

ZENCO BETA

USER MANUAL



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FCC Declaration of Conformance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions:

1. This device may not cause harmful interference
2. This device must accept any interference received, including interference that may cause undesired operation. Changes or modification not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

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1 DEFINITIONS AND WRITING CONVENTIONS

This manual contains warnings and precautionary statements to help prevent personal injury and/or damage to the Zenco system when properly followed. Safety and special notices, including the symbols described in [Table 1](#), are presented in boxes throughout the guide.

Table 1: Safety and Hazard Symbols




	This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations.
	WARNING: Indicates a hazardous situation that could result in death or serious injury if not avoided. Do not proceed until all specified conditions are fully understood and met.
	CAUTION: Indicates a hazardous situation that could result in minor or moderate injury if not avoided. Ensure all specified conditions are fully understood and met before proceeding.

Table 2: Terminology Used in this Manual

Acronyms relating to the components of the Zenco unit can be found in [Table 4](#).

TERM	DEFINITION
Chromatography	A method used primarily for separation of the components of a sample. The components are distributed between two phases; one is stationary while the other is mobile. The stationary phase is either a solid, a solid-supported liquid, or a gel/resin. The stationary phase may be packed in a column, spread as a layer or distributed as a film. The mobile phase may be gaseous or liquid.
Column Volume	The total volume between the upper and lower frits. Column Volumes can be found in their respective qEV user manual.
Method	The programmable workflow used to run a qEV column.

2 SAFETY AND HAZARDS

2.1 Safe Use Requirements and Specifications



Users must thoroughly review the complete User Manual before assembling, setting up, or operating the Zenco system, and keep it readily accessible during operation. Operate the system strictly as outlined in the documentation to avoid potential hazards that could result in personal injury or equipment damage. Adhere to the safe use requirements specified in Table 3. Using the equipment in an unspecified manner may compromise the protection it provides.

Table 3: Safe Use Requirements and Specifications

SAFE USE REQUIREMENT		SPECIFICATION
Operating Temperature	Indoor Use	4-40 °C
Humidity		0-90%
Flow Rate		0-100 mL/min
Maximum Pressure		10 bar Maximum Pressure with Back Pressure Valve (BPV): 5 bar
Sample/Buffer Viscosity		0.35 - 5 cP
Ingress Protection		IP51
Pollution Degree Rating		2
Power Requirements		100-240 V AC 50/60 Hz Power Consumption: 250 W
Inlet	Inlet Tubing Quantity	8
	Inlet Tubing Diameter	Outer Diameter: 3.175 mm Inner Diameter: 1.59 mm
	Pressure Range	0 ~ 5 MPa
	Pump Flow	100 mL/min
Outlet	Outlet Tubing Quantity	8
	Outlet Tubing Diameter	Outer Diameter: 1.6 mm Inner Diameter: 0.75 mm
	Pressure Range	0 ~ 5 MPa

Do not attempt to run the qEV Zenco outside of these conditions.

Liquid will be in contact with the following materials as it flows through the Zenco instrument. Please ensure that the materials are compatible with your application.

- ▶ PEEK: Polyetheretherketone
- ▶ PTFE: Polytetrafluoroethylene
- ▶ PSS: Polyphenylene Sulfide
- ▶ POM: Polyoxymethylene
- ▶ TA2: Titanium (commercially pure grade 2)
- ▶ PCTFE: Polychlorotrifluoroethylene
- ▶ FFKM: Perfluoroelastomer

- ▶ EPDM: Ethylene-propylene diene monomer
- ▶ Stainless steel grade 316L
- ▶ Glass

2.2 Hazards

The qEV Zenco is a laboratory product, however if any biohazardous samples are present then adhere to current Good Laboratory Practices (cGLPs) and comply with any local guidelines specific to your laboratory and location.



The Zenco unit itself does not contain any potentially hazardous chemical materials. However, the Zenco is used alongside qEV columns, which are delivered containing < 0.1% ProClin 200 or < 0.1% sodium azide*, both of which are harmful if swallowed or in contact with skin. When the appropriate qEV column is used with the Zenco unit, the user is potentially exposed to a dilute solution of sodium azide or ProClin 200* from the column and/or waste hose outlet. When using 0.5 M NaOH for cleaning the Zenco unit, follow cGLPs and wear appropriate Personal Protective Equipment (PPE) when handling these substances.

*Izon is transitioning from the use of sodium azide to ProClin 200 for the storage of qEV columns. For information on how to identify which storage buffer is in your column, visit support.izon.com

Please review the following guidelines and precautions prior to each use of qEV columns, especially if flushing with an antimicrobial buffer before storage (recommended).

Prevention

- ▶ Do not get into eyes, on skin, or on clothing.
- ▶ Wash skin thoroughly after handling.
- ▶ Do not eat, drink, or smoke when using this product.
- ▶ Wear protective gloves and clothing; follow general laboratory precautions.

Response

- ▶ IF SWALLOWED: immediately call a POISON CONTROL CENTRE/Doctor.
- ▶ IF ON SKIN: gently wash with plenty of water.
- ▶ Remove immediately any contaminated clothing and wash before reuse.
- ▶ Collect any spillage and dispose of appropriately.

For more information, see the SDS Documentation for qEV columns: support.izon.com/qev-columns-safety-data-sheet-sds

Disposal of Biohazardous Material

Be sure to adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location regarding use and disposal.

General Precautions

- ▶ Always wear laboratory gloves, coats, and safety glasses with side shields or goggles.
- ▶ Keep your hands away from your mouth, nose, and eyes.
- ▶ Completely protect any cut or abrasion before working with potentially infectious or hazardous material.
- ▶ Wash your hands thoroughly with soap and water after working with any potentially infectious or hazardous material before leaving the laboratory.
- ▶ Remove watches and jewellery before working at the bench.
- ▶ The use of contact lenses is not recommended due to complications that may arise during emergency eye-wash procedures.
- ▶ Before leaving the laboratory, remove protective clothing.
- ▶ Do not use a gloved hand to write, answer the telephone, turn on a light switch, or physically engage with people who are not wearing gloves.
- ▶ Change gloves frequently.
- ▶ Remove gloves immediately when they are visibly contaminated.
- ▶ Do not expose materials that cannot be properly decontaminated to potentially infectious or hazardous material.
- ▶ Upon completion of the tasks involving potentially infectious or hazardous materials, decontaminate the work area with an appropriate disinfectant or cleaning solution (1:10 dilution of household bleach is recommended).

Dispose of the following potentially contaminated materials in accordance with laboratory local, regional, and national regulations:

- ▶ Biological samples
- ▶ Reagents
- ▶ Used reaction vessels or other consumables that may be contaminated



For safe usage, please carefully review the following warnings concerning fire, electrical, chemical and mechanical hazards.

Fire and Electrical Hazards

This instrument runs on 100-240 V AC voltage. Stop operation if there is a smell of burning, if electricity leaks (e.g., buzzing when touched), if water ingresses into the instrument, the supply voltage flashes (indicating unstable power supply), electrical parts display damage, or if the instrument fails to function as expected. Maintenance and repair must be carried out under the guidance of Izon support staff when the instrument is unplugged.

Chemical Hazards

Ensure that the correct tubing and connectors have been used for all components, and that these are installed without leaks before running the instrument. The use of incorrect tubing (including incorrect tube diameter) and connectors can result in increased pressure which can damage components and pose a danger to the operator. Leaking connections can result in exposure to potentially dangerous solutions (e.g., NaOH). Ensure that fittings are not over-tightened.

Ensure that tubing entering the waste container is secure (e.g., beaker clips) and that the waste container does not overflow. If this does occur, follow the appropriate hazardous waste cleanup procedures for the substances found in your waste container.

When operating the Zenco with hazardous chemical or biological agents, please ensure your safety by wearing appropriate protective clothing, glasses, and gloves that are resistant to the substances used. Take care to avoid spillage. Always consult the Safety Data Sheets (SDS) for the chemicals you are working with and follow local and national regulations for safe operation and maintenance of this product. Safety Data Sheets relevant to Izon products can be found at <https://support.izon.com/safety-data-sheets>

Mechanical Hazards

Due to high pressure and the potential risk of unsecured tubing, safety goggles should be worn when operating or in the vicinity of a Zenco.

The back pressure valve (BPV) should not be used with pressures over 5 bar and is suitable only for flow rates of less than 10 mL/min.

Blockages can result in pressure increases which can damage components and pose a danger to the operator. Ensure all solutions are properly filtered before using with the Zenco, and/or that all inlet tubes have a filter attached.

Do not allow tubes to kink as this restricts flow and will result in pressure increases which can damage components and pose a danger to the operator. To mitigate this, ensure that appropriate length tubing is used between each component.

Transport & Disposal



Exercise caution and use proper manual handling techniques when lifting or moving the Zenco as it is heavy and may cause injury if incorrectly handled.



The qEV Zenco system contains electrical materials; it should be disposed of as unsorted waste and must be collected separately, according to the European Union Directive: Waste Electrical and Electronic Equipment. The user is fully responsible for ensuring that the obsolete Equipment and/or Consumables are recycled or disposed of in accordance with this and/or any other relevant laws and regulations in the countries where the instrument is being recycled or disposed of. Contact your local Izon Science representative for more information.

3 INTRODUCTION TO THE qEV ZENCO

3.1 Intended Use

The qEV Zenco is an automated chromatography system designed for use with larger qEV columns, including the qEV2, qEV10, qEV100, and custom qEV columns. It accommodates sample volumes ranging from 2 to 400 mL per run.

The system offers programmable functionality, enabling users to customise workflows according to specific sample needs. Its software provides real-time data feedback and visualisation, allowing for continuous monitoring of run progress. Additionally, the qEV Zenco features a flowpath diagram that simplifies the setup and execution of workflow protocols.

This instrument is for use by professional personnel only who are familiar with operating bioprocessing equipment and managing biological materials.

For verification of the entire system, it is recommended that cGLPs are followed to ensure reliable analyses.

4 INSTRUMENT SPECIFICATIONS

4.1 Instrument Layout

Table 4: Components of the Zenco Unit

NUMBER	ABBREVIATION	NAME
1	UV	Ultraviolet sensor
2	-	Column holder
3	Cond	Conductivity sensor
4	AS	Air sensor
5	P1	Pressure sensor
6	-	Pump
7	BPV	Back pressure valve
8	-	Indicator lights
9	V3	Outlet valve
10	pH	pH sensor
11	V2	Flow director valve
12	V1	Inlet valve
13	-	PP centrifuge tube
14	-	Power switch
15	-	Power socket
16	-	Serial port
17	-	USB

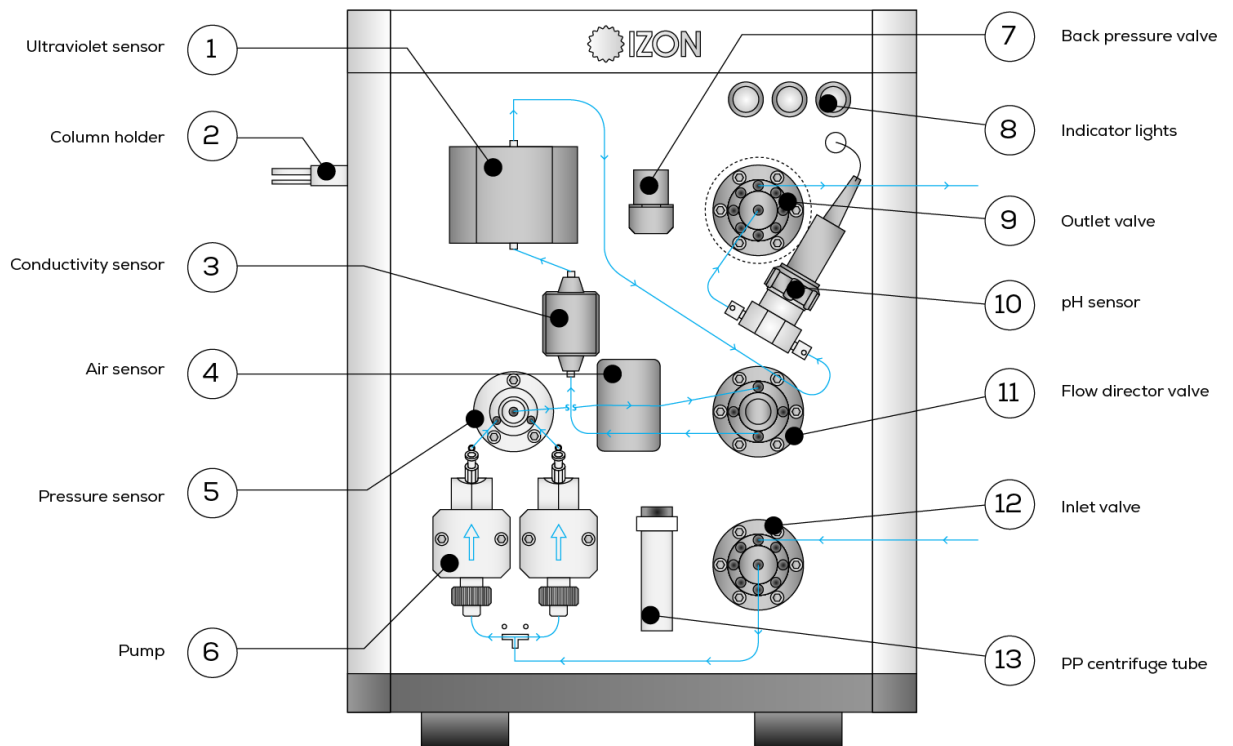


Figure 1: Components of the front of the Zenco Unit. The flow path is represented in blue.

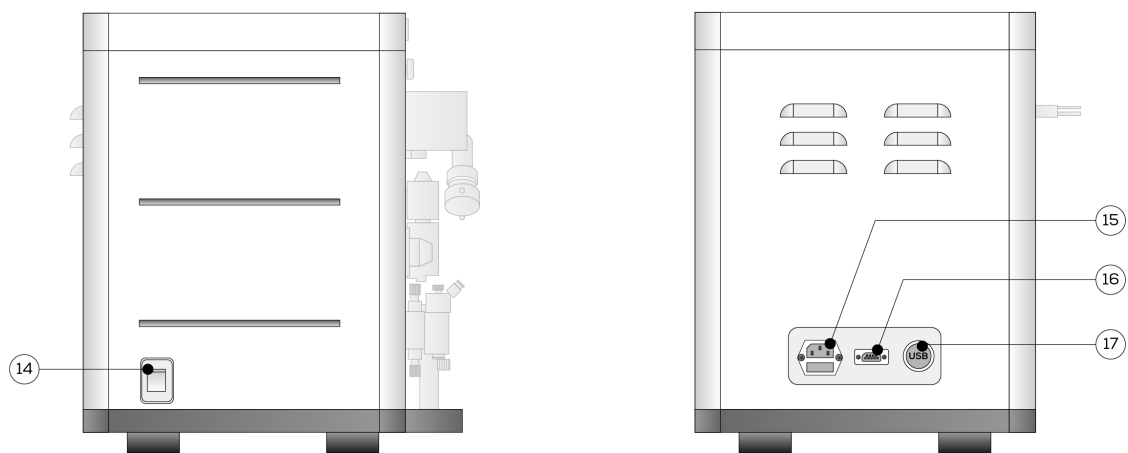


Figure 2: Components on the side and rear of Zenco unit.

In addition to the Zenco unit, additional components are required for operation. A full list of provided components can be found in [Table 5](#) on page 12.

Table 5: Full List of Provided Components and Quantities

NUMBER	NAME	NUMBER	NAME
1	qEV Zenco x 1	11	1/8 PTFE tubing x 2 m
2	pH probe x 1	12	1/16 PTFE tubing x 2 m
3	20 mL injection syringe x 1	13	1/16 PEEK tubing x 1 m
4	USB stick x 1	14	10-32 PEEK fitting cap x 12
5	Encryption key x 1	15	1/4-28 PEEK fitting cap x 8
6	Tubing cutter x 1	16	10-32 PEEK fingertight connector x 5
7	Communication cable x 1	17	1/4-28 PEEK fingertight connector x 5
8	Power cord x 1	18	Ferrule (1/8 PEEK) x 5
9	Pre-made inlet tubing (850 mm 1/8 PTFE tubing, 1/8 PEEK fingertight connector, & 1/8 PEEK ferrule) x 4	19	10 µm inlet filter x 7
10	Pre-made outlet tubing (800 mm 1/16 PTFE tubing & 1/16 PEEK fingertight connector) x 4	20	10-32 PEEK male fingertight connector x female luer lock (located in the pump head)

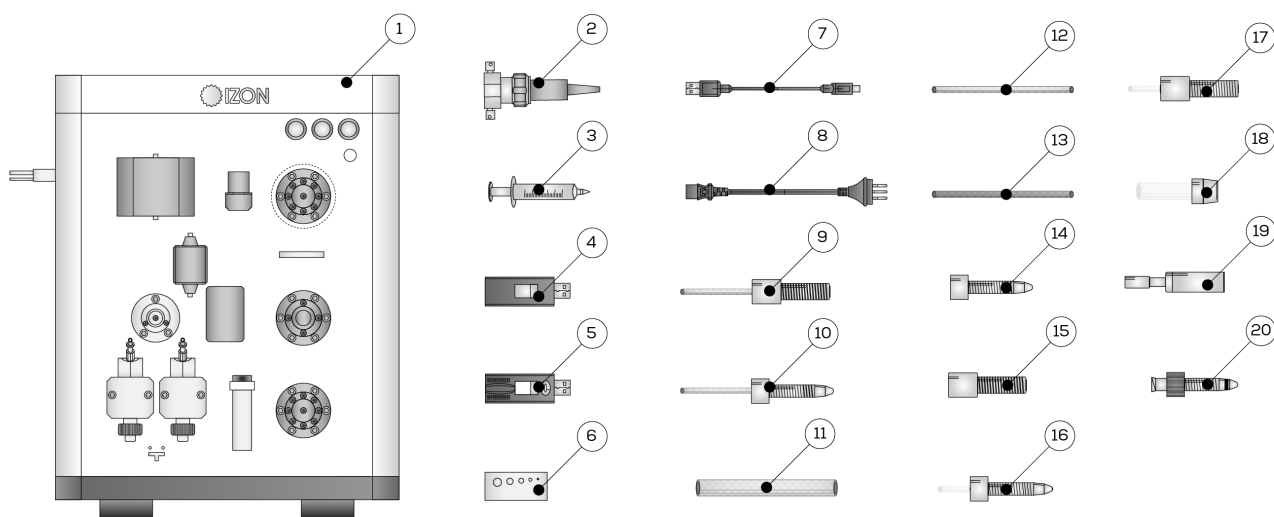


Figure 3: All components provided with the Zenco.

The following components are required for operation and/or maintenance of the instrument but are not provided:

- ▶ Computer with Windows 10 or above for updating the qEV Zenco operating firmware
- ▶ qEV column (qEV2, qEV10, qEV100, or qEV custom columns are compatible)
- ▶ Freshly prepared, filtered (0.22 μm) and degassed buffer. Refer to your qEV user manual for more information about buffers
- ▶ Freshly prepared 0.5 M NaOH solution for column cleaning
- ▶ Freshly prepared 1 M NaOH solution for system cleaning
- ▶ 20% EtOH
- ▶ DI water
- ▶ Large vessel for waste collection

5 ASSEMBLY AND SETUP INSTRUCTIONS

5.1 Assembling and Installing the qEV Zenco

- ▶ For indoor use only and to be used within the rated conditions noted in [Section 2.1: Safe Use Requirements and Specifications](#).
- ▶ Take care not to spill any fluids on electrical parts during operation.
- ▶ Make sure the power supply box is positioned away from fluids.
- ▶ Position unit so it can be quickly and easily disconnected from the mains power.
- ▶ Check the local supply meets the AC input requirement given in the specification in [Table 3](#).



The Zenco must be installed into Earth Grounded Protected Outlets ONLY. To minimise the influence of external noise from the environment, position equipment away from electrical switching gear and interfering equipment.



Izon instruments are only to be operated with Izon supplied leads and power supplies. Failure to use the correct power supply may result in invalid operation.

1. Unpack the Zenco and box contents.



We recommend that you keep the box and packaging materials in case the instrument needs to be returned for servicing.



If you choose not to keep the materials, please recycle them wherever cardboard recycling services are provided.

2. Place the Zenco onto a stable and level laboratory bench.
3. Ensure instrument is turned off by flicking the power switch located on the side of the unit to the "off" position.
4. Plug in the power cable provided into the socket at the back of the unit.
5. Plug in the other end of the power cable into a wall socket.
6. Turn on the unit.
7. Connect the USB cable to the instrument and the computer.

5.2 Unit Setup and Maintenance

The qEV Zenco is an automated chromatography system used in conjunction with larger qEV columns, which utilise size-exclusion chromatography. It is crucial that the system is set up correctly before operation.

Installing the Tubing



To ensure a secure finger-tight connection in the Zenco system, please follow the instructions below.

1. **Cut Tubing Cleanly:** Use the tube cutter supplied for a straight, smooth cut.
2. **Insert Tubing:** Slide the tubing into the fitting until it protrudes ~1–2 mm beyond the end.
3. **Align and Tighten:** Align the fitting with the port carefully to avoid cross threading and hand-tighten clockwise until snug.
4. **Inspect and Test:** Verify the tubing is not pinched or stressed and test for leaks under operating pressure.
5. **Avoid overtightening** to prevent damage.

Inlet tubing: The 1/8 PTFE tubing connects to the Inlet Valve (V1) with 1/4-28 fingertight connectors. The number of tubes to connect is dependent on your method. All inlets must be plugged with connectors, even if tubing is not required.

Outlet tubing: The 1/16 PTFE tubing connects to the Outlet Valve (V3) with 10-32 fingertight connectors. The number of tubes to connect is dependent on your method. All inlets must be plugged with connectors, even if tubing is not required.

V2 tubing: For guidance on how to connect your qEV column to the flow director valve (V2), please refer to [Section 6.4: Preparing your qEV Zenco for qEV Column Isolation](#).

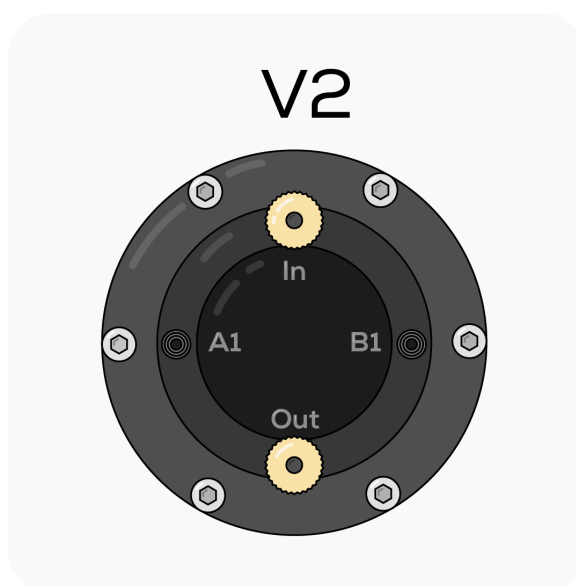


Figure 4: Illustration of flow director valve (V2)

Pump Maintenance

To set up the pump, remove the 25 mL centrifuge tube from the front of the instrument, add 15 mL of 20% ethanol and re-attach the centrifuge tube.

Weekly maintenance requires replacement of the 20% ethanol attached to the pump. The volume should be topped up if it falls to less than 10 mL.

Connecting and Disconnecting the Back Pressure Valve



The back pressure valve (BPV) is only to be used for flow rates below 10 mL/min (i.e., when fine control is required) and pressures below 5 bar. For cleaning and maintenance, ensure the BPV is disconnected. Do not use the BPV during priming and cleaning processes.

Connecting the BPV: Use 1/16" PEEK tubing with 10-32 fingertight connectors to connect the BPV to the UV detector and to the pH detector.

Disconnecting the BPV: Remove the tubing between the back pressure valve and both the UV detector and the pH detector. Connect the UV detector to the pH detector using 1/16" PEEK tubing.

Zenco Unit Indicator Lights

The qEV Zenco unit has three lights/buttons located on the top right corner, as shown in Figure 1.

- ▶ Pressing the green Run button will resume a run.
- ▶ Pressing the red Stop button will pause a run.
- ▶ The R/P/S light indicates whether the qEV Zenco is currently running, paused or stopped. This is reflected as green for running, orange for pause, and red and stopped.



If the qEV Zenco R/P/S light is orange or red and you have not intentionally paused or stopped the system, please check the flow path to identify whether any component is compromised/needs rebooting.

5.3 Zenco Software

Software Installation Instructions

To access the software installation instructions, please head to support.izon.com/how-do-i-download-the-qev-zenco-software

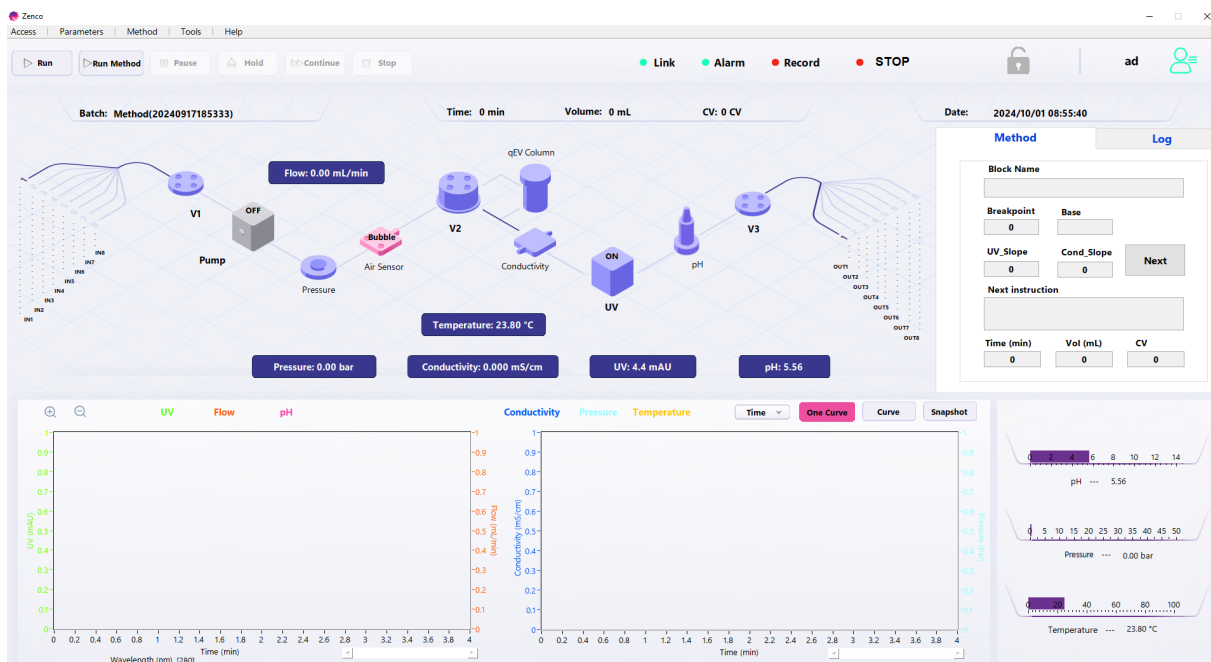


Figure 5: qEV Zenco software main screen.

First Software Login

1. Insert the encryption lock USB provided with the instrument into the computer's USB port.
2. Click on the Zenco icon and use the default login:

Username: ad

Password: Zenco123

3. Click on Parameters and then Settings, then Super settings (third tab), as shown in Figure 6.

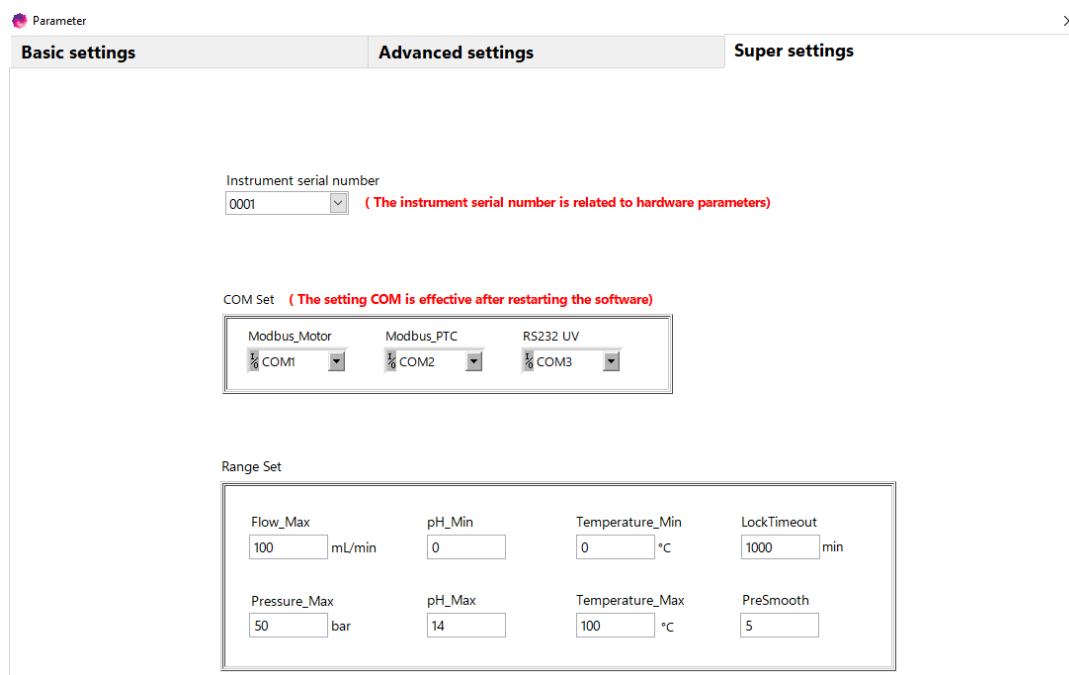


Figure 6: Super settings within the Parameter controls.

4. Set the serial number to the serial number of your Zenco instrument.
5. Set up user logins by heading to Access, then User Setup.
6. Modify the default login by selecting the ad user and clicking Edit if desired and add new users if desired using New.

Passwords are not set by default for new users and cannot be changed in User Setup. To change or add passwords for new or existing users, navigate to Access then to either Change Login Password or Change Signature Password to change the login or signature password respectively.

Software Indicator Lights

The software shows 4 alert lights directly above the flow path diagram, as shown in Figure 7:

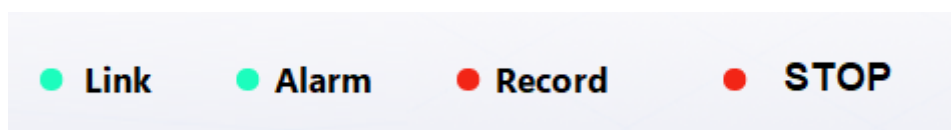


Figure 7: Software indicator lights.

Link

- ▶ Green indicates connections are functioning normally.
- ▶ Red indicates communication failure. If no connections are loose, click Parameters and then Settings and check that the instrument port numbers are set as follows: Modbus_Motor to COM1, Modbus_PHC to COM2 and RS232 to COM3. If this does not fix the issue or the port numbers are not wrong, restart your computer and try opening the software again.

Alarm

- ▶ Green indicates that the instrument is functioning normally.
- ▶ Red indicates an alarm has been sounded for the instrument. Please check the Log tab for further information.

If the system alarms, a pop-up dialog will display the alarm information. Click OK to dismiss this pop-up and pause or stop the run if required. To check for more information on the alarm click Tools and then MessageAlarm.

Record

- ▶ Green indicates that data is being recorded.
- ▶ Red indicates that data is not being recorded.

Stop

- ▶ Green indicates that the system is set to Run or Hold.
- ▶ Yellow indicates that the system is paused.
- ▶ Red indicates that the system has been stopped.

Audit Trail and Data Management

The audit trail for the instrument can be accessed via Access and then Audit Trail. For data management, click Tools and then DataManager. Data can be exported. Historical data can be accessed through Tools and then History.

Liquid Flow Path Overview

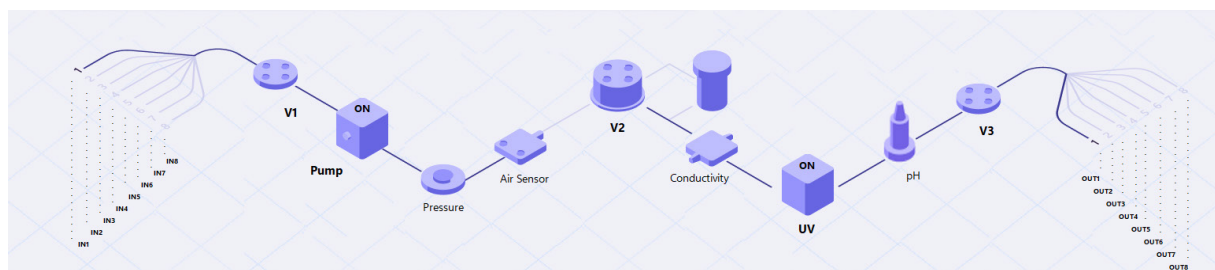


Figure 8: Flow path diagram.

- ▶ The inlet tubing connects to the inlet valve (V1), and then passes through the pump.
- ▶ From the pump, two tubes connect to the pressure sensor (P1).
- ▶ After this, a single tube exits P1 and passes through the air sensor before entering V2 via the channel labelled 'IN'.

- ▶ Next, the flow goes either through the column (forwards through A1 to B1 or backwards through B1 to A1) or directly exits OUT of V2 (bypass).
- ▶ Whether the flow passes through a column or not, the flow next passes through the conductance and then the UV sensor, followed by the pH sensor.
- ▶ The flow enters the right side of the pH sensor and exits the left-hand side.
- ▶ The flow will then enter the outlet valve (V3) before exiting through the outlet tubing into a designated vessel.
- ▶ The back pressure valve (BPV) can be connected between the UV and pH if required (see [Section 5.4: Connecting and Disconnecting the Back Pressure Valve](#)).

6 OPERATING INSTRUCTIONS



All solutions, including samples, must be filtered before use with the Zenco instrument. Buffers should be filtered at 0.22 µm.

6.1 Priming the Pump

1. Place all inlet tubing ends into the buffer solution.
2. Attach the 20 mL syringe provided to one of the two luer lock fittings protruding from the top of the pump.
3. Slightly loosen the screw fitting and use the syringe to aspirate the tubing until liquid enters the syringe.
4. Re-tighten the screw fitting and remove the syringe.
5. Repeat with the other luer lock fitting. This is essential for smooth operation of the pump.

6.2 System Cleaning

The Zenco system should be cleaned before initial use, between runs, and at the end of the day.

1. Prepare the required amount of 1 M NaOH.
2. Place all inlet tubing into the NaOH and all outlet tubing into the waste vessel.
3. Use the manual control instructions (see [Section 6.3: Manual Controls](#)) to pump the NaOH through the instrument, with V2 set to bypass, at 50 mL/min



It is crucial to have V2 set to bypass for this step to avoid 1 M NaOH from entering the column.

4. Each inlet position must be run for 5 minutes with the NaOH and each outlet position must be run for 10 minutes. Pause the pump after priming each flow path.

6.3 Manual Controls

Manual control of flow is used for the priming and cleaning of the instrument and the tubing. It can also be used for running your protocol if you wish, though the method editor is designed to streamline this process and increase reproducibility.

If you are running your protocol through manual control, you can set your column volume by clicking on Parameters > Settings. Select the Basic settings tab, shown in [Figure 9](#), and enter the Column Volume of your qEV column – this value can be found in your respective qEV User Manual.

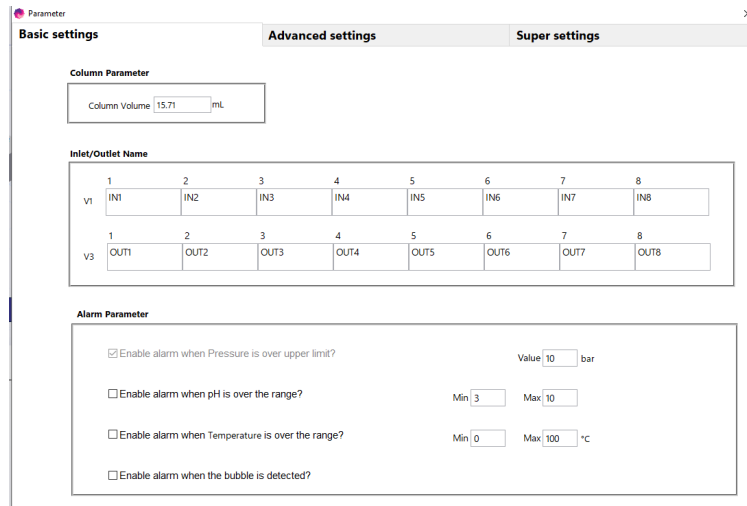


Figure 9: Basic settings within the Parameter controls.

To set the flow using the Flow Path diagram:

1. Set the inlet position by clicking on the Inlet Valve V1 icon and set the appropriate inlet position. Click OK to set the position.
2. Set the Flow Direction Valve V2 by clicking on its icon and selecting whether to use forward, reverse or bypass (bypasses the column). Click OK to set the position.
3. Set the Outlet Valve V3 position by clicking on its icon and setting the appropriate outlet position. Click OK to set the position.
4. Set the flow rate by clicking on the Pump icon and entering the required flow rate, as shown in Figure 10. If the BPV is to be used for fine control, set the flow rate to a maximum of 10mL/min. Click OK to set the flow rate.



When operating at a flow rate above 70 mL/min, limit use to a maximum of 2 hours at a time to prevent excessive pump wear. Flow rates of 70 mL/min or lower are suitable for continuous operation.

Set SystemPump



FlowRate(mL/min) [0~100.00]

0.00

0 for Stop

OK

Cancel

Figure 10: Set system pump pop-up

5. Click Run to open the Manual Mode Run Confirmation box.

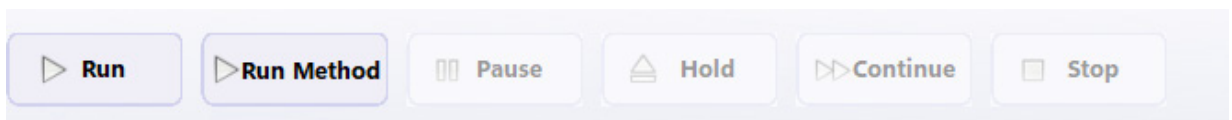


Figure 11: Manual control buttons.

- In the Run Confirmation box shown in Figure 12, select whether you want to save data by ticking or unticking the save data box. If you wish to save data, ensure you have entered a file name.

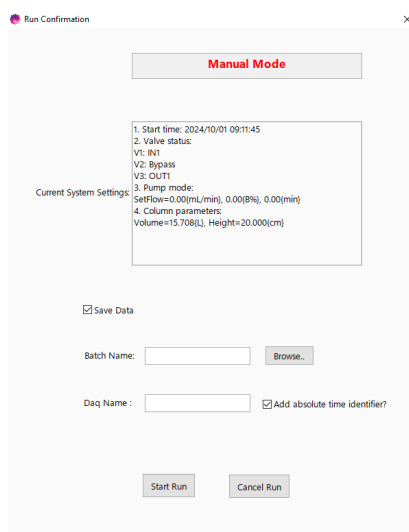


Figure 12: Manual mode run confirmation pop-up.

- Select Start Run to begin.

Other Manual Controls

- ▶ Pause will stop the system running temporarily but does not end the run. It can be re-commenced.
- ▶ Hold will not stop the system running but will prevent movement to the next step if a method is being run.
- ▶ Stop will stop the run and system entirely.

If desired, the software can be locked during a run by clicking on the lock symbol. The user will have to login to regain access.

A Timer can be set using Tools and then Timer which allows you to enter a Time or Volume which should be reached before the system pauses. Click Start to begin timer. This can be useful when running the system manually. The Stop button can be used to stop the timer early.

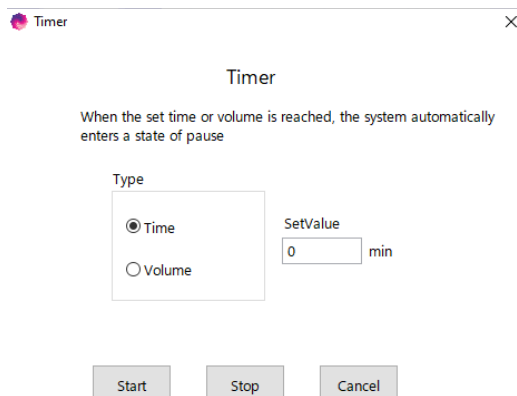


Figure 13: Timer pop-up.

6.4 Preparing your qEV Zenco for Use with qEV Columns

1. Attach your qEV column using the clips (if using qEV2 or qEV10) on the side of the Zenco or place your column in an appropriate stand.
2. Using 10-32 fingertight connectors, prepare an appropriate length of 1/16" PTFE tubing to reach the top of the column to A1 on V2, and another appropriate length of 1/16" PTFE tubing to reach the bottom of the column to B1. Do not connect the tubing to the column yet.
3. Add the Male Luer Lock x Female 10-32 fingertight connector to the end of the tubing from A1 and the Female Luer Lock x Female 10-32 fingertight connector to the end of the tubing from B2.

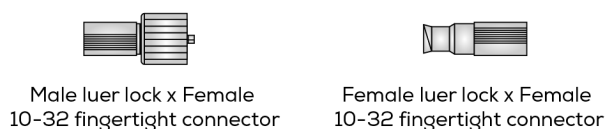


Figure 14: Illustration of the two types of luer lock x fingertight connectors required to connect your qEV column to the Zenco unit.

Prime Inlet Tubing

1. Place all inlet tubing ends into buffer
2. Read [Section 6.3: Manual Control](#) instructions if needed.
3. Set a flow rate of 30 mL/min.
4. Ensure that V1 is set to the first inlet tube you wish to prime and V3 is set to your waste outlet tubing. Set V2 to bypass.
5. Begin to manually flow buffer until your first inlet tube has been filled.
6. Switch to the next inlet tube and repeat the process until all inlets have been primed.
7. For the final inlet, allow buffer to run through the entire system until it exits through the waste outlet before stopping the run.

Prime Column Tubing

1. Place the ends of the tubing that are connected to A1 and B1 (i.e., the tubing that will connect to the column) into the waste container.
2. Ensure the selected inlet position is the tube which goes into your buffer.
3. Set V2 flow direction to forward and click OK.
4. When buffer has exited the tube attached to A1, select Pause.
5. Remove the connector cap and then unscrew the qEV column cap and fill the column with buffer until it reaches the upper frit (i.e., is level with the top edge of the column). Screw the column connector cap back on, forcing buffer up through the connector junction.

6. Attach the tube from A1 to the top of the column using the luer lock fitting.
7. Click on V2 again to set the flow direction to reverse, press OK.
8. When buffer has exited the tube attached to B1, select Pause.
9. Remove the qEV column bottom cap and attach the tube from B1 in its place, using the luer lock fitting.

6.5 Set Upper Pressure Limit and Leakage Test

Set Alarm Upper Pressure Limit



Ensure you have set an upper pressure limit alarm and checked for leaks before continuing

1. Click on Parameters and then Settings. In the Basic Settings interface, set the alarm upper limit to 10 bar and tick "Enable alarm when Pressure is over upper limit".
 - a. When the pressure exceeds 80% of the set value, the flow rate will be reduced to 90%;
 - b. When the pressure exceeds 90% of the set value, the flow rate will be reduced to 80%;
 - c. When the pressure exceeds 95% of the set value, the flow rate will be reduced to 50%;
 - d. When the pressure reaches the set value, the pump will be stopped until the pressure drops to lower than 80% of the set value.



Set the alarm upper limit to 5 bar if the BPV is to be used.

2. Next, select Advanced Settings and, set the UV Wavelength Settings to 280 nm and then press Set Wavelength to detect proteins (both free and EV-surface proteins). The UV detector must be turned on for 30 minutes before use in a run.

Check for Leaks

Run a manual flow check of buffer in the forward and bypass directions at 10 mL/min to ensure that there are no leaks at any connections. If any leaks are detected, please tighten the relevant connectors. Once no leaks have been detected, press Stop. If leaks continue to be detected, please contact Izon Support for further guidance.

Your Zenco is now set up for a run and you can proceed to setting up the method via the method setup page.

6.6 Setting Up a New Method



The setup described throughout this section is for typical qEV column use.

1. Click on Method in the top menu, followed by Create New Method > Create Blank to open the method editor page.
2. In the **Method Details** tab, enter the name of the method (e.g., qEV100 20 nm). You can also enter a description of the method in the Description text box.

- ▢ Method Details
- ▢ Method Parameters
- ▢ Column Priming
- ▢ Sample Isolation
- ▢ Column Cleaning

Create New Method

Name

User

Date created

Description

Estimated Time min

Figure 15: Method details tab.

3. In the **Method Parameters** tab, select which base unit you wish to use. This will either be time, volume, or column volume (CV). The base unit selected at this stage will flow through to additional steps. In the Pressure Upper Limit box, set 10 bar as the upper pressure and ensure that the Enable Pump Limiting feature is ticked. Enter the CV for the relevant qEV column to be used. Ensure the UV wavelength is set correctly. Select whether you would like to skip entering a new sample ID for queued methods.

- ▢ Method Details
- ▢ Method Parameters
- ▢ Column Priming
- ▢ Sample Isolation
- ▢ Column Cleaning

Method Parameters

Base Unit Time Volume CV

Pressure
Upper Pressure limit bar Enable Pump Limiting

Column
Column Volume: mL

UV
UV Wavelength nm
(190-400)

Sample ID
 Skip entering a new sample ID for queued methods

Figure 16: Method parameters tab.

4. In the **Column Priming** tab, select which inlet will draw the buffer to prime your column. Select the outlet which will go to waste. Enter your desired flow rate and volume. Set the flow direction to Forwards for column priming. Select whether you wish to "auto zero" the UV sensor during this step.

- ▢ Method Details
- ▢ Method Parameters
- ▢ Column Priming
- ▢ Sample Isolation
- ▢ Column Cleaning

Column Priming

Inlet

Outlet

Flow rate mL/min

Volume mL

Flow Direction

Automatically zero UV after priming

Figure 17: Column priming tab.

- In the **Sample Isolation** tab, enter the relevant fluid details. This will include which inlet will draw your sample. Set the sample load volume. Select the buffer inlet. Select the flow rate (equivalent to gravity for optimal purity, refer to [Table 6](#) below). Select the waste outlet. Select the flow direction to Forward for column priming. Next enter the collection details. Select whether the parameters are predetermined. Next, enter the buffer volume. This can be found in the relevant qEV user manual. Select whether you would like to collect the buffer volume and which outlet it will exit from. Enter your desired fraction volume and the number of fractions you wish to collect (between 1-7). Lastly, select which outlet your first fraction should exit from, additional fractions will exit from the numerically consecutive outlets.

Figure 18: Sample isolation tab.

- The final stage of the method setup is the **Column Cleaning** tab. Select your waste outlet and set the desired flow rate and flow direction (Forward). Next, set the pre-clean flush inlet and pre-clean flush volume. Then select the inlet for the cleaning solution and the volume. Select the post-clean flush inlet and post-clean flush volume. Please note that the flow rate can be higher than gravity for column cleaning.

Figure 19: Column cleaning tab.

- Your method is now ready to be saved by clicking Save at the top of the screen.
- Select Back to return to the homepage.

Table 6: Recommended Flow Rate Equivalent to Gravity for qEV Columns

qEV COLUMN SIZE	RECOMMENDED FLOW RATE
qEV2	3 mL/min
qEV10	4 mL/min
qEV100	18 mL/min

6.7 Starting a Run with a Method

1. Ensure that your Zenco is properly connected to the correct required inlets and outlets as specified by the method which you want to run.
2. Select Run on the homepage and then tick run method and then select the saved method that you wish to run. Press OK. Finally, click start to begin running the method.
3. Refer to [Section 7: Live Data and Data Analysis](#) for guidance on visualising and exporting data.

6.8 Ending a Run

1. At the end of the run, ensure that all outlet tubes are emptied.
2. Refer to [Section 6.2: System Cleaning](#) for end of day cleaning instructions.
3. A run of the cleaning protocol using DI water should be done at the end of each day's use to prevent salt crystallisation.
4. Turn the power switch to the "O" position to put the the system into standby mode. If the power cord of the instrument is unplugged from the socket or the power socket is turned off, the instrument will enter storage mode.

7 LIVE DATA AND DATA ANALYSIS

7.1 Data Visualisation During a Run

1. Select One Curve or Two Curves to display one or two graphs respectively.
2. Select Curve and select the desired display parameters ticking the box associated with them. You must select at least two parameters per graph.
 - a. Customise the appearance of the parameters using the Graph, Width, Style and Color options.
 - b. The Y-axis tab is used to determine whether you wish to use auto or fixed scale.
 - c. The X-axis tab allows you to determine whether the x-axis is displayed using the units of Time, Volume or CV. You can also set the zoom mode.
4. After exiting the Curve pop-up, you can use the + and – symbols to manually scale the graph(s) to show the data as desired.
5. A snapshot of the current graph view can be taken by clicking Snapshot, entering a descriptive name and clicking OK.

7.2 Data Analysis

1. Click Tools and History and then the Chromatography Analysis tab.
2. Ensure that the column height parameter is set correctly.
3. Select the desired Analysis Signal and X-base and enter the following parameters:
 - ▶ Height Threshold is used to filter out shorter peaks and artifacts.
 - ▶ Width Threshold is used to filter our narrow peaks and artifacts.
4. Baseline Adjustment is used to determine the baseline of the curve. As the baseline may drift, the appropriate baseline for the required section of the trace should be inputted. This can be done either automatically or manually.
5. Click Auto Compute to automatically mark the peak position and use a green line to indicate the calculated baseline. The calculated values will be displayed in the table.
6. Click Data Export to export your data as a CSV file.
7. Signature can be used to sign your analysis using your login.
8. To compare curves between runs, go to the Curve Comparison tab.
9. To generate a PDF report, use the PDF Report tab and modify the settings there to your needs.

8 CALIBRATION AND TROUBLESHOOTING

8.1 Calibration

If calibration is required for any or all components of the Zenco, please follow the relevant instructions below.

pH Calibration

1. Click on Parameter and then Calibrate.
2. Prepare a neutral, acidic and alkaline standard, each with known pH.
3. Enter the pH of your reference standards with neutral in CP1, acidic in CP2 and alkaline in CP3.
4. Place the pH probe into CP1, wait 5 minutes, and then click Calibrate CP1.
5. Once calibration for CP1 has been completed, remove the probe from the CP1 standard and rinse with DI water.
6. Repeat steps 4-5 for CP2 and CP3.
7. The pH will now be calibrated.
8. Finally, recheck with a sample of neutral pH to check calibration.

Conductivity Calibration

1. Click on Parameter and then Calibrate.
2. Prepare solutions with conductance in $\mu\text{S}/\text{cm}$ in accordance with the specified 4-point linear calibration at the top of the conductance section on the open Calibration dialog box.
3. Enter the conductance of your reference solutions to confirm which solutions are assigned to which calibration point (CP).
4. Replace the threaded connector at the IN channel of V2 with a female luer lock x male 10-32 fingertight connector from one of the pumps. Replace the UV detector outlet with a 10-32 hand screw connector. Connect a 1/16" PTFE tubing to the UV detector outlet, placing the tubing ends into a waste vessel.
5. Clean and empty the tubing before each calibration point.
6. Inject 15-20 mL of 84 $\mu\text{S}/\text{cm}$ conductivity standard solution into the IN channel of V2, input the conductivity value of the standard solution in the CP1 input box. Click CP1 calibration and wait for completion.
7. Use 1413 $\mu\text{S}/\text{cm}$, 12.88 mS/cm , and 111.3 mS/cm conductivity standard for CP2, CP3, and CP4 respectively following the same instructions as step 6.
8. The conductivity will now be calibrated.
9. Test the conductivity using a solution of known conductance.

Temperature Calibration

1. Place the test water at the ambient temperature for at least 1 hour, and start the calibration when the temperature fluctuation does not exceed 1 °C.
2. Place the tubing connected to V1 into the test water, and the tubing connected to V3 into the waste tank.



Ensure the BPV is disconnected before increasing the flow rate.

3. Set the flow rate to 70 mL/min and start the pump.
 1. Click on Parameter and then Calibrate to open the Calibration dialog box.
 2. After five minutes, enter the measured temperature value in the Temp Calibrate Value box.
 3. Click Calibration, and wait until the completion of the calibration.

Pressure Calibration

1. Place the tubing connected to V1 into DI water.
2. Connect a pressure tester to the outlet of the pressure sensor with 15 cm long PEEK tubing.
3. Place the outlet of the pressure tester in the waste vessel.
4. Before calibration, ensure tubing is dry. Use a syringe to extract any residual liquid from the pressure flow cell and inside of the tubing.
5. Click on Parameter and then Calibrate.
6. Click Zero Calibration and wait until the completion of the calibration.
7. Set the flow rate to 8 mL/min and start the pump.
8. Adjust the pump speed to 0.2 mL/min once the tubing is filled with liquid.
9. Block the outlet tubing of the pressure tester in order to increase the pressure to 10 ± 1 bar.
10. Stop the pump.
11. After stabilising for 3–5 minutes, read the value of the pressure tester and enter it into the P1 Actual Valve box.
12. Click Calibration and wait until the completion of the calibration.

Valve Zeroing

1. Click on Parameter and then Calibrate.
2. In the Valve calibration window, click the button to self-zero each valve.
3. If this is successful then it will display “Home is found”.

8.2 Troubleshooting

No Power Displayed When System is Powered On

1. Check that the power supply cables are not loose.
2. Unplug and re-plug the power cord.
3. Reboot the system.
4. Check if the cooling fan is running.

5. If the cooling fan is not running or if the reboot does not fix the issue, please get in touch with Izon Science Support

Alarm Occurs When the Zenco is First Turned On

1. Check that the Link light is green. If it is red, a communication error is occurring.
2. Check whether the cable between the computer and the Zenco is loose.
 - a. If the cable is not loose, proceed to step 3.
 - b. If the cable is loose, turn off the instrument at the wall, tighten the cable connections and restart the Zenco.
3. Check the system log on the right-hand side of the main software interface.
 - a. If the error is not a valve error or if no error is shown, please contact Izon Science Support.
 - b. If Valve error is displayed, proceed to step 4.
3. Navigate to Tools and then MessageAlarm to identify which valve has failed.
4. Restart the instrument and use the manual controls in the Flow Path diagram to determine whether you can manually switch the valve to each position.
5. If this does not resolve the issue, please contact Izon Science Support.

Alarm Occurs When the Zenco is in Use

1. Pause the run.
2. Navigate to Tools and then MessageAlarm to identify the cause of the error.
3. If the error relates to a valve or pump, use the Flow Path diagram to attempt to manually run the valve or pump.
4. If the error persists, please contact Izon Science Support.

The Pump Cannot be Started

1. If the instrument was not sufficiently cleaned after the last use, salt crystals may have formed in the pump. If this is the case, proceed to step 2. If this is not the case, proceed to step 3.
2. Run DI water through the pump, bypassing the column, for 10 minutes at 50 mL/min to dissolve any remaining salt crystals. Verify if the pump now functions normally using manual controls.
3. Air may be getting into the pump. Loosen the air vent connector at the pump head and draw liquid out with a syringe. Tighten the connector again and verify if the pump now functions normally using manual controls.
4. If the pump still cannot be started, please contact Izon Science Support.

Pump Rinsing Buffer (20% EtOH) Not Circulated

The membrane of the check valve may be blocked or damaged. Please contact Izon Science Support for further instructions.

The UV Reading Exceeds 7000 mAU

1. Salt crystals may have formed in the optical path of the UV flow cell.
2. Flush the system with DI water for 10 minutes at 50 mL/min with the column bypassed.
3. Check whether the UV reading returns to normal levels. If this does not occur, please contact Izon Science Support.



Please note that if DI water is run through the system to fix a blockage part way through a run, the system must be fully flushed with buffer before the flow is returned from bypass to forward. This is to ensure that DI water is not passed through the qEV column.

8.3 Maintenance

Daily Maintenance

System

1. Clean and wipe the surface of the instrument to prevent crystallised salt corroding the equipment.
2. Check the system tubing and connectors for any breakage or leakage.
3. After use, the flow path should be rinsed with water followed by 20% ethanol.
4. Solutions and samples must be filtered before use.

System Pump

1. Check for any leakage around the pump head. If the pump head has leakage or the flow rate is inaccurate, remove the pump head and inspect the O-ring for damage.
 - a. If the O-ring is not damaged, wash with water to remove any impurities.
 - b. If the O-ring is damaged, please replace it.
2. When refilling the buffer reservoir, it is necessary to remove any remaining bubbles in the pump head to prevent inaccuracies in the flow. This can be done by running the Zenco system until all bubbles are removed. Ensure the qEV column is removed prior to running and V2 is set to bypass to prevent any bubbles from entering the qEV column.
3. The pump system is equipped with a pump head for liquid extraction. To extract liquid, loosen the luer lock x fingertight connector on the front of the pump head and use a syringe to remove any liquid.

Weekly Maintenance

System Pump

1. Replace pump cleaning fluid (20% ethanol) once per week.
2. If the volume of pump cleaning fluid significantly increases or decreases (by more than 5 mL), inspect the pump head seal ring. If leakage or wear is found, please replace the seal ring.
3. For additional information, please refer to [Section 5.2: Unit Setup and Maintenance](#).

Monthly Maintenance

1. Clean the system with 1 M NaOH solution regularly every month. Refer to [Section 6.2: System Cleaning](#) for more information. Rinse the system with water in the same manner as the cleaning steps.
2. Inspect the pump head to ensure proper function. Set the flow rate to 10 mL/min and start the pump. If the outlet flow is obstructed and showing intermittent water flow, the pump head may be blocked or letting air in. Refer to [Section 8.2: Troubleshooting](#) and follow **The Pump Cannot be Started** instructions.

Semi-Annual Maintenance

Pressure Detector

1. Conduct a zero calibration by following the Pressure Calibration instructions described in [Section 8.1: Calibration](#).

UV Detector

1. Inject 10% surfactant (e.g., Decon90, Deconex11, DBS25, SDS, etc.) into the UV flow cell with the syringe. Leave for 20 minutes and then rinse with water.
2. Inject absolute alcohol or 1 M NaOH into the UV flow cell. Leave for 20 minutes and then rinse with water.
3. Check the accumulated lamp usage time by referring to the advanced settings. If lamp usage time exceeds 2000 hours, please replace the lamp.

Preventative Maintenance Advice

System Pump

- ▶ If the operating time is greater than 1000 hours, replace the seal ring, inspect the check valve, fix screws, and clean the diaphragm.
- ▶ If the operating time is greater than 2000 hours, replace the worn parts such as the diaphragm and the check valve.
- ▶ If the operating time is greater than 10,000 hours, replace the pump head.

Valve

- ▶ Open the valve head and clean the inner hole to avoid clogging and affecting the flow rate as necessary.

UV Detector

- ▶ If the operating time is greater than 2,000 hours, clean and replace the optical fibre, and replace the deuterium lamp.
- ▶ If the operating time is greater than 4,000 hours, replace the seal ring and replace the flow cell as necessary.

Back Pressure Valve

- ▶ Clean the BPV as necessary by connecting it to the Zenco unit and flushing it with water at 10 mL/min for 5 minutes.

9 CONTACT US

Additional support material is available at support.izon.com

If you have any questions that are not answered on the support portal, or your instrument requires repairs/maintenance, please contact our support staff via the online support portal by raising a support ticket.

When reporting Zenco issues to Izon support, please provide the serial number of the Zenco, which can be found on the rear of the Zenco as in [Figure 20](#).



Figure 20: The Zenco serial number and compliance sticker.

