. Based on Shimadzu test parameters, carryover for the SIL-20AHT/10ACHT is typically 0.005% or less.

Figure 3 shows a chromatogram and reproducibility of retention time and peak area of 7 alkyl phenones under steep gradient conditions and a 1-minute run time. Prominence UFLC provided excellent reproducibility on all compounds with a 2µl injection. When using instruments designed for high-pressure operation, it is conceivable that there may be problems with the durability of maintenance parts, ease of maintenance and quality of analysis. In the case of a high-pressure autosampler, the longevity of the high-pressure valve seal is shortened. Prominence UFLC uses Shimadzu's own proprietary high-precision 6-port valve, which achieves

Compounds	Retention Time		Peak Area	
	Average (min)	%RSD	Average	%RSD
Acetophenone	0.916	0.089	312,670	0.059
Propiophenone	1.398	0.058	315,739	0.045
Butyrophenone	2.111	0.030	336,428	0.063
Valerophenone	3.341	0.031	296,609	0.040

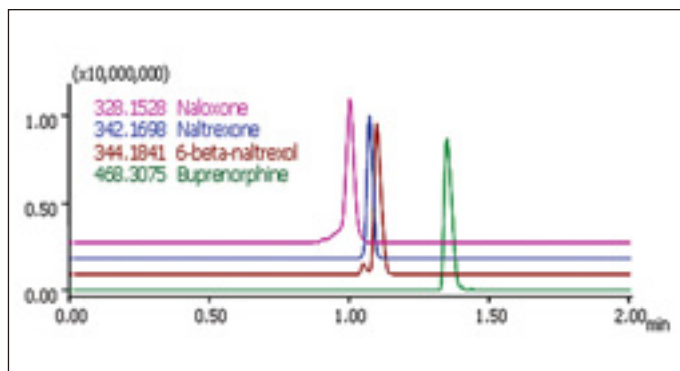**Table 1:** Reproducibility for four alkyl phenones after finishing 100,000 cycle endurance Test (5µL injection, n=6).

Figure 4: Analysis of Opioid Antagonist and its Metabolites using Prominence UFLC with the LCMS-IT-TOF.

Chromatographic Conditions

|	
Column:	Shim-pack XR-ODS (2 mm i.d. × 30 mm, 2.2 μm)
Mobile Phase:	A: 0.1% FA – Water
Mobile Phase:	B: 0.1% FA – Methanol
Time Program:	4%B (0 min) – 100%B (0.5 min) – 4%B (0.51 min)
Flow Rate:	0.5 mL/min
Oven Temperature:	50°C

0.3% RSD or less area repeatability in 100,000 cycle endurance tests (based on Shimadzu test parameters) as shown in *Table 1*. As shown, Prominence UFLC conclusively results in significantly faster analysis with high reliability.

Flexibility

Prominence UFLC can be used for a broad range of applications, including conventional HPLC, or set up as a column switching system or a semi-preparative system. The wide flow rate range (100nL-10mL/min) and high delivery resolution of the pump (3.7nL), combined with the reproducibility of the autosampler, offer superior performance for both UFLC and conventional LC, so usage is not restricted to UFLC analyses.

Furthermore, Prominence UFLC excels as a front end for MS and MS/MS. *Figure 4* shows high-speed, accurate MS^n analysis of Opioid Antagonist and its metabolites utilizing the LCMS-IT-TOF (Shimadzu). With the auto MS^n function, the IT-TOF with Prominence UFLC produced MS/MS data with high accuracy in a 2-minute cycle time.
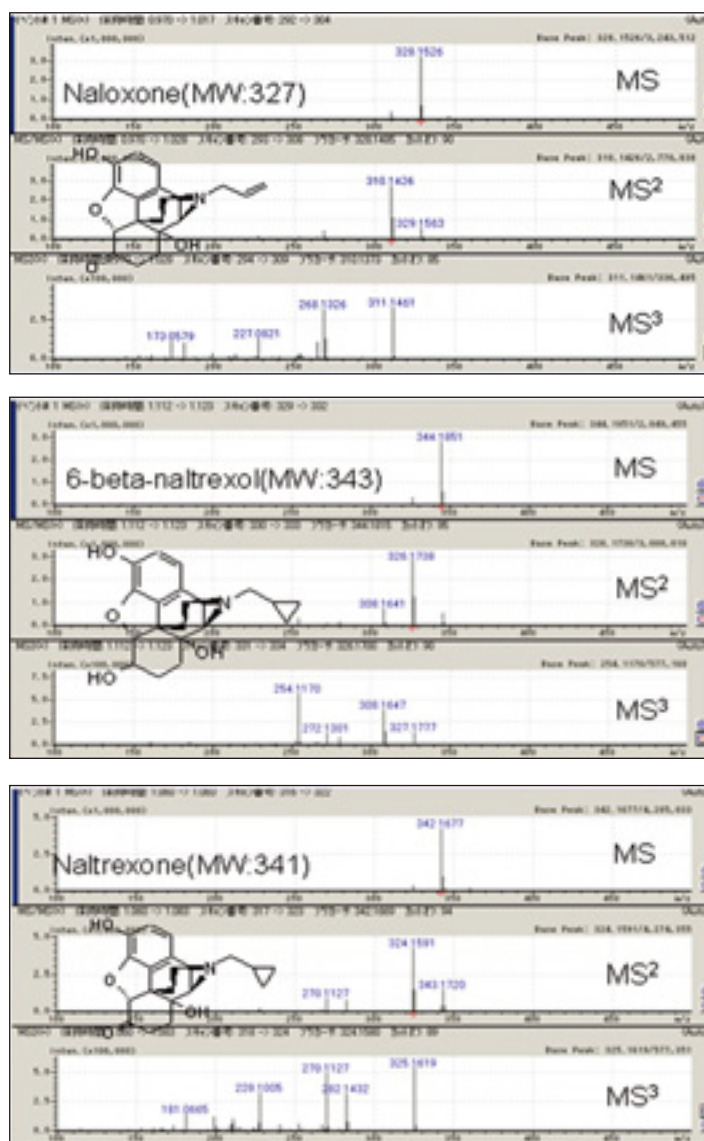
High-speed/high-accurate MS^n offers identification of target compounds in the field of impurity analysis, biomarker search and metabolite profiling.

Conclusion

Prominence UFLC with XR-ODS columns enables users to shorten analysis time drastically and easily without extremely high pressure, while maintaining the high separation efficiency of conventional columns and system performance features such as reproducibility, carryover and durability. Prominence UFLC is applicable for a wide range of customers as a highly reliable, and flexible, fast LC system that does not require specialized components. ⊕

LCMS-IT-TOF

Developing an empirical formula prediction tool that takes into account MS and MS^n spectra with high mass accuracy and mass resolution.

Formula Predictor Software

Overview

Predicting a candidate list based on MS and MS^n data takes into account a number of variables, including mass accuracy and mass resolution of the experimentally derived pseudo-molecular peak and related fragment ion data generated using MS^n spectra (or complement ion data) together with conventional chemical rules (such as nitrogen, electron configuration, DBE range, H/C ratio).

As the hybrid ion trap (IT) time of flight (TOF) system generates MS and MS^n spectra with a low mass cut-off, the prediction tool set can use the fragment ion data to reduce the list of potential candidates and remove invalid formula.

Although the main variables are mass accuracy and isotopic distribution for the MS spectra, the results also highlight the importance of fragment ion data in filtering candidates to remove invalid formula and helping to identify the correct empirical formula with a higher degree of confidence without the need for sub-ppm mass accuracy data.

Introduction

High mass accuracy mass spectrometry is achievable on a number of technology platforms, including time of flight mass analyzers, hybrid ion trap and quadrupole TOF's and FT-MS. Such technologies are playing an increasing role in pharmaceutical drug characterization and metabolite identification to help identify or confirm empirical formula assignments. Recently, software tools have been applied to modeling theoretical isotopic distributions and mass accuracy data with experimentally derived data together with sets of chemical or statistical rules to help increase the probability of assigning the correct empirical formula. In this paper, we describe the development of an empirical formula prediction tool that takes into account MS and MS^n spectra with high mass accuracy and mass resolution.

Methods

To evaluate and develop the prediction tool set, a small library of pharmaceutical compounds was evaluated on a hybrid ion trap time of flight mass analyzer (LCMS-IT-TOF, Shimadzu Corporation, Kyoto, Japan) depicted in *Figure 1*.
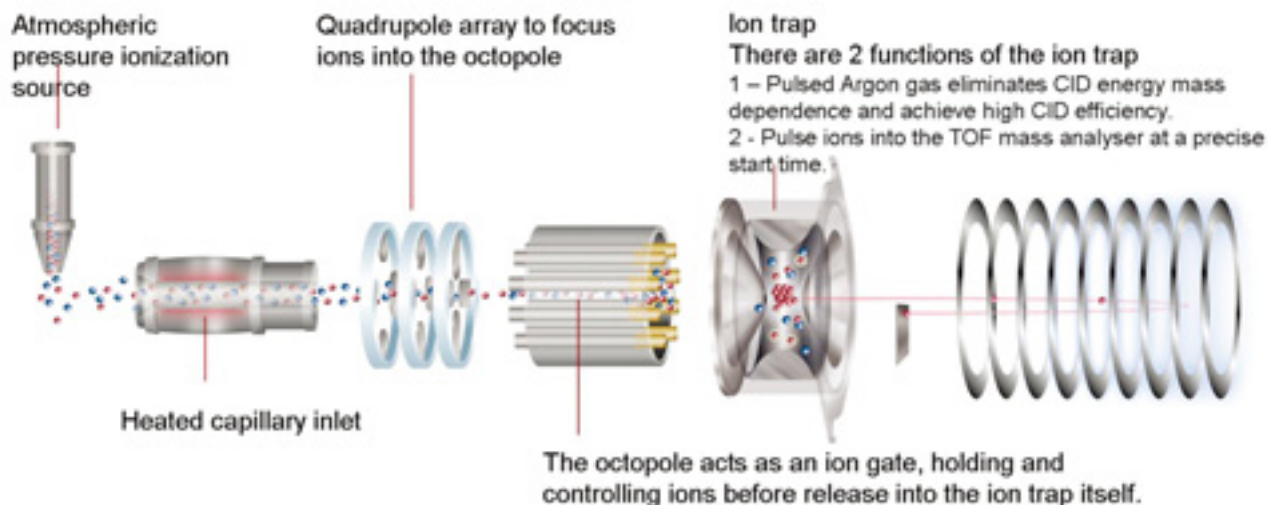
Atmospheric pressure ionization source

Quadrupole array to focus ions into the octopole

Ion trap
There are 2 functions of the ion trap
1 – Pulsed Argon gas eliminates CID energy mass dependence and achieve high CID efficiency.
2 - Pulse ions into the TOF mass analyser at a precise start time.

Heated capillary inlet

The octopole acts as an ion gate, holding and controlling ions before release into the ion trap itself.

Figure 1: Schematic of the LCMS-IT-TOF mass spectrometer.

The software was designed to support
3 stages in data processing:

Stage 1: MS spectrum data
Considers the molecular ion (or adduct) and mass accuracy.

Parameters for MS spectrum data
To increase the likelihood of identifying the correct empirical formula, conventional filters are applied, including double bond equivalency (DBE) range, electron configuration, hydrogen carbon (HC) ratio, nitrogen rule, active elements, adducts, mass tolerance, & chemical rules settings.

Stage 2: MS^n spectrum data
This stage considers MS^n fragment ions generated for the molecular ion of interest and calculates the theoretical complement ion for each fragment ion.

Parameters for MS^n spectrum data
Invalid formula are filtered out using the fragment ion data. This technique is applied recursively through the various stages of MS^n data, allowing elemental composition of MS^4 fragment ions to filter the MS^3 precursors' formula generated using elemental composition; MS^3 ions are used to filter MS^2 ions, and MS^2 ions are used to filter the MS^1 molecular ion.

Stage 3: Isotope fitting of possible formula
This stage generates the theoretical isotopic distribution profile spectra for each of the possible formula generated for a molecular ion using accurate mass and elemental composition.

Parameters for isotope distribution
Least squared fitting routines are applied to pattern matching between experimentally derived data and theoretical distributions. A likelihood scoring algorithm takes into account the closeness of fit to the theoretical isotope data, the variation in mass accuracy and the candidate list filtered using MS^n fragment data. The log transformation ranking score lists more likely candidates with a higher score in the range 0-100.
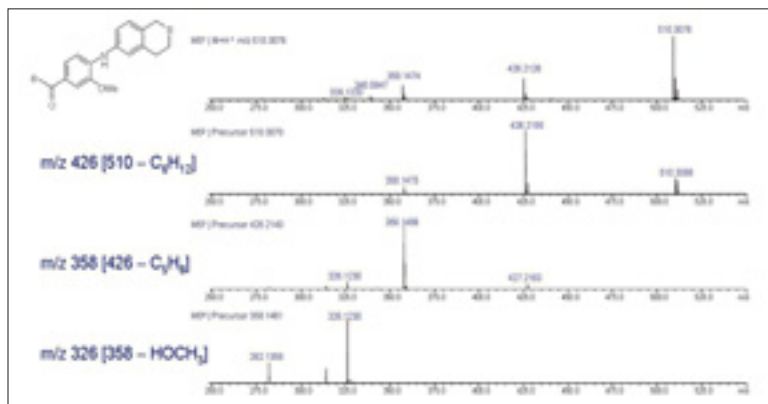
Figure 2: MS^n mass spectra of a new chemical entity. MS and MS^n data were used to predict/confirm likely empirical formula using mass accuracy data on the parent and fragment ions.
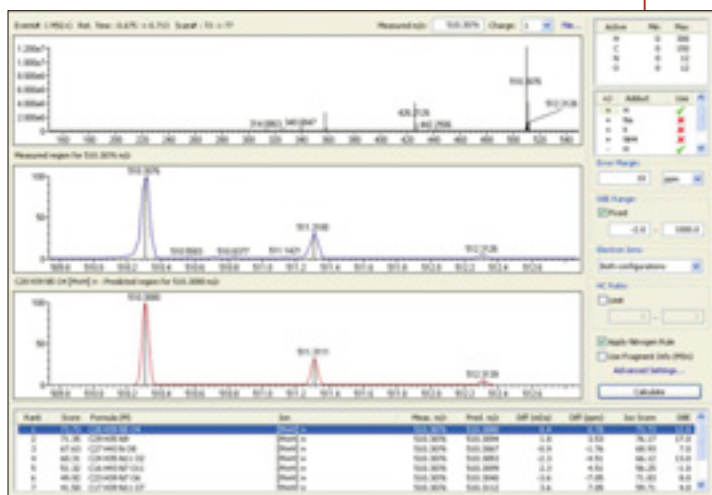
Results

Figure 3: A screen capture of the Formula Predictor software showing results using MS^1 data alone.

Mass accuracy and mass resolution are key parameters in identifying the correct empirical formula. To increase the reliability of the candidate list, the experimentally derived MS data is also compared to the theoretical isotope pattern to provide an Iso score. In this case the empirical formula of the chemical entity was $C_{28}H_{39}N_5O_4$. (The software tool also predicted the same empirical formula as the first hit).
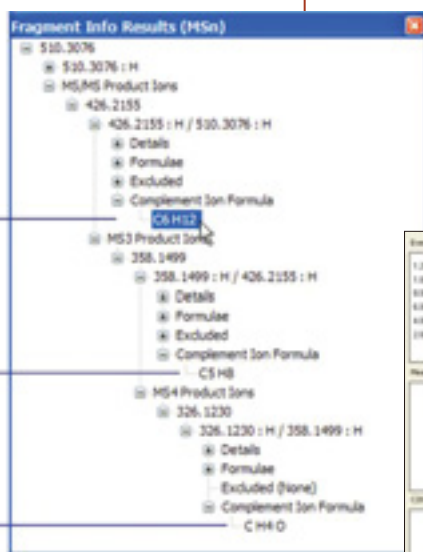
Figure 4: The Fragment Info Results window in Formula Predictor shows the supportive MS^n data.

Using the high mass accuracy of the fragment ions it is possible to exclude possible candidates and identify the correct formula using the fragment ion information.

m/z 426 [510 – C_6H_{12}]
Complement ion correctly identified.

m/z 358 [426 – C_5H_8]
Complement ion correctly identified.

m/z 326 [358 – $HOCH_3$]
Complement ion correctly identified.

Figure 5: A screen capture of the Formula Predictor software showing results using supportive MS^n data.

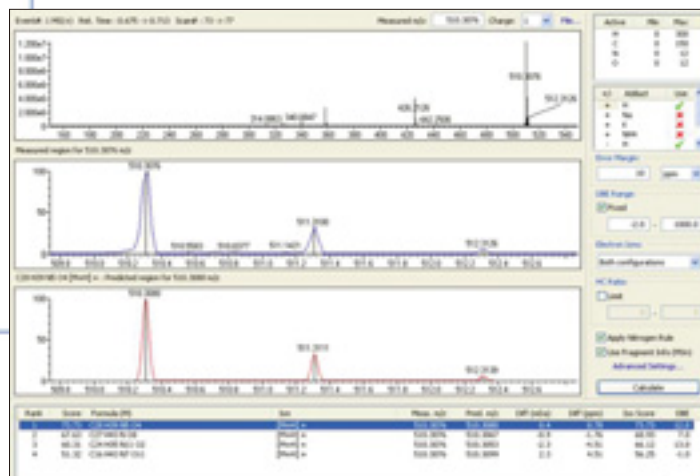The results recalculated using MS^n information reduced the candidate list further.

Figure 6: MS^n mass spectra of a new chemical entity empirical formula C$_{19}$H$_{21}$O$_3$N$_3$S.

MS^n data was used to generate ions at relatively low m/z values to help increase the likelihood of a first hit using MS^n fragment ions.
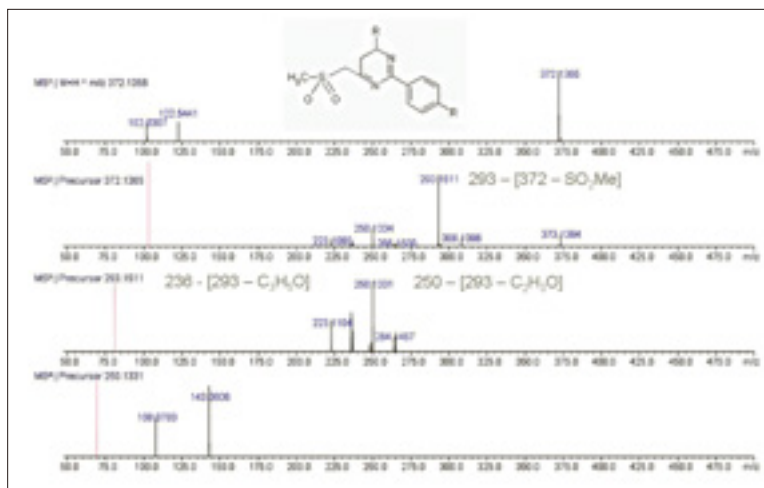

Figure 7: Using only MS^1 data, the true candidate was identified as hit number 16.
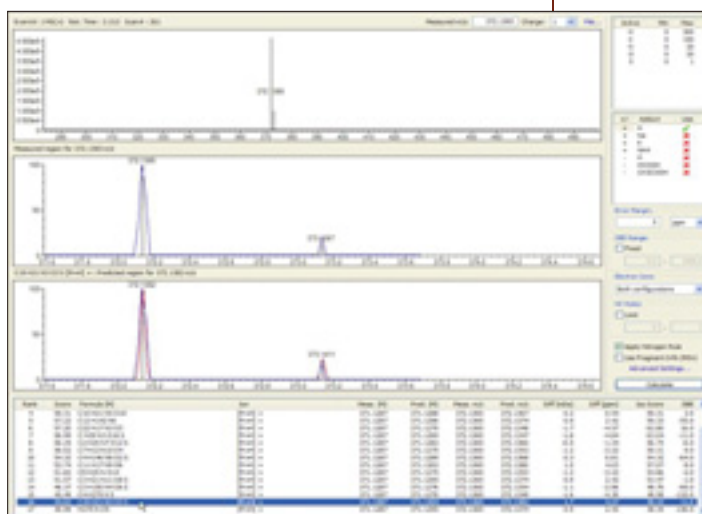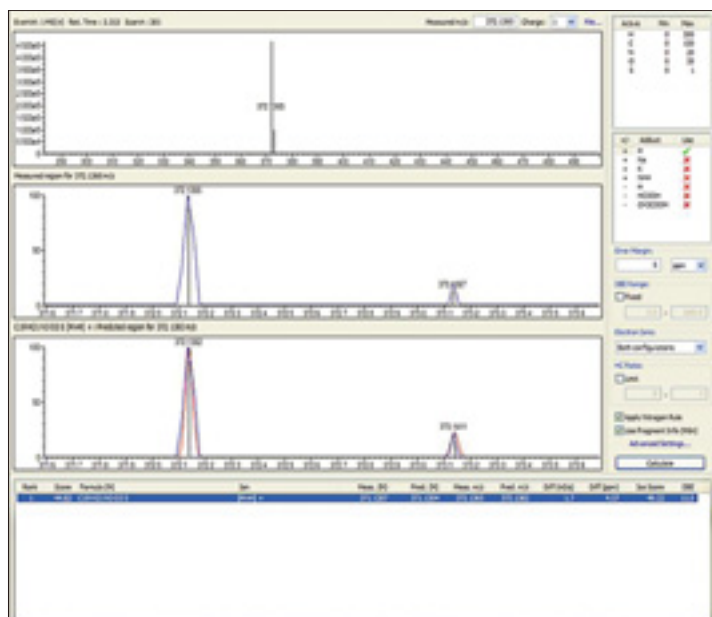
Figure 8: Using MS^n data, the true candidate was identified as hit number 1.

Discussion and Conclusions

Tools to predict empirical formula from accurate mass data typically consider chemical rules and isotope fitting routines. We described the application of MS^n data to help reduce candidate lists and to increase the likelihood of predicting the correct formula.

There are several factors that clearly affect the probability of identifying the true candidate, accurate mass being the most critical.

By providing tools that consider the fragment ion data in addition to pseudo-molecular ion information, it has proved useful in identifying the correct candidate mass with a higher probability than using simply MS data alone. ⊕

WITH INCREASING ENVIRONMENTAL POLLUTION, the development of an environmental monitor with high-throughput capability has increased in importance, since the human body may be exposed to many kinds of environmental pollutants. However, the concentration level of pollutants in the human body may only be a trace amount (down to ppb levels). For sensitive and selective detection, LC/MS is an effective and efficient method for the determination of these pollutants in biological samples such as plasma or serum.

Automated On-line Pretreatment LCMS System

for the Determination of Bisphenol A and 4-Octylphenol in Serum

Efficient sample pretreatment, for example, deproteinization, is critical to achieving reliable results while maintaining LC/MS performance. Off-line pretreatment methods are often limited in that the procedures can be time-consuming and, as a result, constrain high-throughput analysis. To resolve this issue, a number of pretreatment columns have been reported for development of automated on-line sample pretreatments of plasma and serum to achieve robust and reliable system performance with a high recovery yield.

In the last five years, Shimadzu has also reported on an automated sample pretreatment system that uses a unique dilution function of the sample, as well as a novel methylcellulose-immobilized reversed-phase pretreatment column (Shim-pack MAYI-ODS) for the rapid determination of drugs in blood plasma[1-5].

In this report, this system was applied for the determination of endocrine disrupting chemicals (EDCs) analysis such as Bisphenol-A and 4-Octyphenol (Figure 1) for high-throughput monitoring. EDCs possess a high health risk potential. Bisphenol-A (BPA), an estrogenic EDC, is widely used in the production of polycarbonate plastics and epoxy resins, which are used for packaging materials. 4-Octyphenol (4-OP), an alkylphenol, is one of the popular non-ionic surfactants that are used in many detergent formulations. These chemicals (BPA and 4-OP) may enter the body via the gastrointestinal tract as food contaminants.
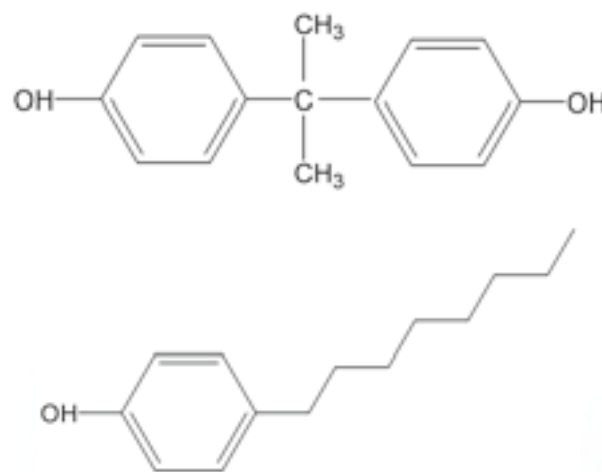

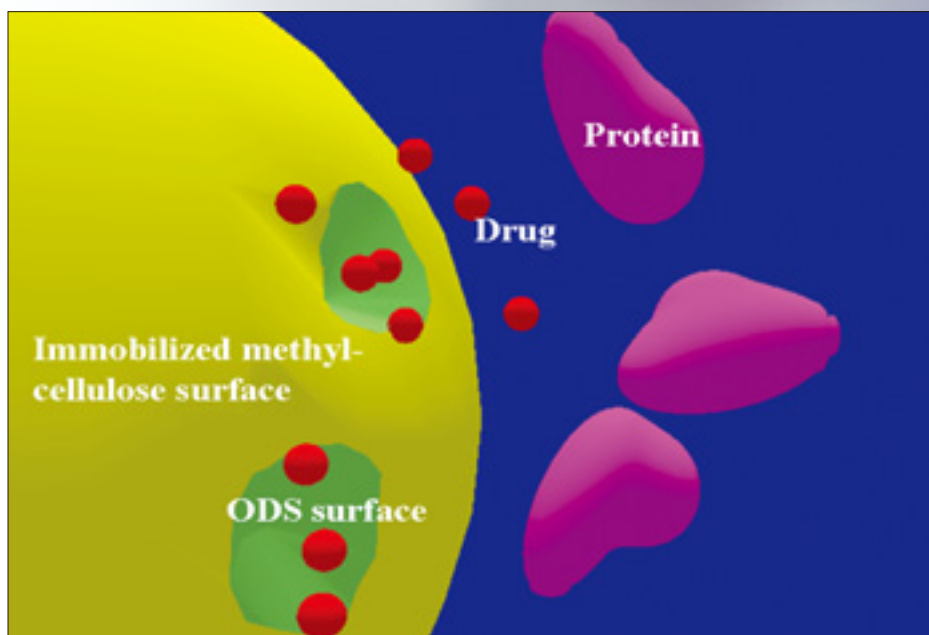**Figure 1**: Chemical structure of BPA and 4-OP.

Figure 2: An image of the pretreatment column.

Figure 2 is an image that demonstrates how the Shim-pack MAYI-ODS works to remove proteins in the sample. The outer surface of this packing material is immobilized by methylcellulose, and octadecyl functional groups (C18) are bonded onto the internal surface. Based on size limitation, proteins are excluded and flushed away from the pre-column, while small target molecules can penetrate into the pores and are retained due to hydrophobic interactions.

Figure 3 shows the flow diagram of this system (called the Co-Sense BA-LCMS system). This system is composed of a two-dimensional LC configuration. The first LC dimension functions as a sample pretreatment step to remove proteins in the plasma, as well as to trap BPA and 4-OP simultaneously by using the MAYI-ODS column. The second LC dimension separates BPA and 4-OP, which are detected by LCMS. In order to maximize the extraction efficiency for these compounds, the composition of the extraction mobile phase, such as the organic solvent content, ionic strength, pH and dilution times, was evaluated. The final analytical conditions are shown in Table 1.

The dilution function improves the recovery rate as shown in Figure 4. During the dilution step, serum
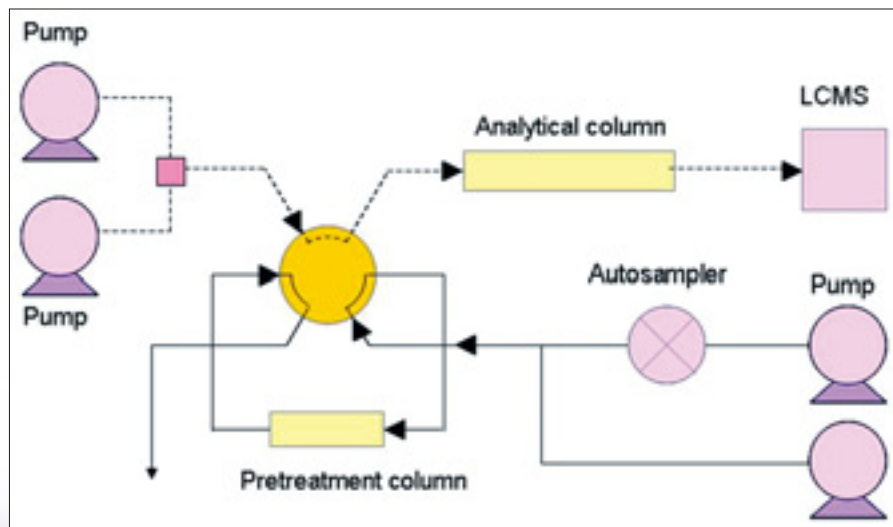

Figure 3: Flow diagram of the Co-Sense BA-LCMS system.

is diluted by the extraction mobile phase to release these analytes from the protein cavity effectively, and the released analytes are retained by the inner surface of the ODS. The dilution rate is also an important factor to improving recovery. In this analysis, 7 times dilution provided the highest recovery. The acceptable results of recovery and precision by using serum sample spiked with 0.5 ng/mL, 50 ng/mL and 500 ng/mL of each analyte are shown in Table 2. The LOD (S/N=3) and LOQ (S/N=10) for BPA in serum were 0.05 ng/mL and 0.1 ng/mL, respectively, while those of 4-OP were 0.1 ng/mL and 0.5 ng/mL, respectively.

CONCLUSION

This system enables us to minimize sample pretreatment time with effective extraction and can be utilized as a routine analysis with a simple and effective method to determine environmental and occupational exposure. ⊕

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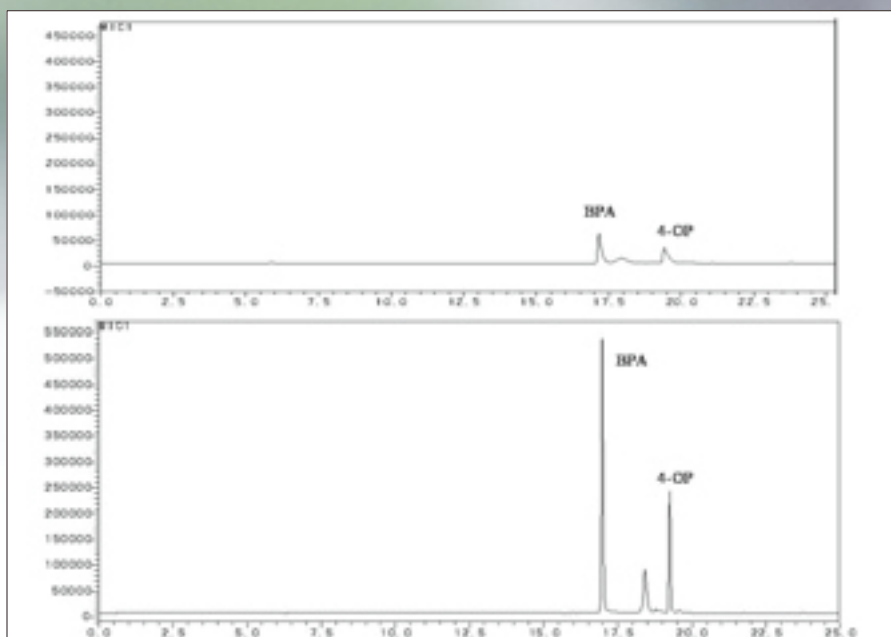
Figure 4: Comparison of Chromatograms – Upper Chromatogram: without dilution line; Lower Chromatogram: with dilution line.

Sample Pretreatment

Column:	Shim-pack MAYI-ODS (4.6mmI.D. x 10 mmL)
Extraction/Dilution Mobile Phase:	Water/Methanol = (95/5, v/v) containing 10 mM ammonium acetate
Extraction Flow Rate:	0.3 mL/min
Dilution Flow Rate:	2.1 mL/min
Injection Volume:	100 µL

LC Conditions

Flow Rate:	0.5 mL/min
Mobile Phase:	(A) Water, (B) Methanol
Gradient Program:	(B) concentration Initial – 30% 3 min – 30% 7 min – 100% 16 min – 100% 16.01 min – 30% 21 min – STOP
Column:	Shim-pack VP-ODS (2 mm i.d. x 150 mmL)
Column Temperature:	40°C

MS Conditions

Ionization:	APCI (400°C) (Negative mode)
Nebulizer Gas:	N2 (2.0 L/min)
Detector Voltage:	1.5 kV
Probe Voltage:	4.5 kV
SIM:	m/z 227 for BPA and m/z 205 for 4-OP

Table 1: Analytical Conditions.

Concentration (ng/mL)		0.5	50	500
BPA	Recovery	88%	101%	90%
	RSD	6.5	2.95	2.16
4-OP	Recovery	83%	100%	86%
	RSD	7.5	4.93	1.72

Table 2: Recovery and RSD of spiked serum samples (n = 5).

Online help: anytime, anywhere

Interactive LC Virtual Advisor

The web-based, interactive LC Virtual Advisor offers 24-hour interactive support for the Prominence HPLC Series. With 24-hour, password-protected access, users can custom-configure virtual systems to match their current or dream HPLC system configurations. The user can then access animated easy-to-follow maintenance procedures specific for their configured Prominence system. Users can also access the Troubleshooting section, which leads them to problem-solving procedures. A newer Advanced Troubleshooting module allows users to diagnose and correct many of the problems encountered with HPLC applications, using a step-by-step logical sequence of questions designed to troubleshoot chromatography issues.

The LC Virtual Advisor offers added convenience and learning opportunity, as the web site includes a reference/education section with a glossary of HPLC-related terms, and an ever-increasing list of other reference applications.

To learn more and to register, please visit **www.shimadzu.com/lc_virtualadvisor**

The LC Virtual Advisor includes a maintenance section that provides animated, easy-to-follow maintenance procedures.