KNAUER

Application Note

▶ Rapid performance verification of AZURA® systems with refractive index detector

Method	HPLC
Keywords	Quality control, system verification, refractive index, AZURA® Analytical HPLC Plus system, OpenLAB® CDS
ID	**VSP0012N**

Summary

This application note is a guideline describing step-by-step a compact **performance verification (PV) of AZURA® Analytical HPLC systems including a refractive index detector,** using a reversed phase (RP) column and a standard test solution. This procedure is recommended for testing newly installed AZURA® Analytical HPLC systems as well as for regular monitoring of system performance.

Introduction

We strongly recommend every HPLC user to check the reliability of their system before running an analysis. The accuracy and reproducibility of a HPLC separation method is strongly dependent on stable flow rates, the precision of detectors, injection valves and autosampler, respectively.

This guideline provides a detailed instruction for testing the HPLC system performance using a standard test solution which is separated on a RP column. Retention time and area of two peaks from the standard test solution are used as parameters for the system performance and provide information about the status of the HPLC system. Five replicates are used for statistical analysis. The verification methods are adapted to the requirements of different AZURA® Analytical HPLC systems.

Note: This performance verification procedure is exclusively developed for refractive index detection mode.

Performance Verification method

KNAUER provides all method parameters as part of the performance verification document for your particular AZURA® Analytical HPLC system.

Note: The PV procedure is developed for standard system configurations which contain a 10 or 20 µl sample loop in the autosampler. Deviations from this configuration may require adaptions to the procedure.

Note: The pictures exemplarily shown in this document are based on an AZURA® Analytical HPLC Plus system and OpenLAB® CDS.

Equipment

Chemicals Double deionized water

Uridin (CAS 58-96-8)
Desoxyuridin (CAS 951-78-0)

The data shown in this application note were produced with the KNAUER PV test mix containing uridin and desoxyuridin. The concentration of uridin and desoxyuridin is 1.0 mg/ml. For the verification procedure it is necessary to separate both substances and to evaluate the data regarding retention time and area of these two peaks.

Note: We recommend producing your own test solution. 100 mg of uridin and 100 mg of desoxyuridin were dissolved in 100 ml water and sonicated for 10 minutes. Aliquots of the test solution can be stored for up to 24 months at -20 °C.

Column Eurospher II 100-5 C18A, 50 x 4 mm with precolumn (order no. 05WE184E2J)

Software OpenLAB® CDS

Accessories 10 µl sample loop for manual injection valves (order no. A05645)
10 µl sample loop for autosampler 3950 (order no. A50078)
20 µl sample loop for manual injection valves (order no. A05646)
20 µl sample loop for autosampler 3950 (order no. A05646)

After installing OpenLAB® CDS and KNAUER drivers, ensure that all power saving functions of the operating system are switched off. Then open the software, create a project and configure the HPLC system.

Note: It is strongly recommended to avoid the usage of space characters within any project names as well as data files or methods with OpenLAB® CDS.

Important note for OpenLAB®

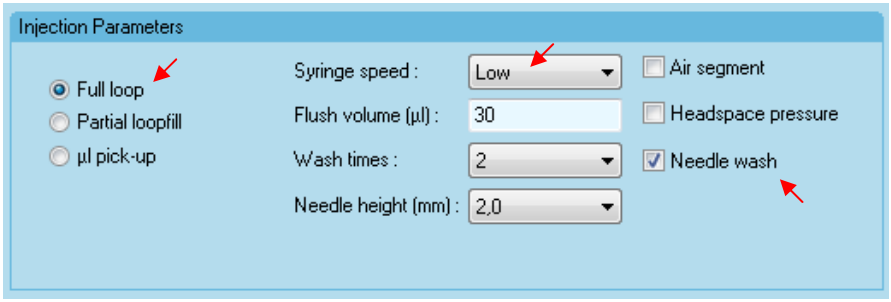
After installing OpenLAB® and KNAUER drivers, ensure that all power saving functions of the operating system are switched off. Then launch OpenLAB® CDS and create a project. Next configure the HPLC system.

Note: It is strongly recommended to avoid the usage of space characters within any project names as well as data files or methods with OpenLAB®.

►Method parameters

Injection

Fig.1: AS 3950

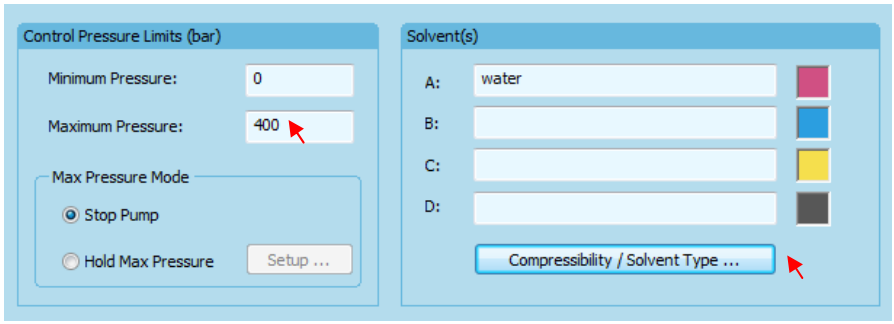


The threshold values for the PV test were determined with the injection parameter *Full loop*. Deactivate the options *Air segment* and *Headspace pressure*. Set the *Syringe speed* to low and use the *Needle wash* option.

Note: In the mode of *Partial loopfill* you can inject up to 50 % of the full loop volume, whereas in the mode of *Full loop* the volume of the entire loop is injected.

Pump

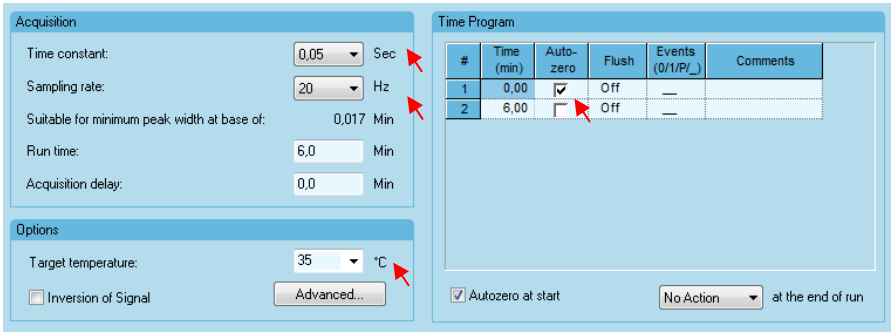
Fig.2: P 6.1L



Applications based on refractive index detection are usually isocratic due to the principle of measurement. Nevertheless, also a high pressure gradient (HPG) or low pressure gradient (LPG) pump can be used. It is important to set the *Maximum Pressure* depending on the column specifications and the *Compressibility factor* (CF) of the solvent.

Detector

Fig.3: RID 2.1L



The detection is performed with a time constant of 0.05 sec and 20 Hz sampling rate. Activate the options *Autozero* and set the *Target temperature* to 35 °C.

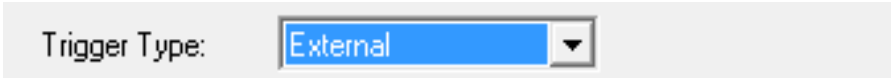
Additional settings

Auxiliary traces

#	Acquire	Trace	Unit
1	☑	Temperature [RID2.1L]	°C
2	☐	Temperature [CT2.1]	°C
3	☐	Leak Level [CT2.1]	%
4	☑	Pressure [P6.1L]	bar
5	☐	Stacked Inj. [AS 3950]	a.u.

It is always useful to record the pressure and the temperature of the detector to acquire further data in case of separation problems.

Trigger



If the HPLC system includes an autosampler the *trigger type* is always *external*.

Integration events

#		Event	Start Time	Stop Time	Value
1	☑	Width	0,000	0,000	0,2
2	☑	Threshold	0,000	0,000	50

It is recommended to use the default settings for the integration parameters *width* and *threshold*. Sometimes it is necessary to define *integration off* to ensure correct baseline behavior.

Peaks/groups

#		Name	ID	Ret. Time	Window
1	☑	Uridin	1	2,62	0,262
2	☑	Desoxyuridin	2	3,99	0,399
3	☐				

After a test run the retention time should be inserted into the peaks/groups table to enable automated peak recognition and peak integration via report function.

►Verification procedure

First steps

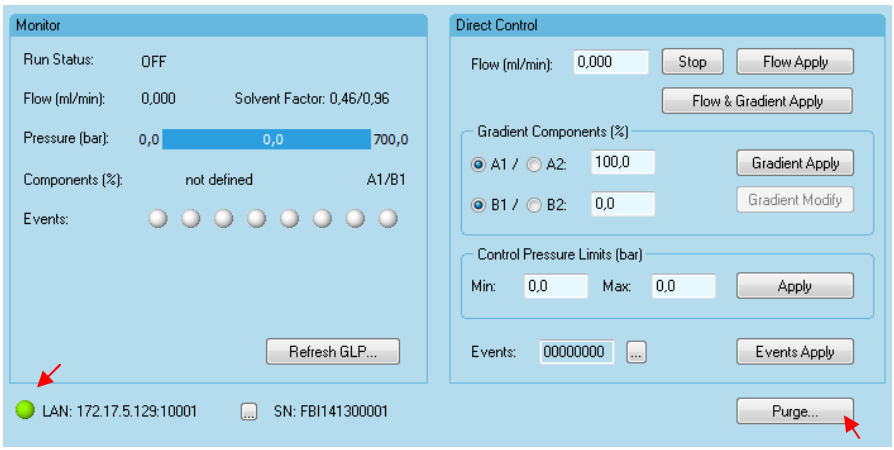
This section describes step-by-step the procedure for validating an AZURA® Analytical HPLC system including a refractive index detector.

First of all enable the detector to warm up the lamp for at least 30 minutes to guarantee best performance. Use the warming-up time to connect channel A with a bottle of fresh double deionized water and to mount the column (Eurospher II 100-5 C18A, 50 x 4mm).

Launch the HPLC system via OpenLAB® CDS, open the verification method and check again the method configuration for the AZURA® Analytical HPLC system.

Note: Check the pressure limits of all HPLC components to prevent damage to your system.

Open *Control* → *Instrument status* to check whether all devices are ready. This is indicated by a green dot in the bottom left corner of the window.



Purge the pump

Always purge the channels you use before starting a method. Open the purge valve and choose the purge function to flush any air bubbles out of the system.

Note: After installation of the HPLC system, maintenance or exchange of capillaries, we recommend flushing the system without column and detector.

Then download the method (*Control* → *Download Method*) close the purge valve and equilibrate the column for at least 10 minutes.

Flush the detector cell

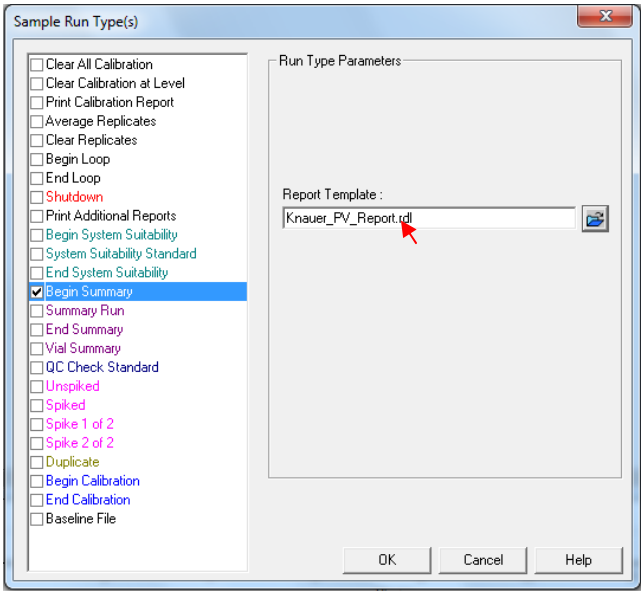
Note: We recommend flushing the detector cell several times after the column is equilibrated with the solvent.

Sequence

Create a new sequence (*File → New → Sequence → Blank.sec*) and enter all required parameters like method, injection volume as well as the corresponding vial position for your test solution. The Run Type should be configured as indicated in the figure below (*Summary Begin*, *Summary Run* and *Summary End*).

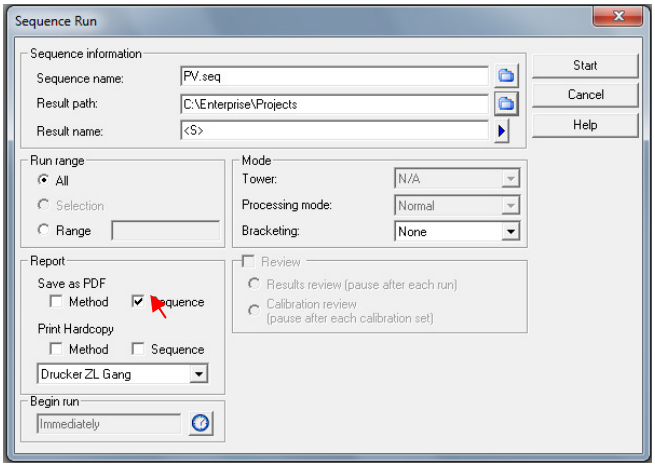
Run #	Run Type	Level	Conc	Custom	Reps	Vial	Volume	Sample ID	Method	Filename
1	Summary Begin	0	n/a	Unconfigur	1	1A1	10	2015-08-21_rep1	PV_method.met	2015-08-21_rep1.dat
2	Summary Run	0	n/a	Unconfigur	1	1A1	10	2015-08-21_rep2	PV_method.met	2015-08-21_rep2.dat
3	Summary Run	0	n/a	Unconfigur	1	1A1	10	2015-08-21_rep3	PV_method.met	2015-08-21_rep3.dat
4	Summary Run	0	n/a	Unconfigur	1	1A1	10	2015-08-21_rep4	PV_method.met	2015-08-21_rep4.dat
5	Summary End	0	n/a	Unconfigur	1	1A1	10	2015-08-21_rep5	PV_method.met	2015-08-21_rep5.dat

Report



The report template is defined on the right side of the window if you choose the *Begin Summary* option. You can choose your preconfigured advanced report template (see below).

Start the sequence



Note: If you activate the report option *Sequence* you will find the report as a pdf file in the "Result path" folder.

Threshold values

The given threshold values are specific for KNAUER analytical HPLC systems including a refractive index detector such as AZURA® RID 2.1L. For a standard system configuration as described above, we recommend to accept values of % RSD of < 0.5 % regarding retention time and < 1.0 % regarding peak area to pass the PV test.

Note: KNAUER provides specific limiting values together with the PV method. They can vary depending on the system configuration.

How to create an Advanced Report with OpenLAB®?

This short description will help you to create an Advanced Report in OpenLAB® for calculation of verification parameters. The arrows indicate the obligate selections.

→ *File* → *New* → *Report* → *Blank.tpl*.

Right Click → *Table Wizard* → *Sequence Summary Table*.

Follow the wizard and select the parameters *retention time and area* for verification. In the next selection windows choose:

→ *Named Peaks that were detected* (deactivate the other options)

→ *Data Filename*

→ *down*

→ *yes*

Finish the wizard and save the report template.

Troubleshooting

If the system does not pass the PV test, check the following points to locate the cause of the issue. Compare the pressure trace with former successful PV tests to ensure that the pump is working properly. Check the RID 2.1L temperature trace (if available) to see whether the temperature is stable or out of limits. Compare also the peak shape with former runs. Deviations can indicate that capillary connections are not suitable for a successful PV test. For more information please visit our homepage, consider KNAUER HPLC troubleshooting guide and FAQs on www.knauer.net or contact us via email or phone.

Note: Keep in mind that the methods are optimized for new columns and certain parameters e.g. retention time may vary after a certain amount of injections or the usage of alternative eluents.

Conclusion

With this procedure we can offer a robust and fast method to validate the reproducibility of the performance of a KNAUER AZURA Analytical HPLC system including refractive index detector.

We recommend running a PV test at least once a year or whenever a part of the HPLC system is exchanged or varied.

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