UFLC XR

The SIL-20AXR is a total volume injection type (direct injection) autosampler that, in addition to a high-speed injection function and high-precision function for injecting trace quantities, features an improved high-pressure valve that increases the pressure resistance (max. allowable pressure: 66 MPa). The internal volume of the high-pressure valve was reduced to suppress sample diffusion, which is especially suited to high separation analysis. The 20ACXR includes a sample cooler that allows samples in sample racks to be stored at a constant temperature between 4 °C and 40 °C.

	Rack Changer	Rack Changer /c
Sample cooling	No	Yes (see below for details)
Number of samples processed	1152 (12 microtiter plates or deep well plates with 96 wells each)	1152 (12 microtiter plates or deep well plates with 96 wells each)
Operating conditions	4 - 35°C, 20-85% humidity	4 - 35°C, 20-85% humidity
Power requirements	50 VA, 50/60 Hz	350 VA, 50/60 Hz
Dimensions	W 425 x H 420 x D 500 mm	W 425 x H 420 x D 500 mm
Weight	25 kg	32 kg

Rack Changer/c sample cooler specifications

System	Direct cooling system (environment conditions: room temperature below 30°C with humidity less than 70% when the cooler temperature is set to 4°C), dehumidification system built-in
Temperature setting range	4 – 40°C (can be cooled down to 4°C when the room temperature is below 30°C and the humidity is below 70%)
Temperature accuracy	± 6°C

Method	Variable sample injection volume and total volume direct injection
Allowable Operating Pressure	66 MPa
Injection Volume Range	0.1 µL to 50 µL (0.1 µL to 100 µL with option)
Number of Samples Processed	175 (1-mL vials), 105 (1.5-mL vials, 70 for 20AC), 50 (4-mL vials), 192 (two 96-well MTP/DWP), 768 (two 384-well MTP/DWP) Besides these, ten 1.5-mL vials can be used

Injection Volume Precision	Max RSD 0.3 % (normally max 0.2 %: depending on specified conditions)
Cross-Contamination	Max 0.005 % (depending on specified conditions, without rinsing, normally max 0.0035 %)
Needle Rinsing	Freely settable, before or after sample injection
Operational Temp. Range	4 - 35 °C

In order to investigate minute quantities of impurities in such areas as pharmaceuticals and pesticide residues in food products, both the ability to separate and detect trace compounds more reliably and increased analysis efficiency are required.

Utilizing features of the Prominence UFLC, which offered high speed as well as high analysis accuracy and reliability, thereby increasing analysis cost-efficiency, we have developed the Prominence UFLCXR, which achieves greater data quality by means of higher separation performance.

While maintaining the high analysis accuracy and reliability cultivated with the Prominence series by using a high-speed separation column and optimizing the system, we have increased separation performance to a level greater than that of conventional LC.

The Ultimate in High Separation Performance

The ability to reliably perform the high-separation, high-sensitivity detection of trace compounds is required. Improving separation performance by simply increasing the column length also increases the analysis time, which reduces analysis efficiency.

With the Prominence UFLC, high-speed analysis while maintaining a high degree of analysis accuracy and reliability is possible and with the Prominence UFLCXR, greater separation performance is attained. In order to achieve the ultimate in high separation performance, column efficiency has been improved by optimizing the balance between the particle diameter of the XR-ODS and XRII-ODS high-speed separation column and the column length, and the system has been optimized to attain the highest possible level of column performance.

The results obtained in the analysis of seven alkylphenones are shown below. The Prominence UFLCXR demonstrated a level of separation performance equivalent to that of at least a 250-mm (5-μm) column. The analysis time was a quarter of that required by a general-purpose LC, indicating that both high separation performance and high speed were achieved.

High-Sensitivity Detection of Trace Components

The combination of the Prominence UFLCXR and a Shim-pack XR-ODS II column (length: 150 mm) achieves a level of separation performance equivalent to that of at least a 250-mm column containing packing material with a particle diameter of 5 μm. The wide dynamic range of the SPD-20A detector enables the high-separation, high-sensitivity detection of fine peaks, such as those obtained for minute quantities of impurities in pharmaceuticals, and thereby enables highly reliable analysis in which the slightest presence of impurities is not missed. Also,

the low-carryover SIL-20AXR autosampler supports high-accuracy analysis.

The results obtained in the analysis of a minute quantity of an impurity in cefazolin are shown below. This impurity was sufficiently separated from other impurities, and although its peak was only one thousandth the height of the peak obtained for the main component, the wide dynamic range ensured that it was detected properly.

Evaluation of Minute Quantities of Impurities in Cefazolin

Column: Shim-pack XR-ODS II (150 mm (L) x 3 mm (i.d.))
Column temperature: 40 °C
Mobile phase: Phosphate buffer solution / acetonitrile, gradient elution
Flow rate: 0.9 mL/min
Detection: 245 nm

Attaining Higher Separation Performance than the Prominence UFLC

In general, the aldehydes and ketones found in indoor locations and the atmosphere are subjected to HPLC analysis with pre-column derivatization performed using a 2,4-DNPH (2,4-dinitrophenylhydrazine) reagent. In this case, 13 aldehydes and ketones were analyzed with the Prominence UFLCXR. In comparison with general-purpose LC, relatively high speed and separation performance were attained. Also, separation was achieved in roughly the same analysis time required for the high-speed Prominence UFLC with greater reliability.

Analytical Conditions
Mobile phases: A: Water/tetrahydrofuran, B: Acetonitrile, gradient elution
Column temperature: 40°C
Flow rate: 1.0 mL/min
Detection: 360 nm

Excellent Reproducibility and Low Sample Carryover

Of the basic performance specifications required of analytical instruments, high reproducibility and low sample carryover are particularly important, and are essential for high-separation, high-sensitivity analysis.

With Prominence, these needs were addressed at an early stage, and superior technology was cultivated for all the fundamental elements. This included a high-performance pump, an autosampler capable of a high level of injection reproducibility, even for minute quantities, and an injection mechanism that prevents sample carryover. These technologies have all been inherited by the Prominence UFLCXR, and support high-separation, high-sensitivity analysis. A high level of basic performance supports true high-speed, high-separation analysis.

The SIL-20A(C), which has an established reputation for low sample carryover, has been joined by the SIL-20A(C)XR, which has high-pressure-resistance specifications. Its sampling needle incorporates special surface- treated technology and the utmost consideration has been given to the structure of the needle seal and material of flow channel parts. With this autosampler, there

is practically no carryover even for chlorhexidine, which is a representative example of a highly adsorptive basic substance.

The SIL-20A(C)XR uses the direct injection method (total-volume injection method), in which the mobile phase passes through the interior of the sample needle, washing out adsorptive components . This makes it possible to attain excellent reproducibility of no greater than 0.3% RSD, even in the injection of minute quantities.

Injection volume	Area value	
	Average value	%RSD
1μL	37596	0.148
2μL	75249	0.097
5μL	188382	0.026
10μL	375846	0.021

XR-ODS II High-Separation Column

The Shim-pack XR-ODS, which was developed with the aim of increasing the speed attained under standard column pressure conditions, has been joined by the Shim-pack XR-ODS II, which was developed in order to facilitate high-speed, high-separation analysis for a wider range of applications, such as those involving methanol mobile phases, by using a new longer column.

The Shim-pack XR-ODS II is a high-separation analysis column that is suited for use in combination with the Prominence UFLCXR and that can also be used for high-temperature analysis.

Reducing the particle size of the packing material to an optimum level and unifying the particle diameter help suppress flow resistance and ensure excellent stability. As with the Shim-pack XR-ODS (the pressure resistance for the XR-ODS is 30 MPa), quality control is performed in accordance with the standards indicated in the quality assurance document provided with the column.
Particle diameter of packing material: 2.2 μm
Pore size: 8 nm
Pressure resistance: 60 MPa

Example of High-Separation Analysis
The results obtained by performing the high-separation analysis of polycyclic aromatics using the Prominence UFLCXR and the XR-ODS II are shown below. With general-purpose LC, the resolution, RS, for isomers of benzofluoranthene was 1.25, whereas with the Prominence UFLCXR, this was significantly increased to 2.12. With general purpose LC, the analysis time was approximately 50 minutes, whereas the Prominence UFLCXR, it was reduced to approximately half. One advantage of increased speed of analysis is reduced consumption of mobile phase.

Analysis Conditions
Column: Shim-pack XR-ODS II (150 mm (L) x 3 mm (i.d.))

Column temperature: 40°C
Mobile phase: Water/acetonitrile = 35/65
Flow rate: 0.9 mL/min
Detection: 254 nm

The Prominence UFLCXR (pressure resistance: 66 MPa) also helps expand the range of analysis methods that can be used. For example, allowing the use of water/methanol mobile phases with longer columns and enabling analysis to be performed at room temperature.

The results obtained by analyzing the catechins in green tea using a water/acetonitrile mobile phase and a water/methanol mobile phase are shown below. It can be seen that the separation selectivity, as represented by the elution order and the separation between components, differs between the methanol and acetonitrile systems. This example illustrates how the Prominence UFLCXR helps increase the range of available analysis methods, a feature that is useful for method development.

Analysis Conditions
Column: Shim-pack XR-ODS II (75 mm (L) x 3 mm (i.d.))
Column temperature: 40 °C
Mobile phases: Phosphate buffer solution / acetonitrile (top), phosphate buffer solution / methanol (bottom), gradient elution
Flow rate: 1.0 mL/min
Detection: 230 nm