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• The NanoTek Microfluidic Synthesis System is a modular microfluidic chemistry system with the ability to combine both microscale and macroscale process steps. Modular components give the user maximum flexibility for both discovery and clinical applications.

Application Note

Controlling External HPLC Components with a NanoTek

Introduction

The NanoTek Microfluidic Synthesis System provides a flexible system offering the dual benefits of high efficiency and very fast synthesis, useful both for new compound development and production of unit doses of research compounds. Many compounds require HPLC purification. This application note describes how the NanoTek system can be used to control external HPLC equipment by demonstrating how it was used for batch preparation of FLT.

Description

The NanoTek base module is equipped with auxiliary i/o signals, which can be used to control external equipment. There are 14 TTL/CMOS digital output lines, two analog inputs at 12–bit resolution, and one analog output at 12-bit resolution. These signals are controlled from within the NanoTek software, either as individual manual steps or as instructions stored in sequences known as macros.

The HPLC components used are shown in Table 1.

Table 1. HPLC Components

	Manufacturer	Model #
HPLC Injector	Alltech Associates	Alltech Select Pro fluid Processor
UV detector	Knauer	K200, 254 nm
Radiation detector	BioScan Carrol-Ramsey	Bioscan flow count pin diode OR Carrol Ramsey Pin diode
Column	Phenomenex	Luna 5 µ, C18(2), 100 Å, 250 X 10 mm
3-way solenoid valve	The Lee Company	24 VDC Model # LFRX0500650BE
Relay board	Winford	RLY104-12V

The pump flow rate must be set on the front of the unit prior to operation. The HPLC injector, UV AutoZero, and Pump Start/Stop are then controlled by a digital output logic level. This allows the user to control the pump flow, to autozero the UV detector prior to initiating the HPLC run, and to control the position of the HPLC injector valve.

The UV detector has a fixed wavelength to observe absorptions in the HPLC column effluent at 254 nm, which is suitable for most compounds. It can be connected to one of the analog inputs for the NanoTek and the radiation detector can be connected to the other. These inputs can then be read and the user can generate a routine to display from within the Nanotek software, enabling manual cutting of the peak. Alternatively, these detectors can be connected to a commercial HPLC software package to which the NanoTek can send the start signal after the injection port has been loaded.

A final connection to a three-way solenoid valve allows flow from the HPLC system to be directed either to a waste collection container or to a product vial. The solenoid used in the current setup was a 24 VDC Lee Company Model# LFRX0500650BE, three-way valve. An intermediate relay board, rated up to 250V and 15 A, was used to enable the 5V TTL output from the NanoTek to drive the 24V solenoid. This arrangement grants users maximum flexibility in selecting their preferred solenoid valve.

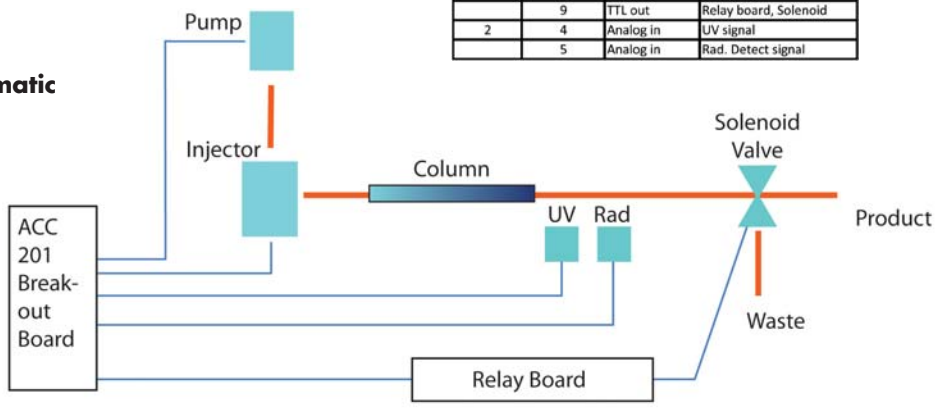
The HPLC components are then connected as shown in Figure 1.

NanoTek Pin Connections

Socket	Pin No.	Signal	Connect to
1	4	TTL out	HPLC Injector
	5	TTL out	UV Auto Zero
	6	TTL out	Pump, run
	9	TTL out	Relay board, Solenoid
2	4	Analog in	UV signal
	5	Analog in	Rad. Detect signal

Figure 1. Connections Schematic

NanoTek
Microfluidic Synthesis System



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Results

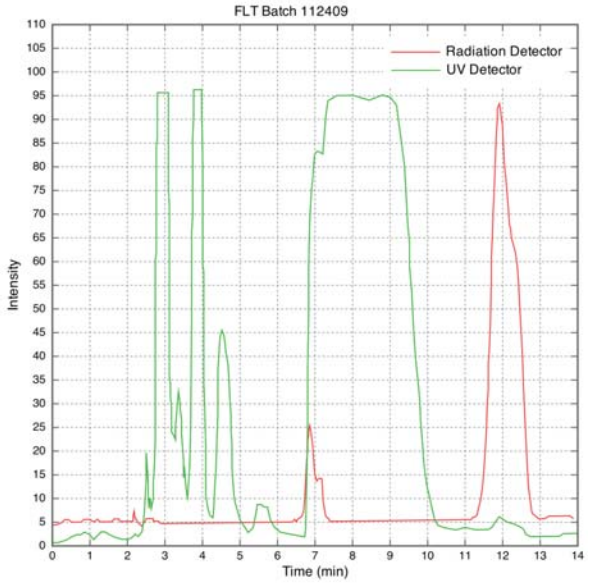
The NanoTek Microfluidic Synthesis System was used for batch preparation of FLT. The full sequence of events, all completed under automated control, is as follows:

1) Clean module, HPLC injector, column, and product delivery line using 70% ethanol/ water.
2) Clean module with a standard acetonitrile cleaning program; clean concentrator modules using the standard concentrator macro.
3) Equilibrate the HPLC injector, column, and delivery line with 8% ethanol/PBS.
4) Dry the [^{18}F]fluoride.
5) Load precursor to loop #1, 40 mg/ml in acetonitrile.
6) Load the dried fluoride in acetonitrile to loop #3.
7) Mix the two solutions in the reactor at 170 ˚C and send reactor output to concentrator 2 with a slow flow of inert gas to remove excess acetonitrile.
8) Hydrolyze with 2 N HCl and neutralize with 3M sodium acetate.
9) Load the hydrolysis reaction mixture to the HPLC injector loop using concentrator 2.
10) Purify on the HPLC and transfer through a sterile filter into the final product vial.
11) Analyze the [^{18}F]FLT.

A typical analysis of the hydrolysis reaction mixture prior to HPLC purification found that approximately 40% of the total activity is [^{18}F]FLT.

A typical trace of the semi-prep purification of the product from the hydrolysis is shown in Figure 2. The use of 8% ethanol in phosphate-buffered saline (PBS) yielded the entire FLT peak in 7- to 8-ml volume and in a formulation suitable for injection

Figure 2. Typical Semi-prep Purification of [^{18}F]FLT



The analytical HPLC trace (after the HPLC semi-prep purification) is shown in Figure 3.

The radiochemical purity of the collected material was greater than 99.9%, and the identity was confirmed by the co-injection of the hot solution with FLT standard. The product was also determined to be suitable for use as an imaging agent in human research, subject to regulatory approval.

Figure 3. Analytical QC of [^{18}F]FLT obtained from semi-prep purification