

„Choroby genetycznie uwarunkowane – edukacja i diagnostyka”

Przygotowanie spektrometru mas do analizy izoform transferyny

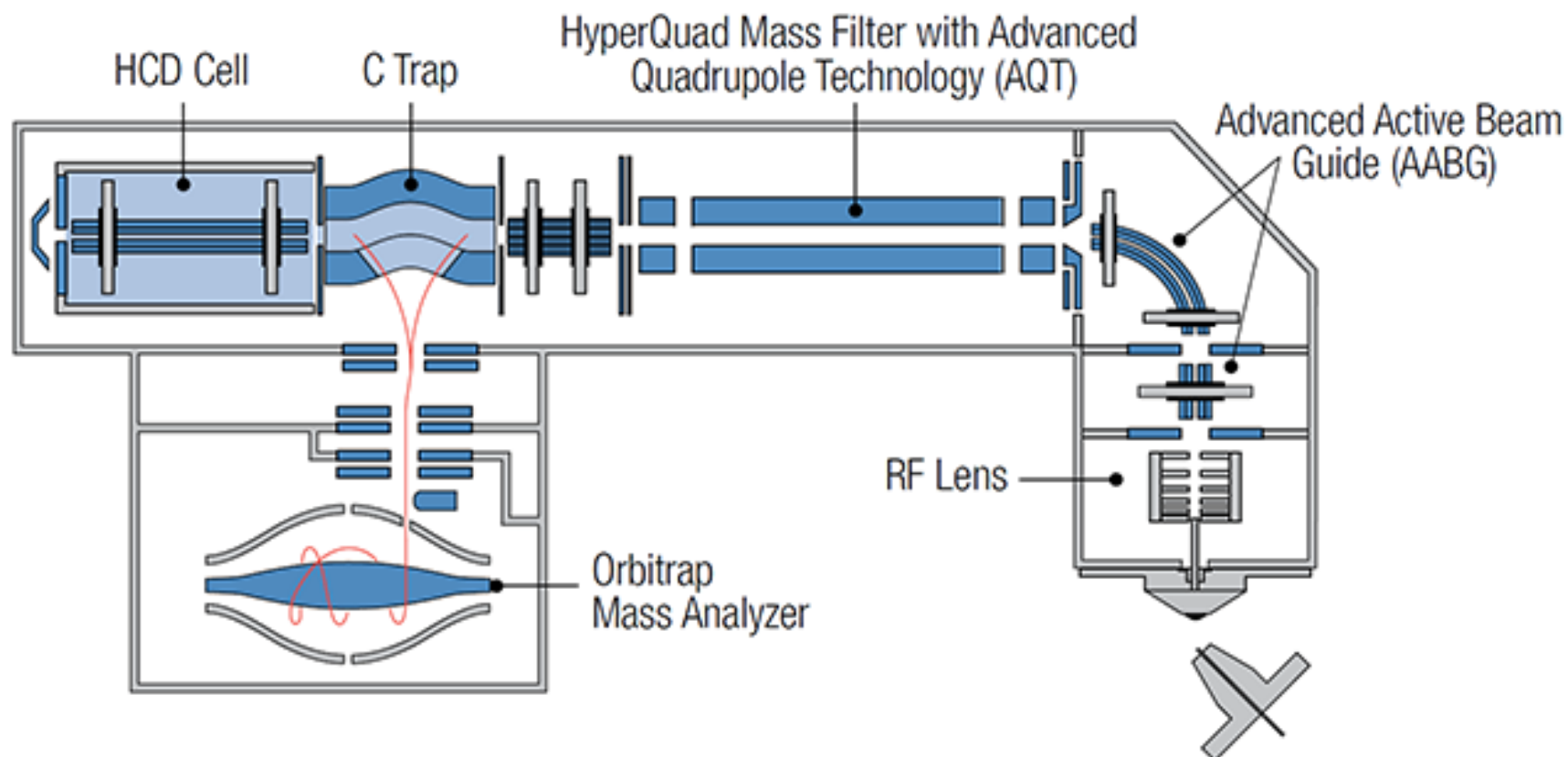
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Warszawa 29 kwietnia 2021 r

Budowa spektrometru



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Przygotowanie linii przesyłowej



Przygotowanie MS do kalibracji



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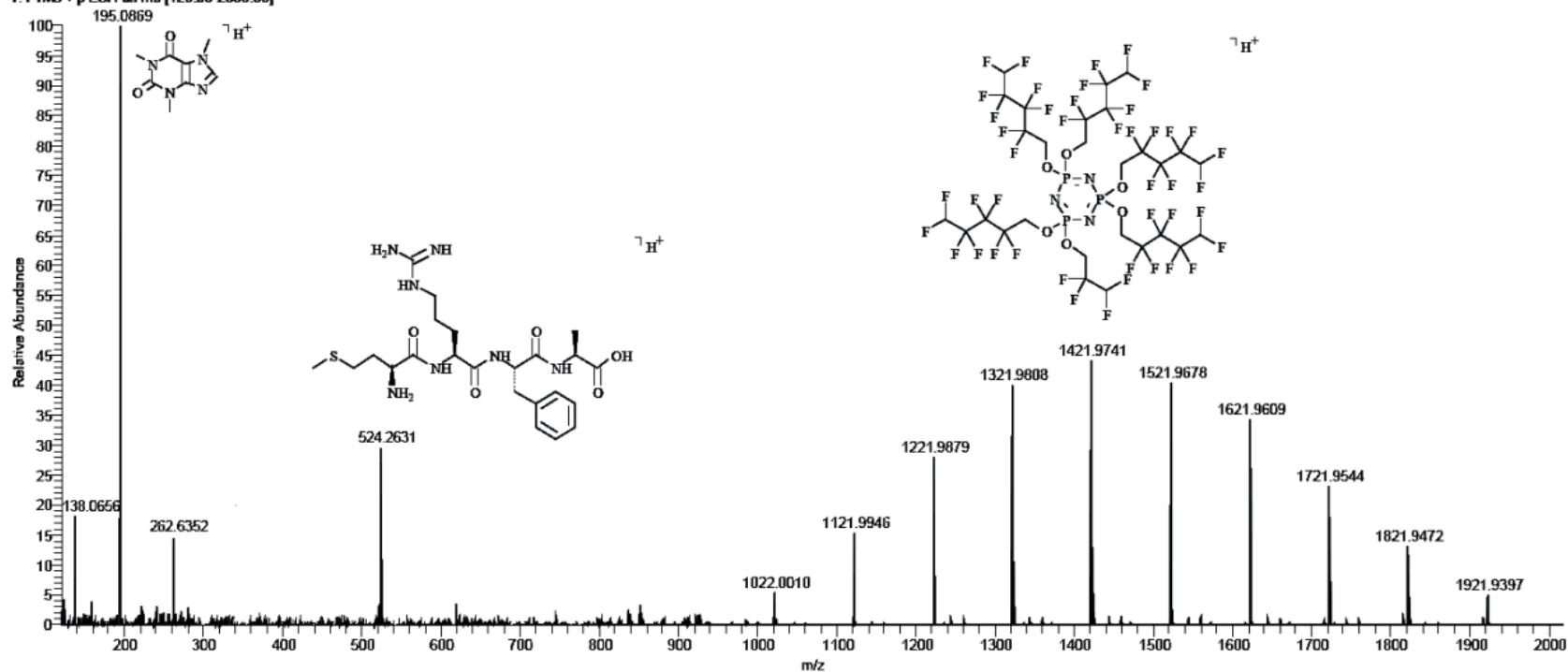
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Kalibracja spektrometru

TFS200_16042501_ORBI_TL180_mz120-2000 #1-100 RT: 0.01-0.79 AV: 100 NL: 1.45E7
T: FTMS + p ESI Full ms [120.00-2000.00]



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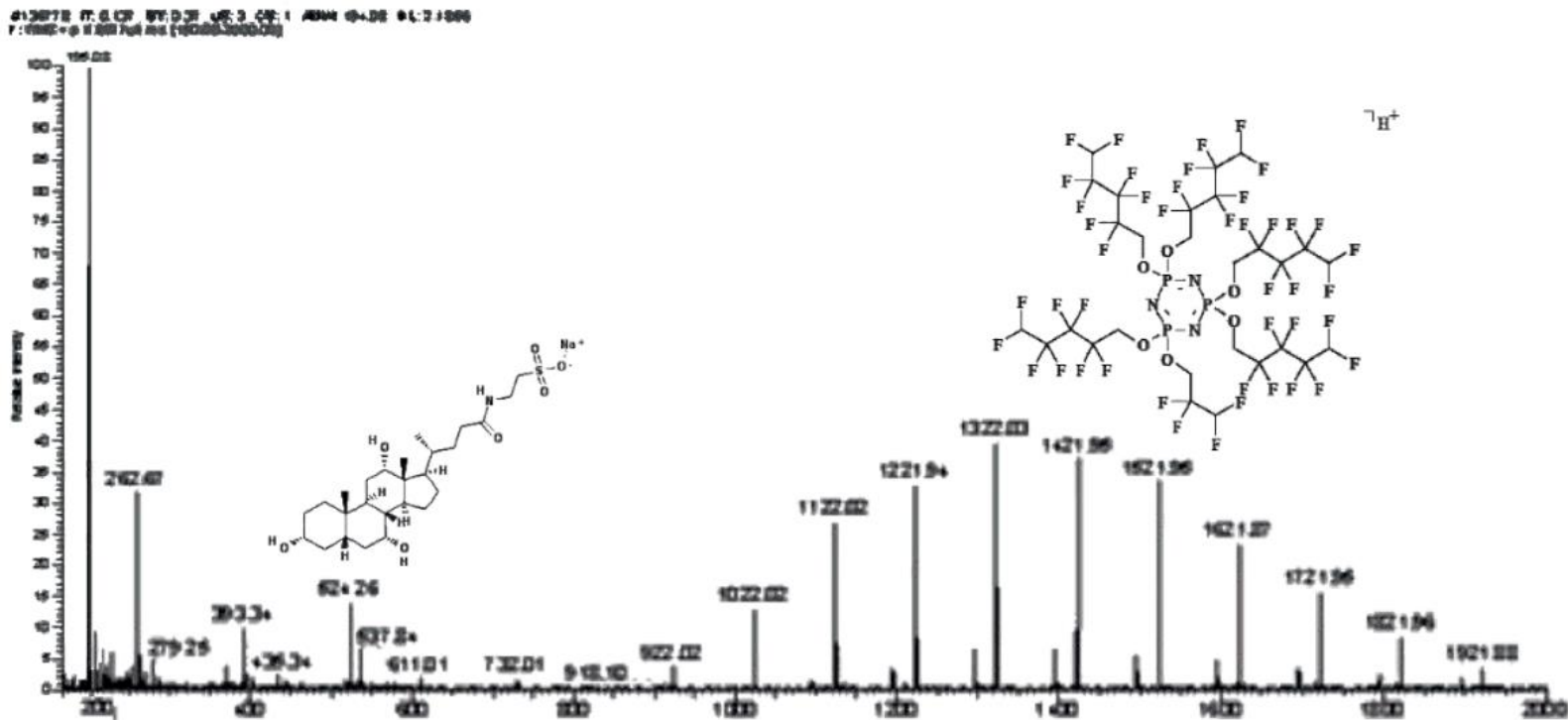


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Kalibracja spektrometru



Kalibracja spektrometru



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Kalibracja spektrometru

Thermo Q Exactive HF — HESI_Installation — operational

File Windows Reports Help

Instrument Control

Scan parameters

History

Scan type: Full MS

Scan range: 150.0 to 2,000.0 m/z

Fragmentation: None

Resolution: 30,000

Polarity: Positive

Microscans: 1

Lock masses: Off

AGC target: 1e6

Maximum inject time: 50

Apply Help ☐ Hot link

HESI source

actual

Sheath gas flow rate: 7 3

Aux gas flow rate: 2 3

Sweep gas flow rate: 0 0

Spray voltage (kV): 4.00 4.02

Spray current (μA): 0.10

Capillary temp. (°C): 320 320

S-lens RF level: 50.0

Aux gas heater temp (°C): 0 31

Source Auto-Defaults...

Apply Help ☐ Hot link

Acquisition

Acquisition state: ready

Progress: 0.00 min

File in use: unknown

Destination file: C:\Xcalibur\data\ESI bez przeplywu 4000V zerowy przeplyw gazu

Method file: by time

Acquisition time: continuously

Mass Traces

Calibrate

Evaluate

Vacuum / Bakeout

Spectrum

Scan: #165806 μS: 1 IT: 50.0 NL: 1.26E4

Type: FTMS + p ESI Full ms [150.0000-2000.0000]

Relative Abundance (%)

m/z

692.016

303.240

410.800

394.419

550.600

914.107

1010.727

1725.483

1834.578

Instrument Status

Instrument

Current Scan

Total Ion Current: 0.04 E6 ions/sec

TIC Variation: 70%

Inject time: 50.00 ms

AGC Target reached: 1%

AGC Prescan Mode: -1

Scan Rate: 0.9 scans/sec

Lock masses

Control

System

Analysis Graph

Performance: Ok

Electronics

ESI Ion Source

Peripheral Devices

Vacuum System

Current Scan

Messages

Priority Source Time Description

13:48:41.398 Calibration failed. Not saving calibration results.

13:48:42.035 Document: C:\Xcalibur\system\Exactive\Instrument\reports\4C52621F8063_Calibration_2021-09-17_13-48-35.pdf generated.

13:49:16.694 Informing instrument about the current data system user FT3NNR2(Thermo)

13:49:16.695 Starting procedure Calibrate()

13:49:16.773 == Running Calibration... ==

13:49:16.776 'Iso Mass/Res. Cal. (neg)(wide iso)' running ...

13:49:21.716 'Iso Mass/Res. Cal. (neg)(wide iso): Signal to noise ratio for 1679.972 too low: S/N 0.0

13:49:21.916 Calibration failed. Not saving calibration results.

13:49:22.564 Document: C:\Xcalibur\system\Exactive\Instrument\reports\4C52621F8063_Calibration_2021-09-17_13-49-15.pdf generated.

13:49:50.790 Informing instrument about the current data system user FT3NNR2(Thermo)

13:49:50.791 Starting procedure ScanHCD(1679)

13:49:50.837 'Scan HCD energy' running ...

13:49:57.534 Procedure interrupted by user.

13:49:57.547 Procedure interrupted by user.

13:50:16.944 [Inspect/PreviewProc] DetectPeaksOnRange timeout, mass range too narrow or ADC clipping?

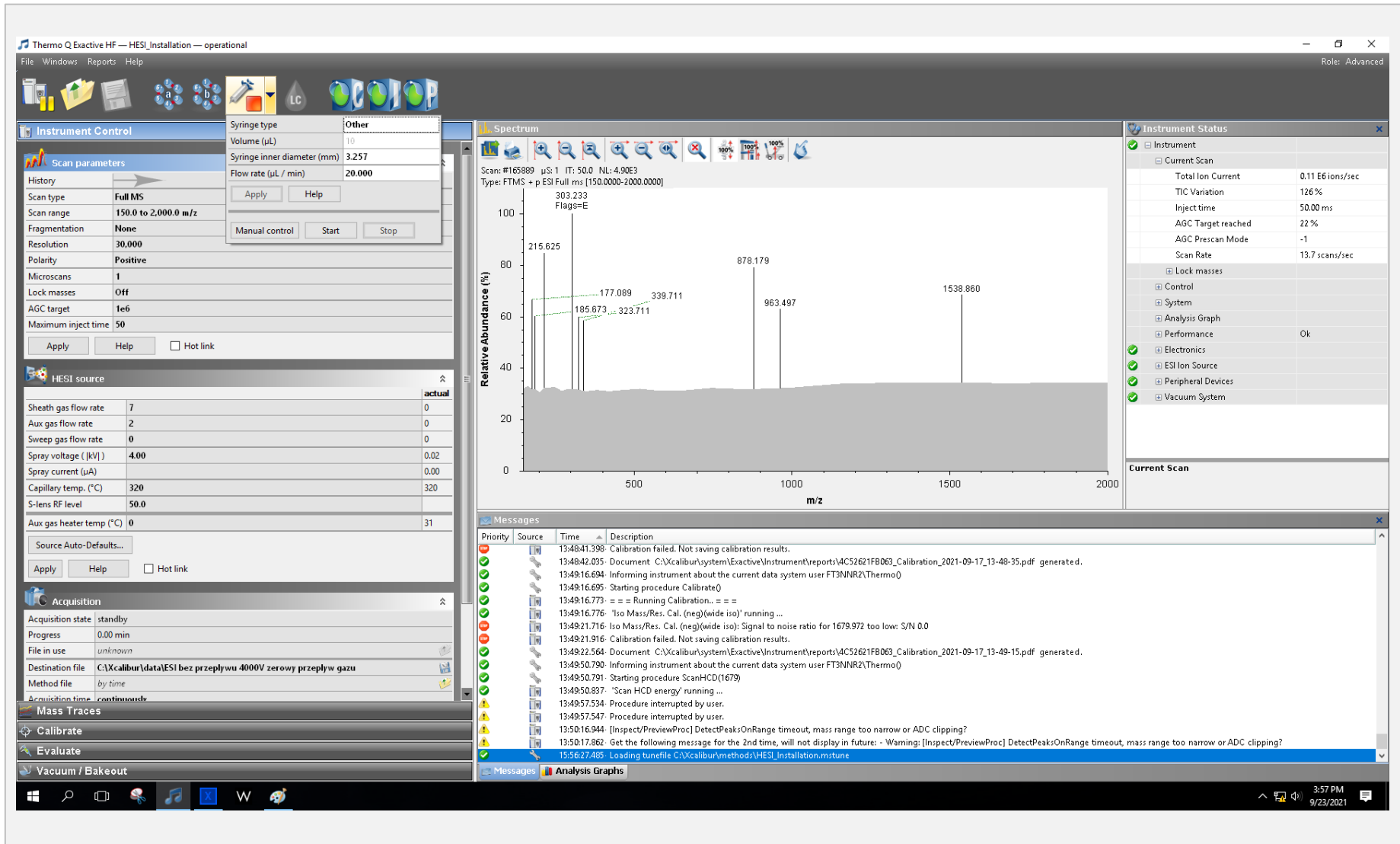
13:50:17.862 Get the following message for the 2nd time, will not display in future: - Warning: [Inspect/PreviewProc] DetectPeaksOnRange timeout, mass range too narrow or ADC clipping?

15:56:27.485 Loading tune file C:\Xcalibur\method\HESI_Installation.ms.tune

Messages Analysis Graphs

3:56 PM 9/23/2021

Kalibracja spektrometru



Ustawienie nano źródła

Thermo Q Exactive Plus — nsi_intact — Peripheral Devices - Syringe pump - stalled

File Windows Reports Help Role: Advanced

Instrument Control

Scan parameters

History

Scan type	Full MS
Scan range	400.0 to 6,000.0 m/z
Fragmentation	None
Resolution	35,000
Polarity	Positive
Microscans	1
Lock masses	Off
AGC target	1e6
Maximum inject time	200

Apply Help ☐ Hot link

NSI source

	actual
Sheath gas flow rate	0
Aux gas flow rate	0
Sweep gas flow rate	0
Spray voltage (kV)	1.90
Spray current (uA)	0.50
Capillary temp. (°C)	346
S-lens RF level	80.0

Source Auto-Defaults...

Apply Help ☐ Hot link

Acquisition

Acquisition state ready

Progress 0.00 min

File in use D:\Data\testy\Reserpina_10.raw

Destination file C:\Xcalibur\data\widmo 250-2500 4000V

Method file by time

Acquisition time continuously

Mass Traces

Calibrate

Evaluate

Vacuum / Bakeout

Spectrum

Scan: #1947685 pS: 1 IT: 19.8 NL: 9.20E6
Type: FTMS + p NSI Full ms [400.0000-6000.0000]

Relative Abundance (%)

m/z

Instrument Status

Instrument

Current Scan

Total Ion Current	25.70 E6 ions/sec
TIC Variation	11 %
Inject time	19.08 ms
AGC Target reached	100 %
AGC Prescan Mode	-1
Scan Rate	7.4 scans/sec

Peripheral...

Performance Status

Warn: Mass...

Mass Calibration...

Base Calibration

Isolation Mass and Res...

Isolation Mass and Res...

Mass Calibration (pos) Spectral Mass...

Spectral Mass... Overdue (2021-09-...

Mass Calibration (neg) Spectral Mass...

Current Scan

Messages

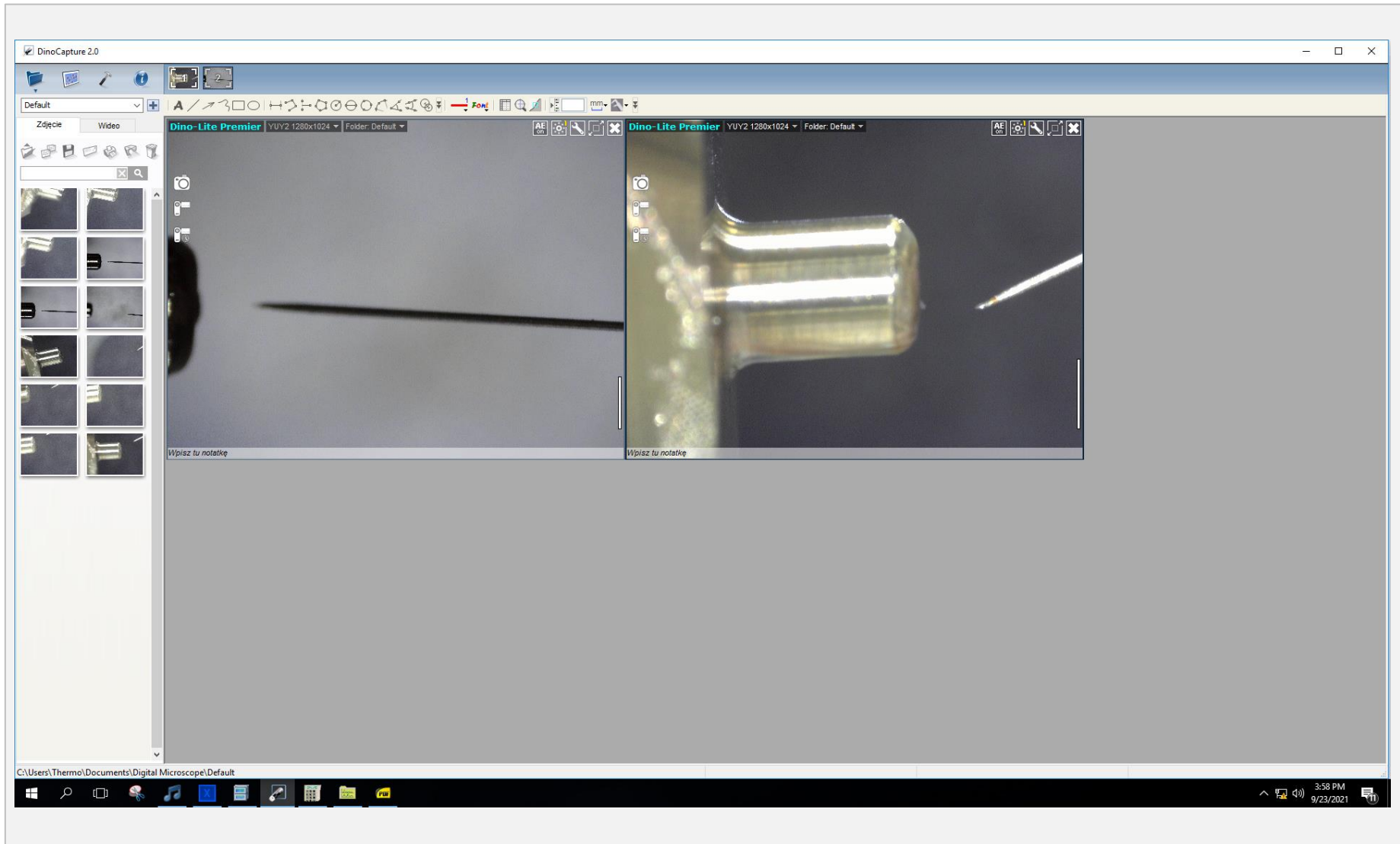
Priority	Source	Time	Description
✓		12:35:55.163	Starting acquisition
✓		12:50:36.746	MS acquisition end. Accumulated MS size: 7381KB
✓		12:50:37.163	Informing instrument about a stopped device of type 3
✓		13:01:49.659	Preparing next acquisition with: <?xml version="1.0" encoding="iso-8859-15" standalone="yes"?> <InstrumentD ... Name>D:\Data\testy\Reserpina_9.raw</RawFileName> <MethodFileName>...
✓		13:01:51.163	Starting acquisition
✓		13:16:34.506	MS acquisition end. Accumulated MS size: 1335KB
✓		13:16:35.172	Informing instrument about a stopped device of type 3
✓		13:16:37.156	Informing instrument about a stopped device of type 0
✓		13:47:40.210	Saving current tune file
✓		13:48:29.874	Preparing next acquisition with: <?xml version="1.0" encoding="iso-8859-15" standalone="yes"?> <InstrumentD ... ame>D:\Data\testy\Reserpina_10.raw</RawFileName> <MethodFileName>...
✓		13:48:31.257	Starting acquisition
✓		14:02:53.014	MS acquisition end. Accumulated MS size: 2888KB
✓		14:02:53.280	Informing instrument about a stopped device of type 3
✓		14:02:55.271	Informing instrument about a stopped device of type 0
✓		15:45:56.357	Loading tune file C:\Xcalibur\methods\HESI_Installation.ms.tune
✓		15:46:16.119	Loading tune file C:\Xcalibur\methods\nsi_intact.ms.tune

3:46 PM 9/23/2021

Ustawienie nano źródła



Ustawienie nano źródła



Ustawienie nano źródła



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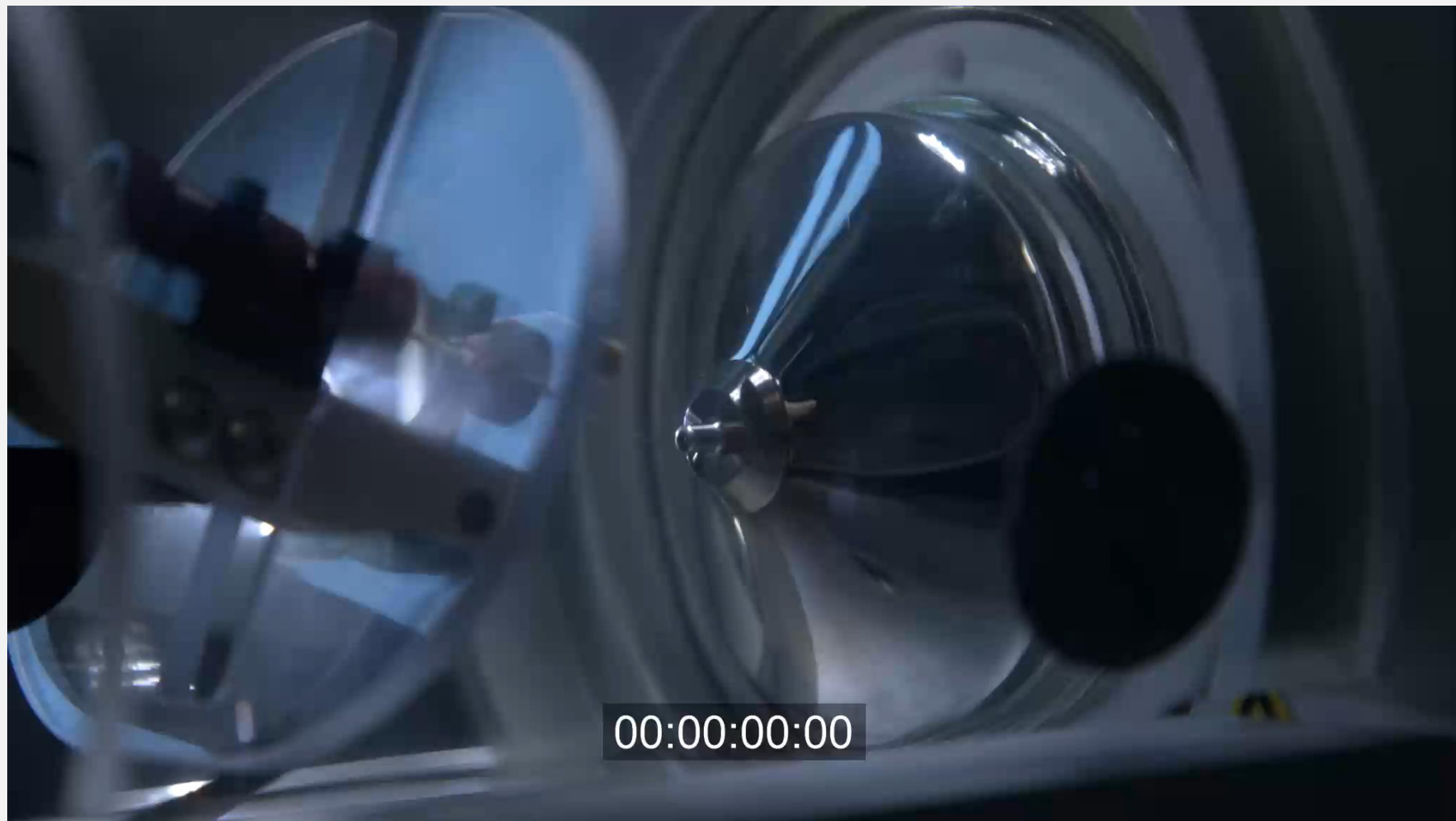


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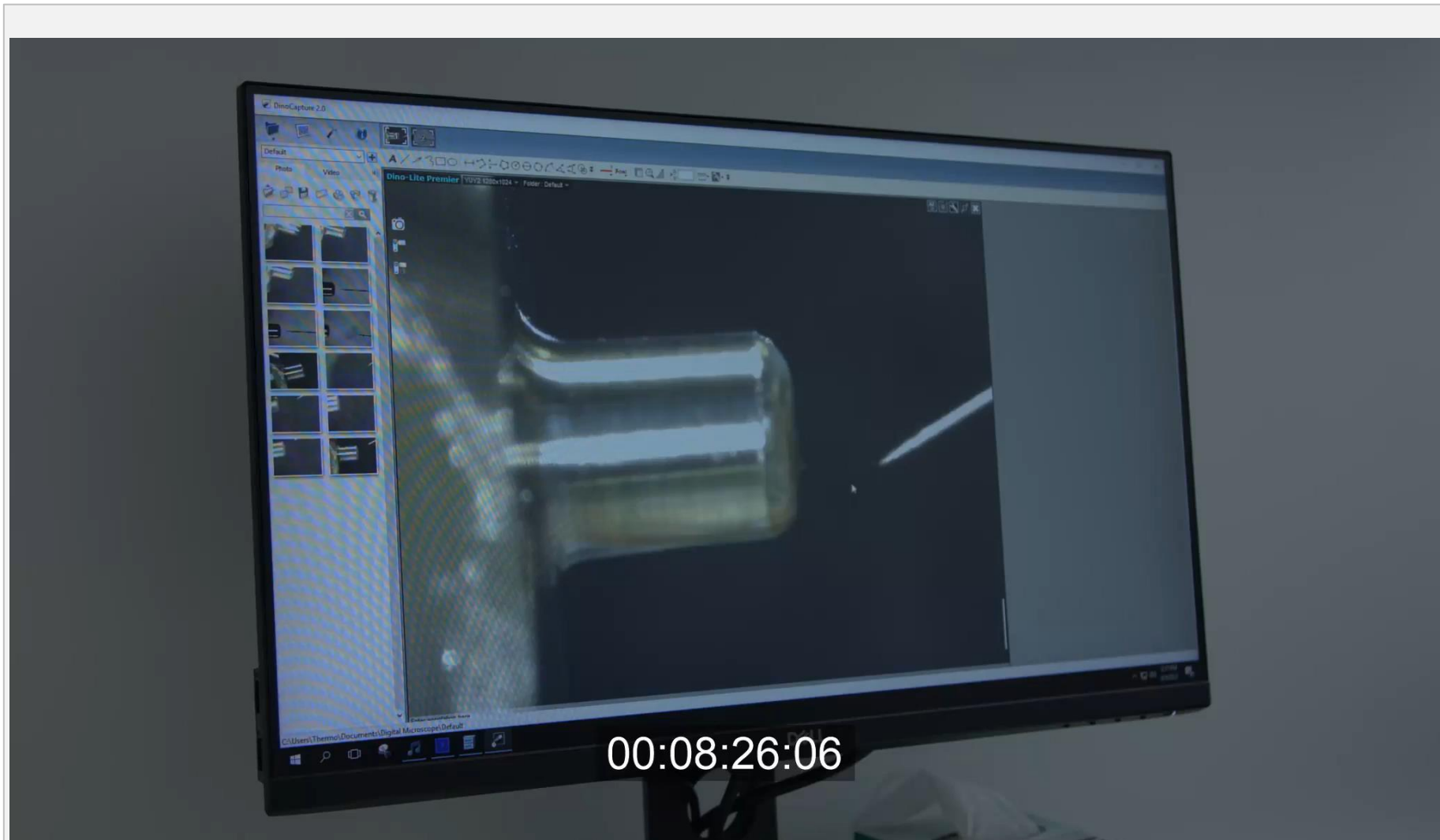
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Ustawienie nano źródła



Ustawienie nano źródła



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Sprawdzenie nanoLC

Direct Control

Release Control Consumables < Autogenerated >

Home PumpModule NCS-3500RS Sampler Audit

Module Status

Connected

Retention Time: Connected

Module Connect

Connect

More Options...

Wellness...

Service...

Qualification...

Relays/Inputs...

Commands

Hold

Stop Flow

Continue

NC_Pump

More Options...

Wellness...

Service...

☒ Motor: On

☐ Purge: Off

Flowmeter

Pressure / Flow

Limits

900 600 400 200 0

1.5 0.0

5.31 [bar] 0.600 [μL/min]

Flow values

A: 300.0 [nL/min] B: 299.7 [nL/min] Total: 599.7 [nL/min]

Settings **Active Display**

% A 100 50 0

% B 100 50 0

50.0% 50.00 [%]

LoadingPump

More Options...

Wellness...

Service...

☒ Motor: On

☐ Purge: Off

Pressure / Flow

Limits

620 400 200 0

2500 1000 0

69 [bar] 12.000 [μL/min]

Settings **Active Display**

% A 100 80 60 40 20 0

% B 100 80 60 40 20 0

% C 100 80 60 40 20 0

100.0% 0.00 [%] 0.00 [%]

Column Oven

Column Details

Nominal Temperature: 35.0 [°C]

Actual Value: 35.0 [°C]

More Options...

Wellness...

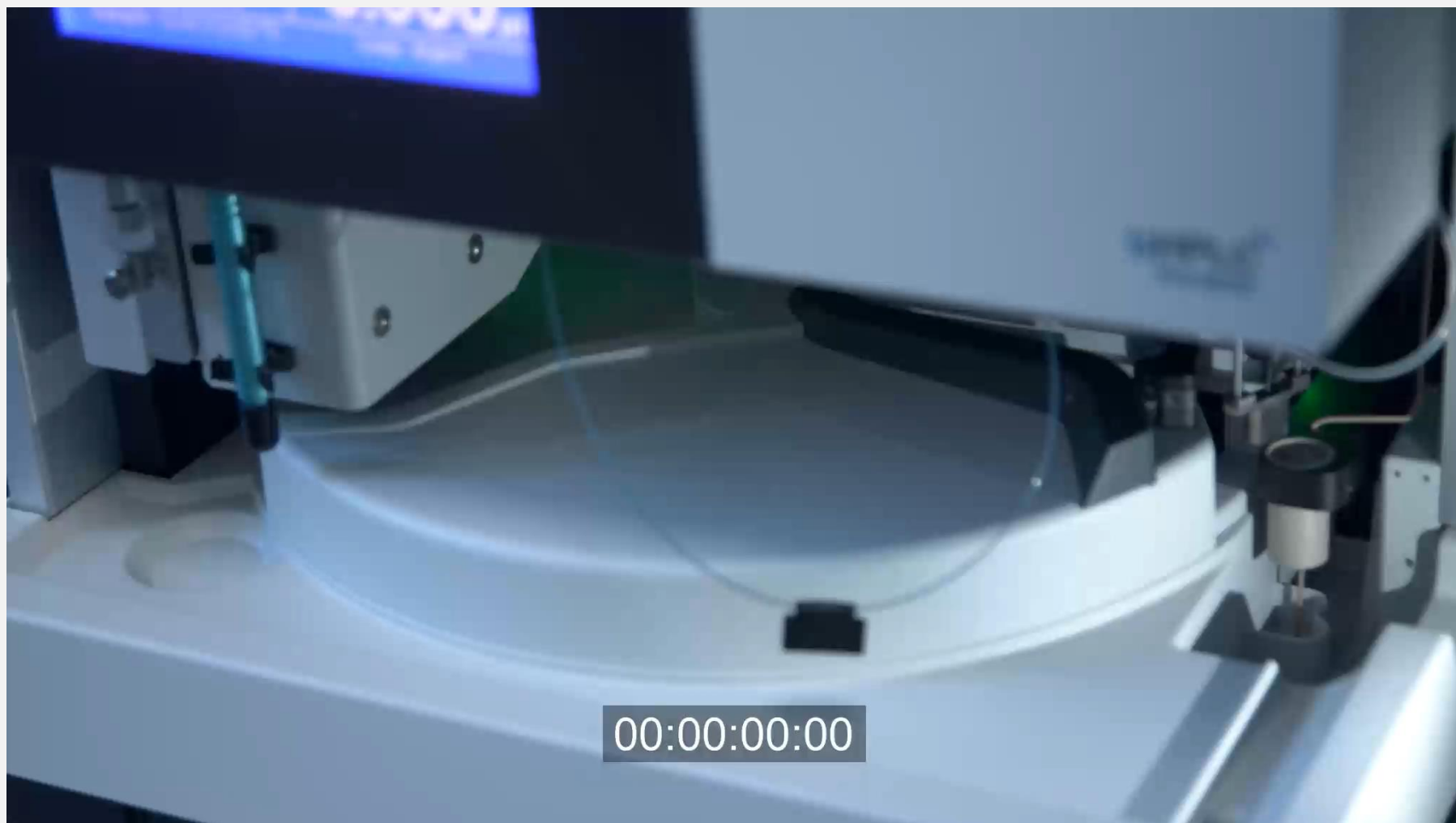
Right Valve

1,2

	Date	Time	Retention Time	Device	Message
1	9/23/2021	3:47:42 PM +02:00		PumpModule NC_Pump	PumpModule NC_Pump %B Value = 50.0 %
2	9/23/2021	3:47:32 PM +02:00		Sampler	WPS-3000 @ USB-08170147 - WPS-3000 - Serial # 8170147 - Firmware Version 4.14.00
3	9/23/2021	3:47:31 PM +02:00		PumpModule	Connection established successfully.
4	9/23/2021	3:47:31 PM +02:00		PumpModule	Connected to NCS-3500RS @ USB-8170776 - Serial # 8170776 - Firmware Version 1.44
5	9/23/2021	3:47:30 PM +02:00		PumpModule	Trying to connect ...
6	9/23/2021	3:47:30 PM +02:00		PumpModule	PumpModule Connected = Connected
7	9/23/2021	3:47:29 PM +02:00			User FRVMMNR2(Thermo) has acquired control over instrument System_1.
8	9/22/2021	9:00:38 PM +02:00		Sampler	Error writing to WPS-3000 @ USB-08170147. Device is not connected.
9	9/22/2021	9:00:37 PM +02:00		PumpModule	Driver has disconnected.
10	9/22/2021	9:00:37 PM +02:00		PumpModule	Error reading from PUMP-3000RS @ USB-8170776. The I/O operation has been aborted because of either a thread exit or an application request.
11	9/22/2021	9:00:37 PM +02:00		Sampler	Error reading from WPS-3000 @ USB-08170147. The I/O operation has been aborted because of either a thread exit or an application request.

Windows taskbar: 3:49 PM 9/23/2021

Umieszczenie próbek w autosamplerze



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