

NOTE:
**THIS IS A REAL SAT PROCOL, BUT IN ORDER NOT TO DISLOSE CUSTOMER
IDENTITY AND SYSTEM PARAMETERS, SOME DETAILS WERE ERASED
AND PHOTOS WERE ALTERED**

PROTOCOL
No. [REDACTED]

**Site Acceptance Test
of**
separlab

**SYSTEM FOR PREPARATIVE
LIQUID CHROMATOGRAPHY**
[REDACTED]

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1. INTRODUCTION

The present Site Acceptance Test (SAT) protocol details the installation and operational test which must be successfully executed before the [REDACTED] can be released for the use and describes test results of SAT.

2. APPROBATION

The approbation of the current SAT protocol is placed under the responsibility of Separlab Ltd.

Date [REDACTED]

Signature:



Stanislav Vozka, Managing Director

3. PURPOSE

The Site Acceptance Test (SAT), is a functional test of the preparative chromatographic system, including equipment, instruments and controls, and verifies of subsystems and whole unit are working correctly.

4. SCOPE

This protocol is applied to
SYSTEM FOR PREPARATIVE LIQUID CHROMATOGRAPHY [REDACTED]

5. PROCEDURE

5.1 DESCRIPTION OF THE INSTALLATION

Separsys [REDACTED] is composed by:

- a [REDACTED] diameter stainless steel column PC 01 which has length [REDACTED] and is equipped by an hydraulic oil cylinder having inner diameter 96 mm. and stroke 350 mm and is feeded by an oil manual pump with a manometer; the column is packed by spherical silica sorbent delivered by the customer
- splitting block for input of liquid to the column consisting of two T pieces, three high pressure ball valves and an electronic pressure sensor
- three [REDACTED] pumps [REDACTED] with frequency changers and connecting pipelines
- UV-VIS detector with remote cell which is connected by optical cables to monitor mobile phase composition
- computer unit with modified software [REDACTED] to set system parameters and to monitor detector signals and pressure in the column

5.2 PROCEDURE AND TESTS

For each type of test, a specific sheet is prepared. (starting from the annex 3 of the current protocol). Each test sheet contains the test scope, the method, the acceptance criteria, the equipment or instruments used to perform the test, the specific test results, the test comments, the test status, the signatories and the dates for the execution by the person who has performed the tests and the person who has controlled them. During the tests, record all the results are written with a blue ink in the corresponding fields of the sheet, under the expected values. According to the noted values correspond to the expected values, indicate the test status with the date and initials.

All the appendices have to be clearly identified with the protocol number, the test number, the test date and the signature of the person who has performed the test. For any correction, strike through with a unique line and add the initials and the date, with blue ink. Note the correct data. Date and sign the correction. If the result does not correspond to the expected and if the error cannot be resolved straight away, a deviation report should be initiate.

5.3 DOCUMENTATION

Documentation delivered with instrument is listen in the Appendix 3. All items are to be found and checked.

5.4 ACCEPTANCE CRITERIA

Each test sheet contains the acceptance criteria to be applied.

5.5 RESULTS EVALUATION

All tests were performed and all criteria were fulfilled. System is accepted.



For Separlab s.r.o. RNDr. Stanislav Vozka

6. APPENDIXES

- 6.1. *PERSONNEL WHO HAVE PERFORMED THE SAT*
- 6.2. *LIST OF EQUIPMENT USED*
- 6.3. *DOCUMENTATION*
- 6.4. *DELIVERY COMPLETENESS TEST*
- 6.5. *COLUMN ASSEMBLING TEST*
- 6.6. *COLUMN AND HYDRAULIC SYSTEM PRESSURE TEST*
- 6.7. *COLUMN PACKING TEST*
- 6.8. *VERIFICATION OF THE WIRING DIAGRAM*
- 6.9. *COMPUTER AND SOFTWARE INSTALLATION*
- 6.10. *PUMPS INSTALLATION AND CONTROL TEST*
- 6.11. *DETECTOR INSTALLATION AND FUNCTION CONTROL TEST*
- 6.12. *SOFTWARE VALIDATION*
- 6.13. *SIMULATED SAMPLE INJECTION TEST*
- 6.14. *STAFF TRAINING*

6.1. PERSONNEL WHO HAVE PERFORMED THE SAT

To allow us to identify the people who have completed the Appendices of this protocol, these people must record their name and the name of the company which employs them in the table set out below (in capital letters) and place a specimen of their signatures and initials in the corresponding columns.

NAME	COMPANY FUNCTION	SIGNATURE	VISA (INITIALS)
			■
Petr Havránek	Separlab Technical director		PH
Milos Boril	Separlab R&D Manager		JK
Stanislav Vozka	Separlab Managing Director		SV

6.2. LIST OF EQUIPMENT USED

Each equipment used to SAT tests must be listed in the table below, indicating, if applicable, the data concerning the calibration of this equipment.

Name of the equipment	Model	Serial no.	Calibration Certificate Reference	Visa/Date
Electric voltage and current measuring device	Solid V12		NA	SV/24.06.10
Mechanical manometer	Bourdon type Hansa Flex O 63 0 – 250 bar	130710	calibrated with certificate	SV/24.06.10
Oil manometer	Bourdon type Power team 0 – 700 bar	4130568971	calibrated with certificate	SV/12.07.10
Length measuring rod	metal type		NA	SV/24.06.10
Small distances measuring device	Extol		NA	SV/24.06.10
Camera	Sony DSLR A 350		NA	SV/24.06.10
Liquid reservoirs 100 l volume, divided by each 10 l			NA	SV/25.08.10
Stop clock	Timex Expedition		NA	

6.3. DOCUMENTATION

Test title: Check of the documentation
Purpose: This section verifies the presence and the organisation of the documentation according to the technical standard in force.
Method: Verify that the documentation necessary for the validation, use and maintenance, and listed below, has been completed. Check that this documentation is organised in accordance with current technical standards.
Equipment: Not applicable.

6.3.1 List of documentation

No.	Specification	Number of items
1	Construction and assembly plans od PC 01 column	1 set
2	Construction and assembly plans of electronic unit SEPAREL 01	1 set
3	Certificates and documentation of materials of column and system parts	1 set
5	Electrical-connection diagrams and installation plans	1 set
6	Instruction manual delivered with the system	1 pc
7	Instruction manual for mobile phase pumps	1 pc
8	Instruction manual for UV detector	1 pc
9	Instruction manual for hydraulic system	1 pc
10	Instruction manual for software	1 pc
11	Instruction manual for USB communication unit	1 pc

Criteria: The documents listed hereafter are present and organised according to the technical standards.	Conforms	Checked by ■ SV
	Yes	Date: ■
Results: Passed	Signatures:	

6.4. DELIVERY COMPLETENESS TEST

Test title: Delivery completeness test
Purpose: This section details the tests which will be carried out to ensure that all components of the equipment were manufactured and delivered in accordance with packing list and production documentation
Method: 1 Delivered assembled parts and subsystems were checked according packing list. 2 Parts and subsystem were unpacked and once more checked according production documentation.
Equipment: Not applicable..

Criteria 1. All the components according packing list are presented. 2. All parts according production documentation are presented.	Conforms Yes	Checked by ■ SV Date: ■
Results: Passed	Signatures:	

6.5. COLUMN ASSEMBLING TEST

Test title: COLUMN ASSEMBLING TEST
Purpose: Demonstrate that all parts fit together, the column pistons are assembled well, hydraulic system is working, piston can be pressed to column tube, column stayin well on legs, all screws are free of tension.
Method: Assemble pistons with seals and supports and end fittings. Assemble output flange with legs. Insert the tube to output piston unit and press piston inside tube using piston rods for upper flange. Assemble upper flange with hydraulic cylinder to the column upper flange by four thread rods with distance tubes. Insert next two rods with distance tubes. Insert upper piston unit on the plastic plate and connect it to hydraulic piston. Connect hydraulic pump to the cylinder. Remove plate and push the piston down by hydraulics. Check if piston is coming smoothly to the column tube. Check oil leakage.
Equipment: DSL camera Sony A350

Record:



Assembled piston unit



Column tube transportation



Column tube inserted to the bottom piston



Column tube assembled to the bottom flange



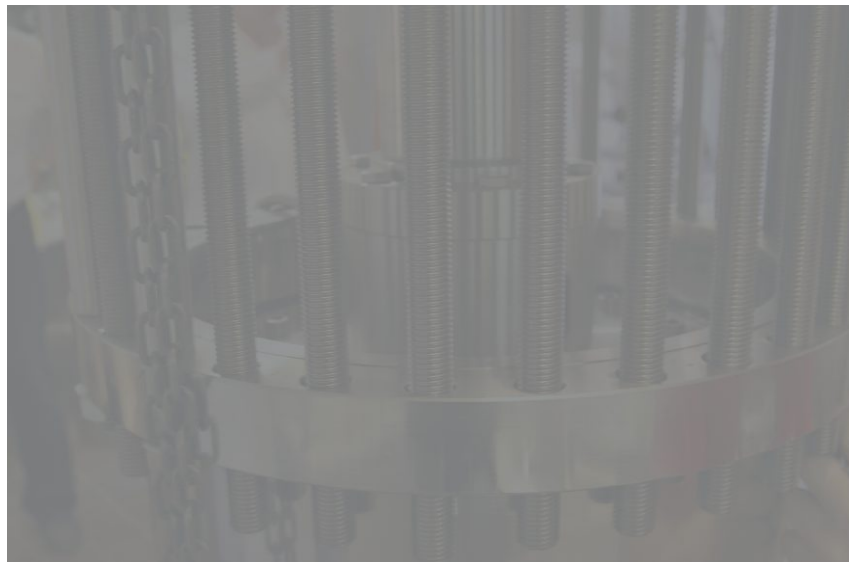
Column bottom part assembled



Column upper flange assembling



Upper piston inserted into column tube



Fully assembled upper column part

Criteria Assembling done without problems. Column in function.	Conforms Yes	Checked by ■ SV Date: ■
Results: Passed	Signatures:	

6.6. COLUMN AND HYDRAULIC SYSTEM: PRESSURE TEST

Test title: COLUMN AND HYDRAULIC SYSTEM: PRESSURE TEST

Purpose:

Demonstrate the column with hydraulic unit is able to work to its maximum pressure without leakage.

Method:

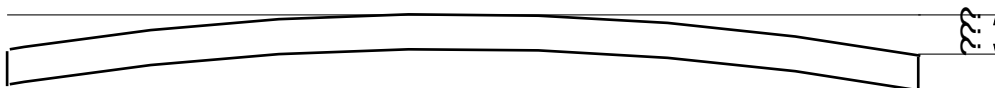
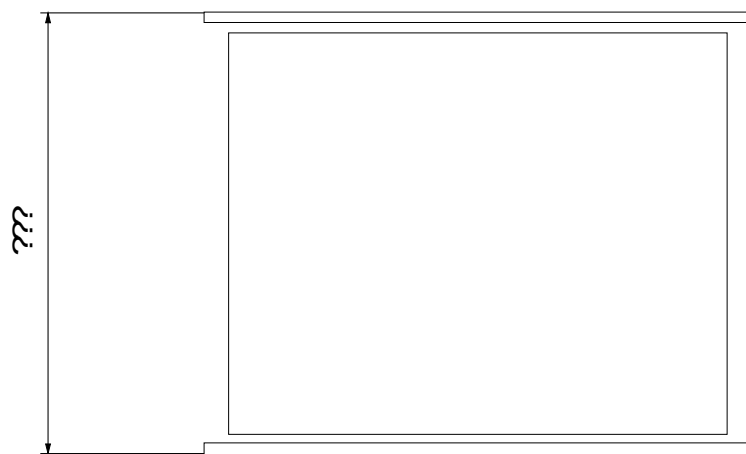
Close output column fitting. Fill full column with demineralized water (70 mm under the edge). Move upper piston inside the column till the time when only water without bubbles is flowing out of the column. Close input fitting. Start pressurize the hydraulic piston to produce 50 bar pressure. Check all possible oil and water leakages.

Measure following distances after each pressure increasing:

- between bottom column and piston flange on six different places equally distanced on the flanges to measure bottom bolts elongation
- measure bottom piston flange deformation according the drawing in two perpendicular directions near to the flange centre
- measure upper piston flange deformation according the drawing in two perpendicular directions near to the flange centre

Check oil and water leakages on all places, mainly on side of piston seals after each pressure increasing

Increase oil pressure in following steps: 100 bar, 200 bar, 300 bar, 400 bar, 470 bar, measure prescribed distances, check the leakage and increase the pressure afters 5 minutes.



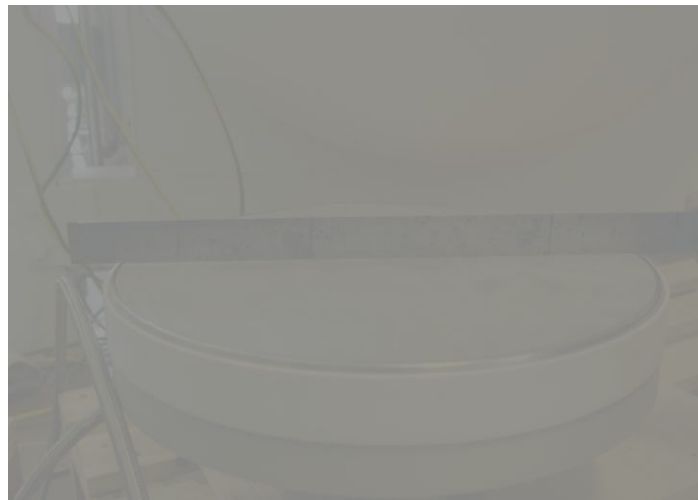
Release the oil pressure in hydraulic circuit then. Wait 120 min. Measure described distances again and compare with prescribed values in followed tables (record page): Deassemble the column and measure deflection of upper piston frit and upper piston support plate.

Equipment:
 Oil manometer
 Length measuring rod
 Small distances measuring device Extol

Record:

Description	100 bar	200 bar	300 bar	400 bar	470 bar	max. value of elongation
bottom column and piston flange, smallest - largest (mm)	■	■	■	■	■	■
bottom piston flange deformation (mm)	no	no	■	■	■	■
upper piston flange deformation (mm)	no	no	no	no	■	■

No leakage of both oil and water was observed.



No frit deformation was observed.



Deformation of piston support was 5 mm. Max allowable deformation is 8 mm. Note: this part is designed to be deformed and increase piston resistance against pressure).

Criteria: No leakage, elongations and deformations OK.	Conforms Yes	Checked by ■ SV Date ■■■■■
Results: Passed	Signatures:	

6.7. COLUMN PACKING TEST

Test title: COLUMN PACKING TEST

Purpose:

Demonstrate the column can be packed with sorbent and prepared for separation

Method:

Assemble the column without upper flange, hydraulics and piston to packing adaptor using proper bolts and sealing tape.

Fill the column with cca 5 lt of prepared sorbent suspension. Check the leakage on output frit. Close output tube.

Fill full unit column – adaptor with sorbent slurry. Check the leakage on the column – adaptor connection. Tighten bolts when necessary.

Wait till next day. Check leakages and sorbent conditions.

Remove remaining liquid from the adaptor.

Remove sorbent when necessary.

Apply vacuum on the output when sorbent is too soft.

Add thick sorbent slurry when necessary.

Assemble upper column part and move piston inside.

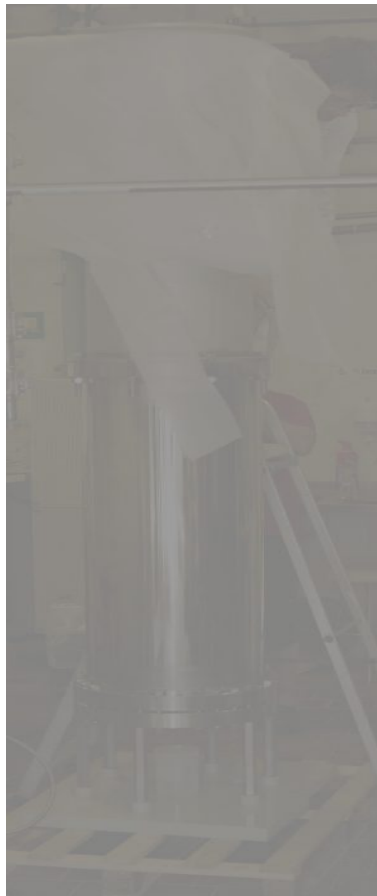
Check the liquid going from upper input on sorbent particles.

Increase oil pressure to 20 bar if possible. Measure piston down movement.

Equipment:



Glass beakers 200 ml.

Record:



Packing – column and adaptor

No leakage on output frit.
 Small leakage on the column – adaptor connection - bolts were tightened than OK.
 Sorbent soft and not enough next day.
 Applied vacuum on the output.
 Added thick sorbent slurry.
 No sorbent particles leakage in input.
 Oil pressure increased to 20 bar. Piston down movement 20 mm.

Criteria Assembling and packing process without problems. No leakage of sorbent through output and input frit No leakage or repairable leakage of liquid and sorbent during packing Possible to reach pressure on oil side when moving piston down.	Conforms Yes	Checked by <div style="text-align: center;">  SV </div> Date: <div style="text-align: center;">  </div>
Results: Passed	Signatures:	

6.8. VERIFICATION OF THE WIRING DIAGRAM

Test title: Verification of the wiring diagram
<p>Purpose: Verify that the supplier has established the protocol of tests and of control with the startup of electrical installation, according to the technical standards. To verify that the electrical connections of the equipment comply with the wiring diagrams, that they are correctly clamped, that the power-supply or connection cables between the different elements of the equipment are correctly labelled, that the different voltages are applied and that the earthing complies.</p>
<p>Method: 1. Cut the main electrical power supply to the equipment. 2. Use a copy of the wiring diagram, mark each connection checked on the diagram, using a felt-tip highlighter pen. Check the entire connections. 3. Check that the different power-supply or connection cables between the different elements of the equipment are correctly labelled according to the technical standards. 4. Verify that the colors of the cables correspond to the technical standards. 5. Re-connect the main electrical power supply to the equipment so as to be able to check the (other) different power supplies of the equipment, using a calibrated multimeter. 6. Check the earthing of the equipment by measuring the resistance between the earthed connection and 3 different points on the equipment, using a calibrated multimeter. 7. Check the cleanliness and the accessibility of the electrical cabinet.</p>
<p>Equipment: Electronic multimeter</p>

Record:

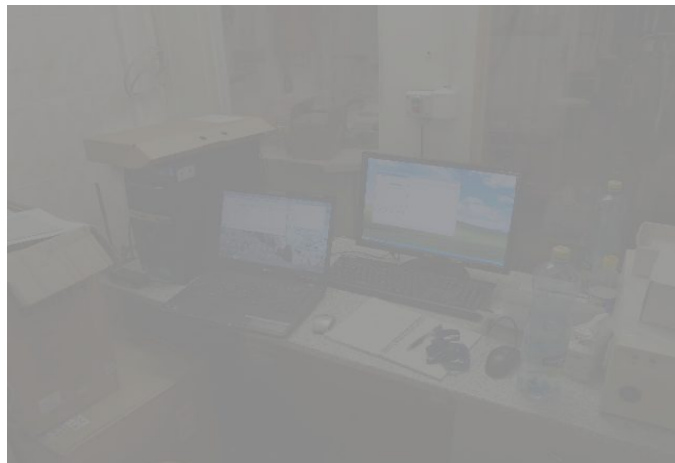
Necessary to set up fuses 25A to the switchboard and 10A before each frequency changer. Done.

<p>Criteria 1. Connections are made according the wiring diagram. 2. Different power-supply or connection cables between the different elements of the equipment are correctly labelled according to the technical standards. 3. The colors of the cables correspond to the technical standards. 4. The earthing of the equipment by measuring the resistance between the earthed connection and 3 different points on the equipment correspond to prescribed standards.</p>	<p>Conforms Yes</p>	<p>Checked by <div style="text-align: center;"> PH </div> Date <div style="text-align: center;"> </div> </p>
<p>Results: <div style="text-align: center; font-weight: bold; font-size: 1.2em;">Passed</div> </p>	<p>Signatures:</p>	

6.9. COMPUTER CONNECTING, SOFTWARE INSTALLATION AND FUNCTION TEST

Test title: COMPUTER CONNECTING, SOFTWARE INSTALLATION AND FUNCTION TEST
Purpose: Demonstrate that a computer designed to control and monitor in the system is in function, all programs are installed and working.
Method: Complete computer set (computer, keyboard, mouse, monitor), start it and install following programs: WINDOWS XP (including a phone registration) [REDACTED] driver for USB – RS232 transducer driver pro graphic cart of computer [REDACTED] microtower driver pro chipset of the computer Check basic [REDACTED] function. Connect a serial line to the electronic unit [REDACTED] a check its function.
Equipment: Any equipment used to the test must be recorded in Appendix 2: List of equipment used.

Record:



Computer installation

Completed computer and installed WINDOWS XP, [REDACTED] driver for USB – RS232 transducer, driver pro graphic cart of computer [REDACTED] microtower and driver pro chipset of the computer. OK and working.
Basic [REDACTED] function OK.
Connected a serial line to the electronic unit [REDACTED]. Function and communication OK.
ECOMAC is able to find pump units.

Criteria: 1. All installed software in function, communication with electronic unit OK.	Conforms Yes	Checked by [REDACTED] MB Date [REDACTED]
Results: Passed	Signature:	

6.10. MOBILE PHASE SYSTEM ASSEMBLING

Test title: MOBILE PHASE SYSTEM ASSEMBLING

Purpose:

Demonstrate the mobile phase system is able to be and reassembled by customer. Demonstrate that pumps can be started from the control box. Demonstrate that the function of oil level switches is OK. Demonstrate that the function of motor termistors is OK.

Method:

Connect the pumps to column splitting unit according general schema by 12 mm O.D. stainless steel tubes.

Deassemble pump motors from pumping heads. Set the flow rate in Ecomac on 0,2 ml/min. Try start and stop both mobile phase delivery pumps and separation solute pump.

Test level switches on all pumps (4 each).

Test pump termistors (switch of – switch on).

Equipment:

Any equipment used to the tests must be recorded in Appendix 2: List of equipment used.

Record:

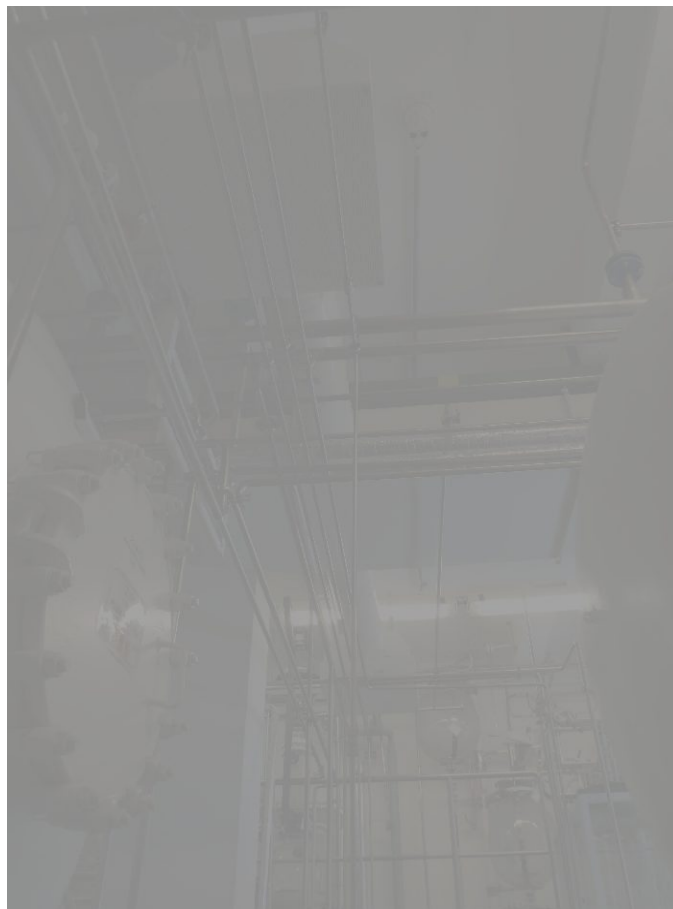
Assembling process



Assembling sample pump connection





Assembling mobile phase pumps connections



Assembling pipeline from mobile phase pumps to column

Connecting the pumps to column splitting unit according general schema by 12 mm O.D. stainless steel tubes - OK.
 Start and stop both mobile phase delivery pumps and separation solute pump -OK.
 Test level switches on all pumps (4 each). Done
 Test pump termistors (switch of – switch on). Done

Criteria Assembling process without problems. Motor start stop function OK Oil level switches function OK	Conforms Yes	Checked by  MB Date: 
Results: Passed	Signatures:	

6.11. DETECTOR AND CELL INSTALLATION AND TEST

Test title: DETECTOR AND CELL INSTALLATION AND TEST
Purpose: Demonstrate the detector is able to monitor absorption of UV light in the cell on the column output.
Method: Connect cell to the column output using DN 10 clamps. Fix cell to the holder. Disassemble UV detector, connect it to net socket, connect it to the computer using proper connector, connect [REDACTED] transducer to the computer and to the control box. Connect the cell to optical cables to the detector in control room. Start [REDACTED] a UV detector. Fill the cell with a mobile phase 1 from the column. Set detector wavelengths to 210, 235, 254 and 280 nm. Start the lamp. Start signal recording (10 min.) , test the function of [REDACTED] marker. Stop recording, save the file. Switch of the detector lamp, switch of the detector. Load the saved file from computer memory to the screen. Switch of all instruments.
Equipment: Not applicable.

Record:

Connected cell. Connected detector, connected [REDACTED] transducer.
Started [REDACTED] a UV detector. [REDACTED] found the detector – OK.
Signal recording (10 min.) OK , test the function of [REDACTED] marker OK.
File saving and loading OK.

Criteria Assembling process without problems. Recording signal without problems. Marker function OK. Files handling OK.	Conforms Yes	Checked by [REDACTED] MB Date: [REDACTED]
Results: Passed	Signatures:	

6.12. SOFTWARE VALIDATION

Test title: SOFTWARE VALIDATION

Purpose:

Demonstrate the software function and do necessary calibrations.

Method:

Complete once more computer set (computer, keyboard, mouse, monitor), start it and install following programs: Reconnect all connectors

- serial line
- detector connector
- Panda connecto

Start WINDOWS XP.

Start [REDACTED] software.

Check if all instruments are visible.

Check basic [REDACTED] function.

Set the flow rate of all pumps in [REDACTED] on 0,2 ml/min.

Check that all mobile phase are connected to pumps.

Reservoir of mobile phase should be filled with mobile phase 1.

Reservoir of mobile phase should be filled with mobile phase 2.

Open bypass valve. Open proper pump valve.

Start mobile phase pumps on the control box in the hall. Stop the flow after 2 min.

Start sample pump on the control box in the hall. Stop the flow after 2 min.

Set flow to 0,5 l/min and start firstly mobiler phase pumps and then sample pump until phases are flowing from bypass valve without bubbles.

Assemble calibrated mechanical manometer to the bypass output.

Open fraction collector valve 1 and start mobile phase pumps and set flow rate to 2 l/min, then 3 l/min, then 4 l/min, then 5 l/min, then 6 l/min, then 7 l/min, then 8 l/min, then 10 l/min,.

Compare pressure on the mechanical manometer with a pressure on electronic unit in computer.

If necessary make the calibration process.

Start mobile phase pumps again and set flow rate to 5 l/min, then 6 l/min, then 10 l/min,.

Measure time necessary to pump 10 l to the calibrated glass container through the column for each flowrate.

Stop mobile phase pumps and start sample pump, use flow rate 3 l/min. Compare the presure and measure the flow rate like described.

Test level switches on all pumps (4 each).

Test pump termistors (switch of – switch on).

Connect UV detector to the computer, Connect the cell to optical cables to the detector.

Check if [REDACTED] found UV detector.

Set detector wavelengths to 210, 235, 254 and 280 nm. Start the lamp.

Start mobile phase pumps with flow 10 l/min.

Start signal recording (10 min.), test the function of PANDA marker.

Stop pump, stop recording, save the file.

Switch of the detector lamp, switch of the detector.

Load the saved file from computer memory to the screen.

Switch of all instruments.

Equipment:

Measuring containers 100 l.

Record:

Software start OK,pumps are visible.

Basic [REDACTED]function OK.

Mobile phase pumps are working.

Sample pump is working.

Pressure calibration table

Flow rate (l/min)	Pressure mechanical (bar)	Pressure electrical (bar)
3	■	■
4	■	■
5	■	■
6	■	■
8	■	■
10	■	■

Calibration of the pressure not necessary.

Flow rate calibration table

Flow rate set (l/min)	Flow rate measured (l/min)
■	■
■	■
■	■
■	■

Flow rate was not calibrated as it was set for flow rate without pressure.

Sample pump gave flow rate 3,2 l/min when 3 l/min was set.

Test level switches on all pumps (4 each) - OK.

Test pump thermistors (switch of – switch on) - OK.

■ found UV detector - OK.

Signal recording (10 min.) OK, test of ■ marker OK.

<p>Criteria All operation without problem. Displayed value (pressure) OK. Se value (flow) OK Recording signal without problems. Marker function OK. Files handling OK.</p>	<p>Conforms Yes</p>	<p>Checked by ■ SV MB Date: ■</p>
<p>Results: Passed</p>	<p>Signatures:</p>	

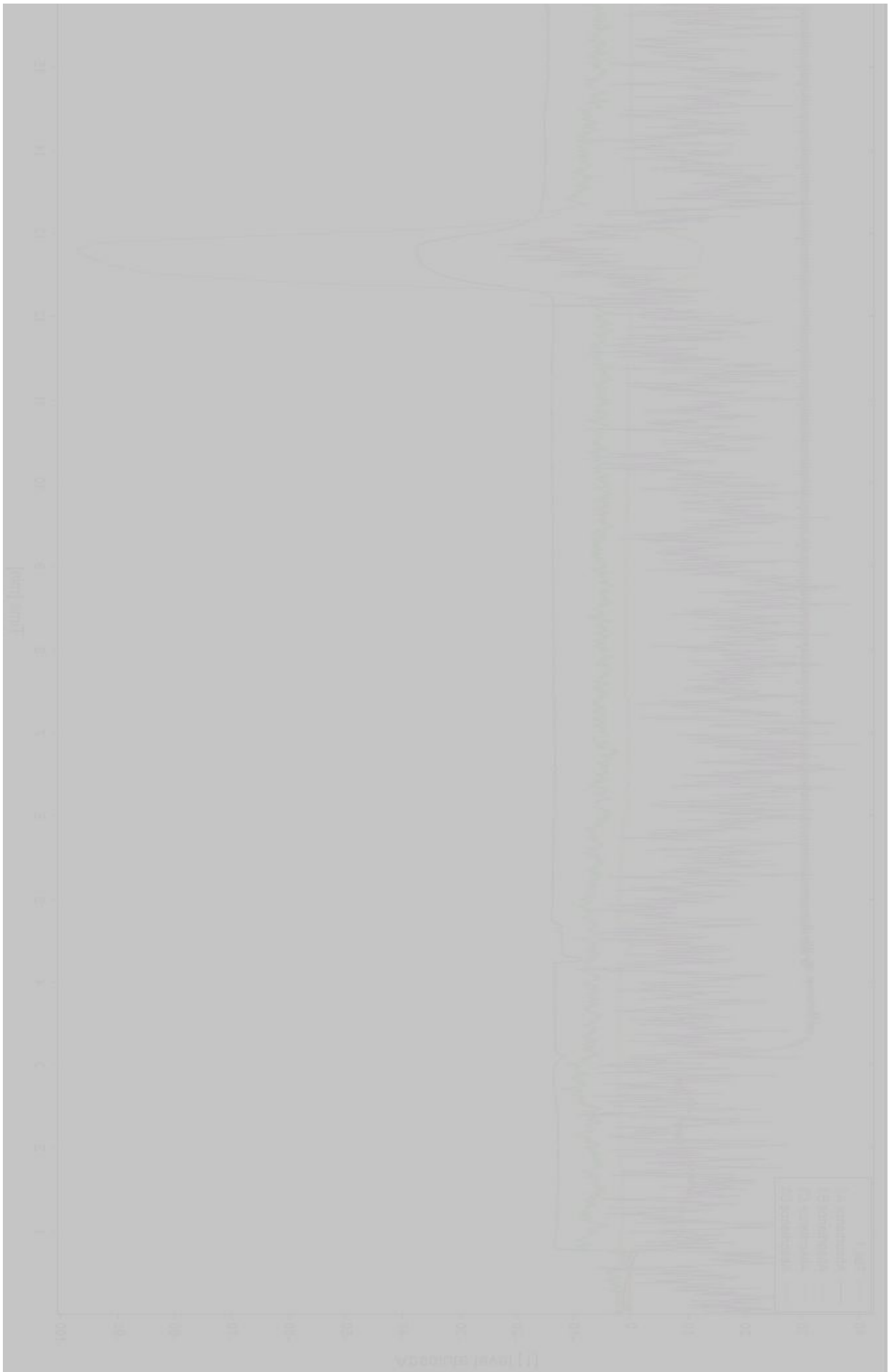
6.13. SIMULATED INJECTION

Test title: SIMULATED INJECTION
Purpose: Demonstrate the full function of the system.
Method: Start the system, start [REDACTED] software, check if all instruments are visible. Reservoir of mobile phase should be filled with mobile phase 1. Reservoir of mobile phase should be filled with mobile phase 2. Set flow of mobile phase to 10 l/min and sample pum flow to 3 l/min. Set oil pressure of the column to 190 bar. Open fraction collector valve 1. Set detector wavelengths to 210, 235, 254 and 280 nm. Start the lamp. Start recording. Start mobile phase pumps for 5 min, then stop. Start sample pump, use flow rate 3 l/min for 2 minutes, then stop. Start mobile phase pumps again. Stop pumps when sample peak is out of the column (15 minutes). Stop recording, save the file. Switch of all instruments.
Equipment: Not aplicable.

Record:

Efficiency of the column correspond with theoretical value. No tailing was observed.

Criteria All operation without problem. Peak without big tailing. Efficiency common for prep. column	Conforms Yes	Checked by [REDACTED] SV Date: [REDACTED]
Results: Passed	Signatures:	



LIST OF INCLINATIONS

6.1. PERSONNEL WHO HAVE PERFORMED THE SAT	NO
6.2. LIST OF EQUIPMENT USED	NO
6.3. DOCUMENTATION	NO
6.4. DELIVERY COMPLETENESS TEST	NO
6.5. COLUMN ASSEMBLING TEST	NO
6.6. COLUMN AND HYDRAULIC SYSTEM PRESSURE TEST	NO
6.7. COLUMN PACKING TEST	NO
6.8. VERIFICATION OF THE WIRING DIAGRAM	NO
6.9. COMPUTER AND SOFTWARE INSTALLATION	NO
6.10. PUMPS INSTALLATION AND CONTROL TEST	NO
6.11. DETECTOR INSTALLATION AND FUNCTION CONTROL TEST	NO
6.12. SOFTWARE VALIDATION	NO
6.13. SIMULATED SAMPLE INJECTION TEST	NO
6.14. STAFF TRAINING	NO