

# Modular Set of Prominence 20A Shimadzu HPLC for Identification of Biomacromolecules by UV-VIS Absorption and Fluorescence Detectors

L. Šikurová\*, M. Zvarík

Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina, 842 48, Bratislava, Slovakia

**Abstract:** High-performance liquid chromatography (HPLC) is a chromatographic technique that can identify, quantify and purify the individual components of a mixture. The present contribution deals with a modular set of Prominence 20A (Shimadzu Co.) HPLC, which was assembled of the following modules and accessories: a system controller, CBM-20A; an auto-sampler, SIL-20AC; a solvent delivery unit, LC-20AD; a degasser, DGU-20A5, a column oven, CTO-20AC; a low-pressure gradient unit; an automatic rinsing kit for Prominence 20A; a semimicromixer, SUS; and a reservoir tray. In addition, Shimadzu's Prominence HPLC system is supplemented with two detectors: an UV-VIS absorption detector, SPD-20A; and a fluorescence detector, RF-10. The entire HPLC system is controlled using the PC software LC solutions, version 1.25 SP1 (Shimadzu). The HPLC set was designed with the aim to identify, quantify and purify the individual biomacromolecules in biological fluids or tissues for possible diagnostics application.

**Keywords:** HPLC, Biomacromolecule, UV VIS, Absorption, Fluorescence

\*Corresponding author: [sikuroval@gmail.com](mailto:sikuroval@gmail.com) (L. Šikurová)

## 1. Introduction

High-performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds. HPLC systems are currently used in a wide variety of fields, in molecular biology, medicine, biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. HPLC is amenable to most analytes and it gives exquisite sensitivity and selectivity. Preparative HPLC is one of the most powerful techniques currently used for the purification of synthesized compounds in medical chemistry laboratories. In addition, therapeutic drug monitoring, screening of doping agents, specification of xenobiotics, and detection of specific oxidized species commonly employ high-performance liquid chromatographs. HPLC technique has also been used to analyze metabolic profiling, to investigate the anti-bacterial activity of plant extracts through quantification of exotoxins, and to identify and quantify the carotenoids, phenolic compounds, isoflavone components and other phytochemicals. Comprehensive, HPLC is, due to its advantages in achieving high resolving power, becoming more and more attractive especially to those who deal with complex samples [1–3].

## **2. Prominence 20A Shimadzu HPLC for identification of biomacromolecules by UV-VIS and fluorescence detectors**

At present, high performance liquid chromatographs are produced by 91 companies; however, a Prominence chromatograph from Shimadzu Corporation features the world's first Web control, fastest sample injection, and the highest detection sensitivity performance to surpass the current HPLC technology. The modular Prominence HPLC system offers exceptional specifications and flexibility for any application. Our laboratory is equipped with Prominence 20A HPLC (Shimadzu Co.) thanks the project implementation: "Centre of excellence for exploitation of informational biomacromolecules in the disease prevention and improvement of quality of life", ITMS 26240120003, supported by the Research & Development Operational Programme funded by the European Regional Development Fund (ERDF). With the aim to separate and identify biomacromolecules in biological tissues and biological fluids, we assembled HPLC system of selected modules. In our laboratory, Prominence 20A (Shimadzu) HPLC system consists of the following modules and accessories: a system controller, CBM-20A; an auto-sampler, SIL-20AC; a solvent delivery unit, LC-20AD; a degasser, DGU-20A5, a column oven, CTO-20AC; a low-pressure gradient unit; an automatic rinsing kit for Prominence 20A; a semimicromixer, SUS; and a reservoir tray. In addition, Shimadzu's Prominence HPLC system is supplemented with two detectors: an UV-VIS detector, SPD-20A; and a fluorescence detector, RF-10 AXL [4].

### **2.1. System Controller CBM-20A**

The CBM-20A is a system controller equipped with a data buffering function and acts as an interface for connecting LC workstations, network-client computers, and analytical instruments via Ethernet. The card-type CBM-20A is used by incorporating it into an LC-20A-Series solvent delivery pump or auto-sampler. It can control up to five units, including the unit in which it is incorporated. Prominence may be controlled over the web from any computer with Microsoft Internet Explorer 6.0.

### **2.2. Auto-Sampler SIL-20AC**

This unit is a total-volume injection-type auto-sampler that enables high-speed injection and multi-sample processing. Comprehensive sample carryover countermeasures make it possible to perform analysis without carry over. The SIL-20AC is equipped with a sample cooler that incorporates a dehumidifying function. Samples can be maintained at a fixed temperature in the range 4 to 40 °C. A rack changer can be used to change the microplates in the auto-sampler's rack and thereby facilitate serial analysis. Up to 12 plates can be mounted in the rack changer. This model incorporates a cooling function.

### **2.3. Solvent Delivery Unit LC-20AD with low pressure gradient unit**

LC-20AD solvent delivery pump system covers a wide application range with optimized performance under any condition. As part of an HPLC system the pump can incorporate the CBM-20A interface to control the entire LC system via the web browser or instrument control software. The LC-20AD pump designed in micro volume double piston technology provides pulsation free solvent delivery from real micro flow applications up to semi-preparative flow rates. The pump is configured as a quaternary low pressure

gradient system (AD). The low-pressure gradient unit LC-20AD is enabling the gradient elution in a compact space with a small void volume. The flow rate ranges from 0.1  $\mu\text{L}/\text{min}$  up to 10 mL/min.

#### **2.4. On-line degasser DGU-20A5**

The LC-20A series degassers feature extremely efficient degassing in a low-volume and compact design. The degassers are powered by the LC-20A series pumps, and the pump control panel can display the degasser vacuum level. The DGU-20A5 is an on-line degasser that uses a fluoroethylene membrane. The DGU-20A5 utilizes five degassing channels for the internal volume of 380 L. The DGU-20A5 also uses an additional channel for degassing the rinse phase of the autosampler. Removing dissolved gases in mobile phases is an important step for ensuring the proper function of pump check valves, and to prevent outgassing in the detector flow cell.

#### **2.5. Column Oven CTO-20AC**

The Prominence Column Temperature Oven (CTO) is a large-capacity forced air circulation device that relies on automatic fan speed control for precise temperature control. The fan speed varies with the set temperature through a quick feedback sensor mechanism to maintain the desired temperature. The CTO-20AC model includes a Peltier device for sub-ambient temperature control up to 10 °C below room temperature. Thus the CTO-20AC, using an electronic cooler, can regulate the temperature in a large range going from 10 °C below room temperature to 85 °C. Whether stand-alone or as part of a Prominence HPLC system, the CTO can provide us with the precise temperature control. Maintaining a constant temperature provides improved reproducibility and separation performance. Elevated column temperatures can reduce analysis times and result in increased sample throughput. Mobile phase viscosity and thus the system pressure are also reduced with increased temperature, resulting in prolonged column lifetimes. The column oven can hold up to 10 analytical columns. With all of these items installed in the heated zone, retention time and chromatographic reproducibility can be maintained regardless of variations in the laboratory environment. The specification of CTO-20AC is in Table 1.

#### **2.6. Automatic rinsing kit for Prominence 20A**

An automatic rinsing kit is an included standard to ensure improved plunger seal life. A special coating on the needle and a low-adsorption valve ensure hardly any carryover, even with basic or hydrophobic compounds. Also, using the optional multi-rinse mode ensures that the optimum rinsing method is selected, even for sample constituents that are difficult to rinse, such as proteins, a clean analysis.

#### **2.7. Achieving Auto-Purge with a Prominence System**

The Prominence system is equipped with auto-purge, a function that is normally available only with all-in one HPLC systems. This makes it unnecessary to open or close the drain valve when, for example, replacing the mobile phase. Combining this function with the startup and baseline-check functions enables a fully automatic analysis with a modular HPLC system.

**Table 1.** Specification of CTO-20AC.

<b>Type</b>	Forced air circulation
<b>Temperature control range</b>	Ambient from 10 °C to 85 °C
<b>Temperature setting range</b>	4 °C to 85 °C in 1 °C steps
<b>Temperature control precision</b>	±0.1 °C
<b>Ambient temperature range</b>	4 to 35 °C
<b>Interior dimensions</b>	220 mm (W) 95 mm (D) 365 mm (H)
<b>Safety measures</b>	User-settable upper temperature limit; thermal fuse equipped to prevent overheating; vapor sensor for leak detection
<b>Fan speed</b>	Automatically regulated in accordance with temperature.
<b>Time program functions</b>	Temperature setting changes; Oven ON/OFF; 320 steps, 0.1-999.9 minutes; Linear temperature regulation

## 2.8. Software

The entire HPLC system is controlled using the PC software LC solutions, version 1.25 SP1 (Shimadzu). LabSolutions software is the unified software platform for all chromatography systems from Shimadzu. The Windows-based graphical control software offers easy understanding of the operations. Exporting data to commercial spreadsheet software is ideal for report generation.

## 2.9. UV-VIS absorption detector SPD-20A

The SPD-20A UV-VIS detector offers an exceptional level of sensitivity and stability. Taking sensitivity to the limit, the SPD-20A has a noise level of  $0.5 \times 10^{-5}$  AU max., making it the world's most sensitive UV-VIS detector. In addition, a temperature-controlled flow cell eliminates inconsistencies caused by changes in absorbance due to shifts in room temperature. This helps increase the baseline stability and the analysis reliability. Using newly developed signal processing technology, the stray-light correction function has been enhanced, and the linearity has been improved. This expanded linearity combines with various noise-reduction technologies to provide users with a wide dynamic range and allows for the analysis of your target compound and minor impurities in a single run. This unit can monitor two components in the UV or VIS range simultaneously (Dual-Wavelength Measurement). Cut-off filter to eliminate the second-order diffraction is automatically activated for 371–700 nm range. The specification of SPD-20A detector is in Table 2.

## 2.10. Fluorescence Detector RF-10AXL

The RF-10AXL fluorescence detector offers the world-class sensitivity, excellent ease of maintenance, and validation support functions. It supports a wide range of applications from conventional analysis to high-performance analysis. Utilizing a newly designed optical system, the RF-10AXL offers the world-class levels of sensitivity. A newly developed ratio system compensates for lamp intensity fluctuation and provides superb noise characteristics. The RF-10AXL features of the enhancements including a new remote lamp on/off function to allow lamp shutdown upon run completion, and a new rugged flow cell design with elevated back-pressure tolerance. The RF-10AXL has a compact,

**Table 2.** Specifications of SPD-20A detector.

<b>Light source</b>	Deuterium (D2) lamp
<b>Wavelength range</b>	190 to 700 nm
<b>Bandwidth, slit width</b>	8 nm
<b>Wavelength accuracy</b>	1 nm max.
<b>Wavelength precision</b>	0.1 nm max
<b>Noise</b>	0.5 <sup>5</sup> AU (under specified conditions)
<b>Drift</b>	1 10 <sup>4</sup> AU/h (under specified conditions)
<b>Linearity</b>	2.5 AU (ASTM standard)
<b>Functions</b>	Dual-wavelength detection in the range 190 to 370 nm and upwards of 371 nm, ratio-chromatogram output, wavelength scanning
<b>Cell</b>	Optical wavelength: 10 mm, capacity: 12 $\mu$ L, withstand pressure: 12 MPa
<b>Web control</b>	Parameter setting, log management, monitor detector output, management of consumable parts, etc.
<b>Buffer memory</b>	Refer to CBM-20A
<b>Operating temperature range</b>	4 C to 35 C

**Table 3.** RF-10AXL Specifications.

<b>Light source</b>	Xenon lamp, 150 W
<b>Excitation/emission monochromators</b>	Concave, blazed holographic grating monochromators, F2.4
<b>Measuring wavelength range</b>	200 650 nm
<b>Spectral bandwidth</b>	15 nm both in the excitation and emission sides
<b>Wavelength accuracy</b>	$\pm 2$ nm
<b>Wavelength reproducibility</b>	$\pm 0.2$ nm
<b>Sensitivity</b>	The S/N ratio is 300 for the Raman line of distilled water (350 nm excitation wavelength and 1.5 sec. time constant)
<b>Detector</b>	Photomultiplier
<b>Cell (volume, pressure, material)</b>	12 $\mu$ L, approx. 20 kg/cm <sup>2</sup> (2MPa) SUS316, Quartz, PTFE, as standard (2 $\mu$ L cell and inert cells are available as option)
<b>Wavelength scanning</b>	Possible both for excitation and emission wavelengths
<b>Time programming</b>	Wavelengths parameters may be programmed in up to 32 steps
<b>Ambient temperature requirements</b>	4 35 °C
<b>Other</b>	Autozero function

modular design and it easily integrates with other Shimadzu LC Series components via fiber optic communication cables for “plug-and-play” convenience in building a customized Shimadzu LC system. The RF-10AXL offers many functions including self diagnostics, maintenance information (lamp lit hours, lamp energy), and span calibration functionality. The fluorescence intensity drops as the temperature rises. A fluctuation of about 1 °C near room temperature may result in approximately 5% intensity fluctuations for some com-

pounds. To prevent this, the RF-10AXL features a temperature-controlled cell with a cooling function. It maintains a constant detector cell temperature, even if the room temperature fluctuates significantly, to ensure superb reproducibility with no drop in sensitivity. Without temperature control, the peak area value dropped approximately by 17% due to the increase in cell temperature when the room temperature changed from 25 °C to 30 °C. With its temperature-controlled cell, the RF-10AXL is not affected by such fluctuations in room temperature. Fast response is required to follow the sharp peaks obtained by ultra-fast analysis. The RF-10AXL permits ultra-fast (10 ms), high-sensitivity multi-component analysis using wavelength switching via a time program (Table 3).

### Acknowledgements

This contribution is the result of the project implementation: "The centre of excellence for utilization of information on bio-macromolecules in disease prevention and in improvement of quality of life" (ITMS 26240120003) supported by the Research and Development Operational Programme funded by the ERDF.

### References

- [1] M. Paige, M. S. Saprino, D. A. Bunyan, Y. M. Shim: *Biomedical Chromatography* **23** (2009) 817–821.
- [2] O. Sagirli, A. Önal, S. Toker, A. Öztunç: *Journal of AOAC International* **92** (2009) 1658–1662.
- [3] C. Herrera, P. M. García-Barrantes, F. Binns, M. Vargas, L. Poveda, S. Badilla: *Journal of Ethnopharmacology* **133** (2011) 907–910.
- [4] <http://www.shimadzu.eu/products/chromato/hplc/modular/prominence/default.aspx>