SEDEX MODEL 80LT

LOW TEMPERATURE EVAPORATIVE LIGHT SCATTERING DETECTOR

OPERATOR'S MANUAL

SEDERE, S.A.S
PARC VOLTA-BP 27
9, Rue Parmentier
94141 Alfortville Cedex
FRANCE

Telephone +33 1 45 18 05 18
Fax + 33 1 45 18 05 25

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Patent Application Number: EP 02 290 580.6 (date Mar. 8, 2002)
Patent Application Number: FR 03 01652 (date Feb. 12, 2003)
Patent Application Number: US 2003/0086092 A1 (date May 8, 2003)
Patent Application Number: FR 03 13706 (date Nov. 24, 2003)
Patent Application Number: EP 1 447 663 A1 (date Aug. 18, 2004)
Patent Application Number: US 6, 936, 174 B2 (date Aug. 30, 2005)
Patent Application Number: FR 06 50372 (date Feb. 2, 2006)
Patent Application Number: FR 07 52752 (date Jan. 19, 2007)
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All information in this manual is subject to change without notice and does not represent a commitment on the part of S.E.D.E.R.E S.A.S. Please consult S.E.D.E.R.E S.A.S if you suspect any error or omission.
S.E.D.E.R.E S.A.S believes that the information in its user manuals is accurate as the date of publication.

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Warnings and Safety Precautions

The following precautions should be followed to minimize the possibility of personal injury and/or damage to property when using the Sedex Model 80 Low Temperature Evaporative Light-Scattering Detector.

1) Read the Operator's Manual thoroughly before you use the detector and keep this manual for future reference.

2) Maintain a well ventilated laboratory. If the mobile phase contains a volatile organic solvent, ensure that the laboratory is well ventilated so that a build-up of vaporized solvent cannot occur.

3) Avoid open flames and sparks. Do not use an open flame and do not use any equipment that can cause sparks in the same room as the instrument.

4) The detector must be plugged into a grounded power line. Make certain that all parts of the instrument are properly connected to a common ground.

5) If the mobile phase includes an organic solvent, use an inert gas to nebulize the mobile phase.

6) The exhaust from the detector should be vented into a fume hood or similar system. Make certain that the output gas does not escape into the laboratory. Take in consideration any solvent filter that could be required by your local environmental laws.

7) The gas pressure should not exceed 4.5bar (67 psi). Make certain that the gas flow is maintained while the mobile phase flows through the detector. If the gas flow is interrupted for extended periods of time, organic solvents could possibly damage the pressure sensor and/or the photomultiplier

8) Do not use corrosive materials (non-exhaustive example: Tetrahydrofuran) that could damage the inner metal surfaces (stainless steel) of the detector.

9) Do not use any liquid or gas (non-exhaustive examples: pure oxygen or hydrogen) that support combustion under temperatures reached by the detector.

10) Access inside the instrument is restricted to a suitably skilled and competent technician.

11) Do not remove the optical head or the photomultiplier tube while the instrument is powered up. This can destroy the photomultiplier.

12) The siphon overflow tube must contain liquid at all times.

13) Do not disassemble the nebulizer or touch any components inside the nebulization chamber. This can lead to the deposition of contaminants that could affect the signal.

14) Do not adjust any components inside the detector unless specifically authorized to do so by your dealer.

15) If the instrument is used in a manner not specified by the manufacturer, the protection ensured by the instrument can be ineffective.

16) The user is responsible for decontamination if hazardous material is spilled on or in the instrument.

17) The user is responsible for detector end of life recycling. You must not discard this electrical/electronic product in domestic household waste. This product is classed as a *Monitoring and Control instrumentation* product. Detector internal parts present no danger for recycling. Make certain than detector has been cleaned to ensure no solvent or solute can remain in detector drift tube.

18) The warning symbols on the instrument indicate the following:

Risk of burn

Electric shock risk

Warning (Refer to user manual for additional information)

Table of Contents

1 Introduction

1.1 The Low Temperature Evaporative Light-Scattering Detector

The ELSD 80 Low Temperature Evaporative Light-Scattering Detector (Figure 1-1) is designed to detect compounds in the eluent from High Performance Liquid Chromatography (HPLC), Gel Permeation Chromatography (GPC), preparative HPLC, Flash Chromatography or Counter Current Chromatography (CCC). It is capable of monitoring eluent flow rates from 100µL/min to 5mL/min. Evaporative Light-Scattering Detection is a nearly universal technique which can detect any non-volatile analyte. Unlike other types of detection mode such as UV Detection, it is not dependent on the absorption of radiation and is not affected by the absorption characteristics of the solvent. Thus, solvents which absorb UV radiation can be used. As the solvent is completely evaporated, a gradient can be performed to optimize the separation.

Figure 1-1: The SEDERE ELSD 80 Low Temperature Evaporative Light-Scattering Detector

The detector is controlled via the keypad and digital LCD display on the front panel. The analog signal output can be sent to a recorder, an integrator or a data station.

As an alternative, the instrument can be controlled by an external computer using the RS-232 port.

The detector includes a nebulization cell, an evaporation tube and a detection chamber. The evaporation tube is heated in order to evaporate the solvent.

1.2 Principle of Operation

There are three discrete steps in the operation of the detector; nebulization of the eluent, evaporation of the solvent and detection of the compound(s) of interest (Figure 1-2).

NEBULIZATION → **EVAPORATION** → **DETECTION**

Figure 1-2: Schematic Diagram of an Evaporative Light-Scattering Detector

Nebulization involves the conversion of the eluent into a fine aerosol. This aerosol is directed to an evaporator to vaporize the solvent, then the mist is irradiated by a light source and the scattered light is measured by a photomultiplier; which is related to the concentration of the compound of interest in the sample.

A cross sectional view of the instrument is presented in Figure 1-3.

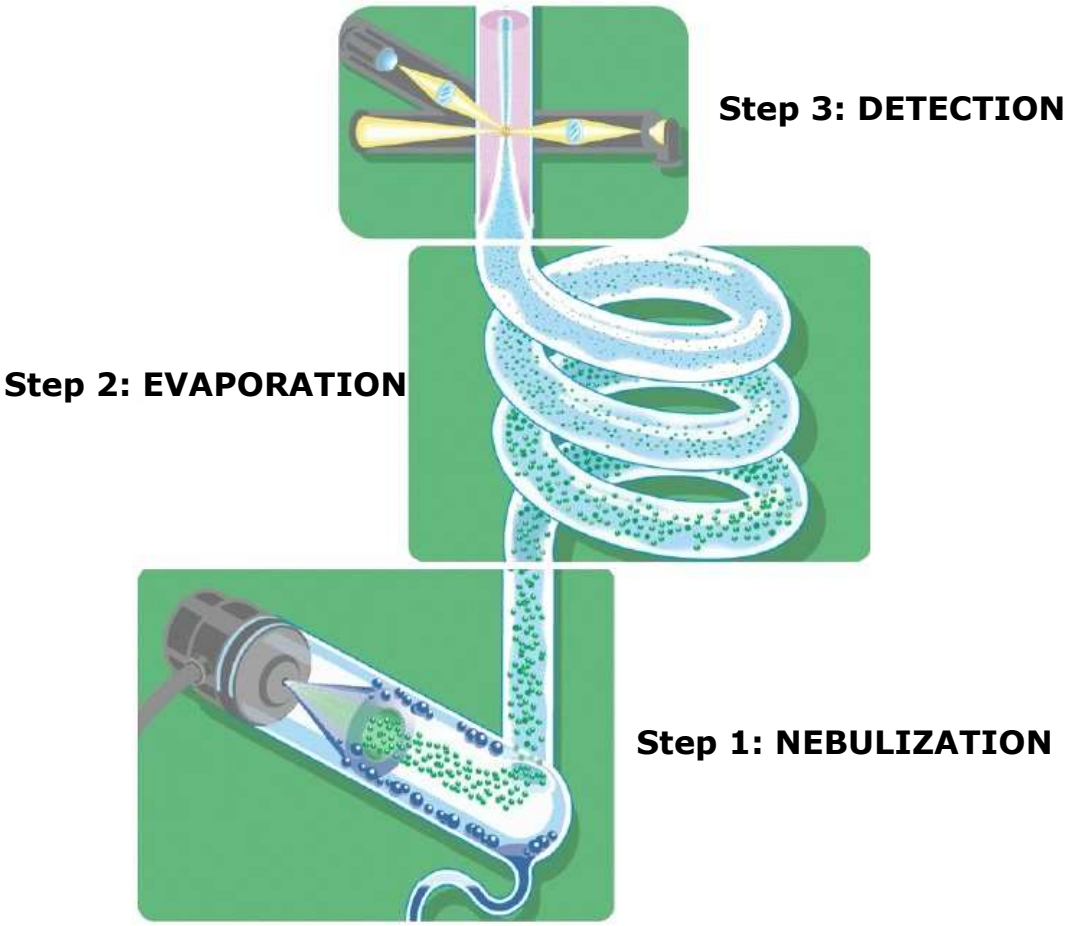


Figure 1-3: Cross-sectional View of the Detector

1.2.1 Nebulization

The eluent from the chromatograph is nebulized by the inlet gas (typically air or nitrogen). At the outlet of the nebulizer, the aerosol travels through a chamber. Large droplets in the aerosol are drawn to a siphon while the fine mist travels to the evaporation tube. The overall design of the nebulizer is shown in Figure 1-4 and the nebulization chamber is shown in Figure 1-5.

Three different nebulizers are available to optimize performance of the detector at different Liquid Chromatography flow rates (see Table 1-1). The user should select the nebulizer to best match the flow rate that will be used with the separation when the detector is ordered (the optimal range for each nebulizer is indicated in Table 1-1). Additional nebulizers are available can be easily installed as described in Section 5.3.5.

Nebulizer	Flow Rate Range	Back Pressure - bar (with water)	Identifying Marks	Part Number
HPLC Nebulizer	100µL/min - 2.5mL/min	4 (1mL/min)	Black Seal 2 Rings White Capillary	**80003**
Combinatorial Chemistry Nebulizer	1.0mL/min - 4.0mL/min	4 (1mL/min)	Red Seal 1 Ring White Capillary	**80008**
Flash Chromatography Nebulizer	100µL/min – 5mL/min	4 (1mL/min)	Black Seal No Ring White Capillary	**80005**

Table 1-1 Nebulizers for the ELSD 80 Low Temperature Evaporative Light-Scattering Detector

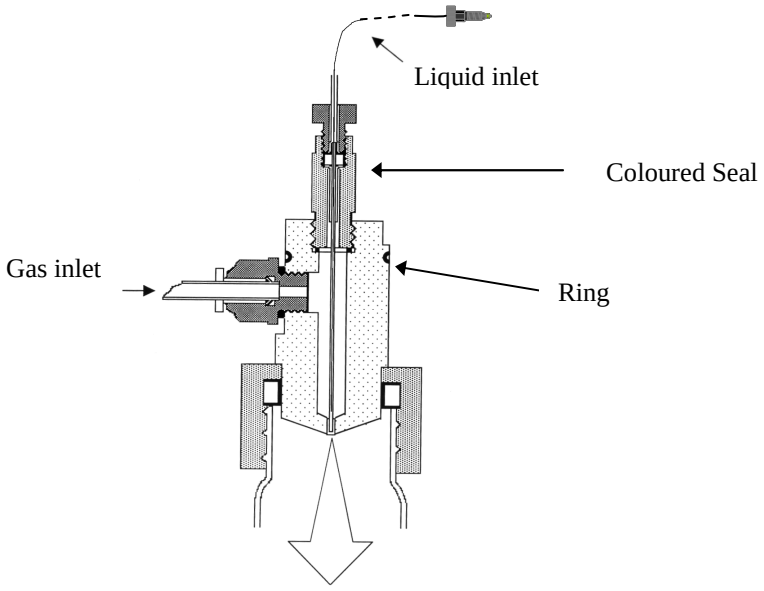


Figure 1-4: Design of the Nebulizer

Figure 1-5: Glassware Chamber

1.2.2 Evaporation of the Solvent

A heated tube is used to evaporate the solvent. The exit of the heated tube leads directly into the detection chamber.

In liquid chromatography, water and organic solvents with low boiling points are typically employed (e.g. CH_3OH, $CHCl_3$, CH_3CN). A typical mobile phase for a reverse phase separation using Evaporative Light-Scattering Detection might be CH_3OH/H_2O (60/40) while a typical mobile phase for normal phase separation might be $C_6H_{14}/CHCl_3$ (60/40).

If acids, bases and salts are used to modify mobile phase to provide the desired separation, they should be able to be readily evaporated, sublimed or decomposed into gases in the evaporation tube. Mobile phase modifiers that are commonly used when an Evaporative Light-Scattering Detector is employed include NH_4OH, $(C_2H_5)_3N$, NH_4 Acetate, NH_4 Formate, HCOOH, CH_3COOH and CF_3COOH.

1.2.3 Detection

The carrier gas transports the microparticles from the heating tube into the detection chamber (Figure 1-6).

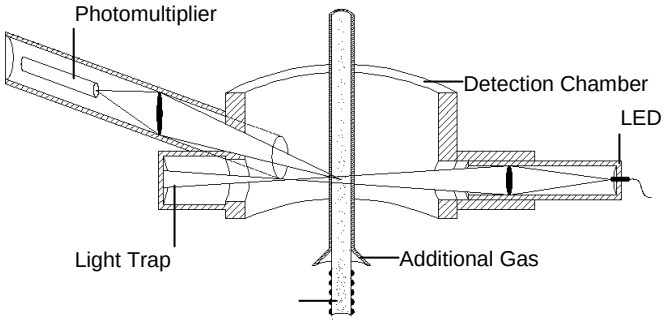


Figure 1-6: The Detection Chamber

The detector chamber contains a Light Emitting Diode (L.E.D.) and a photomultiplier that is positioned at an angle of 120° with respect to the light beam (Figure 1-6). When the carrier gas contains microparticles, the light is scattered and is detected by the off-axis photomultiplier.

The intensity of the scattered light is a function of the mass of the scattering particles and generally follows an exponential relationship, which is shown in equation 1-1.

$$\mathbf{I = k\ m^b} \qquad \textbf{(1-1)}$$

where: **I** is the intensity of light
m is the mass of the scattering particles
k and **b** are constants

A plot of log I versus log m provides a linear response. The values of the constants (k and b) are dependent on a variety of experimental conditions (e.g. the temperature and the nature of the mobile phase).

An inlet to provide additional gas is located immediately before the detector chamber to provide a concentric shield for the carrier gas. This serves to eliminate diffusion of the carrier gas and eliminate contamination of the detection chamber.

1.3 Content of this Manual

This manual is designed to describe the installation, operation, maintenance and troubleshooting of the SEDEX ELSD 80 Low Temperature Evaporative Light-Scattering Detector. It includes:

- *Chapter 2* - **Installation of the Detector**, describes suitable laboratory conditions for the detector and includes information about interfacing the detector to other devices.

- *Chapter 3* - **Start-up Procedure,** describes the role of the various controls and displays on the detector. In addition, this chapter discusses a number of activities to prepare the unit for routine data collection.

- *Chapter 4* - **Operating the Detector**, describes how to operate the Low Temperature Evaporative Light-Scattering Detector. It includes information about starting the unit on a routine basis, collecting data and shutting the unit down.

- *Chapter 5* - **Maintenance and Troubleshooting**, describes a series of activities that should be performed on a periodic basis to ensure maximum performance. In addition, this chapter includes a protocol that can be used to determine the cause of problems that are observed with the instrument.

- A series of appendices are provided which include product specifications, a list of spare parts, Standard Operating Procedures, applications and drivers.

1.4 For Additional Information

The detector is used as a part of an HPLC system and manuals supplied with other components contain important information. For additional information about Evaporative Light-Scattering Detection, the following articles may be of interest:

1. Stolyhwo, A.; Colin, H.; Martin, M.; Guiochon, G. *J. Chromatogr*., Study of the qualitative and quantitative properties of the light-scattering detector. **1984**, 288, 253.

2. Dreux, M.; Lafosse, M.; Morin-Allory, L. *LC-GC Intl*., The evaporative light scattering detector-A universal instrument for non-volatile solutes in LC and SFC. **1996**, 9 (3), 148 and references 1, 3, 11, 22, 23 cited therein.

3. Trathnigg, B.; Kollroser, M. J. *J. Chromatogr. A*, Liquid chromatography of polyethers using universal detectors. V. Quantitative aspects in the analysis of low-molecular-mass poly(ethylene glycol)s and their derivatives by reversed-phase high-performance liquid chromatography with an evaporative light scattering detector. **1997**, 768, 223.

4. Nordbäck, J.; Lundberg, E.; Christie, W.W., *Mar. Chem.*, Separation of lipid classes from marine particulate material by HPLC on a polyvinyl alcohol-bonded stationary phase using dual-channel evaporative light scattering detection. **1998**, 60, 165.

5. Practical SFC and SFE, edited by M. Caude and D. Thiebaut. Chapter 5d ELS detection in SFC p 201-218, M. Lafosse, Harwood Academic Publishers, The Netherlands, **1999**.

6. Fang, L.; Wan, M.; Pennachio, M.; and Pan, J., *J. Comb. Chem*., Evaluation of ELSD for combinatorial library quantitation by RP-LC. ELSD as a universal detector for rapid quantitation in combinatorial chemistry. **2000(2)**, 254.

7. Sims, J.L., *Chromatographia*, Proposed performance qualification and calibration method for evaporative light scattering detectors. **2001**, 53, 401.

8. Petritis, K.; Dessans, H.; Elfakir, C.; Dreux, M., *LC GC Eur*., Volatility evaluation of mobile-phase/electrolyte additives for mass spectrometry. **2002**, 15, 98.

9. Carbohydrate Analysis by Modern Chromatography and Electrophoresis. Edited by Ziad El Rassi. Chapter 30 Carbohydrate Analysis by LC and SFC using ELS detection, M. Lafosse and B. Herbreteau; Journal of Chromatography Library, Vol. 66, p1101-1134; Elsevier, **2002**.

10. Megoulas N.C. and Koupparis M.A., *Critical Reviews in Analytical Chemistry*, Twenty Years of Evaporative Light-Scattering Detection. **2005**, 35, 301.

A more complete bibliography on Evaporative Light-Scattering Detection applications can be obtained from your local distributor.

1.5 S.E.D.E.R.E Location Information

S.E.D.E.R.E has two locations in France:

- Administration is located in Paris.
- Production is located in Orléans.

S.E.D.E.R.E, S.A.S.
9 Rue Parmentier
Parc Volta - BP 27
F-94141 Alfortville, France
Phone: 33 (0)1 45 18 05 18
Fax: 33 (0)1 45 18 05 25
e-mail: manager@sedere.com

S.E.D.E.R.E
Parc du Moulin
841, Boulevard Duhamel du Monceau
F-45160 Olivet, France

Please, visit our web site for additional information or assistance:

www.sedere.com

2 Installation of the Detector

2.1 Overview

This chapter describes how the laboratory should be prepared to optimize the performance of the SEDEX ELSD 80 Low Temperature Evaporative Light-Scattering Detector and indicates how the unit is interfaced to other devices such as the column and the data recording device. When you have successfully installed the unit, refer to Chapter 3 for start-up procedures.

The detector is provided in three different configurations as described in Table 2-1. The first configuration (with HPLC nebulizer) is dedicated to standard HPLC applications, the second one (with CC nebulizer) is dedicated to Combinatorial Chemistry using high eluent flow rates and the third one (with FLASH nebulizer) is dedicated to Flash Chromatography. All models can be provided with RS-232 activated (communication option) to be fully controlled by PC via drivers for Chromatography Software or not. The communication option can be activated afterwards. An accessory kit includes the components indicated in Table 2-2.

Detector Version	Communication Option	115V/60 Hz	230V/50 Hz
ELSD 80 with a HPLC Nebulizer 100µL/min – 2.5mL/min	Without With	**80001** **80001S**	**80000** **80000S**
ELSD 80 with a CC Nebulizer 1mL/min – 4mL/min	Without With	**80401** **80401S**	**80400** **80400S**
ELSD 80 with a FLASH Neb. 100µL/min – 5mL/min	Without With	**80501** **80501S**	**80500** **80500S**

Table 2-1: ELSD 80 Low Temperature Evaporative Light-Scattering Detector Versions

Quantity	Part Number	Description
1	See Table 2-3	Nebulizer
1	**85009-10**	Glass Cell
1	**81000**	Operator's Manual (this manual)
1	**80097**	Start-up kit consists of: 1 Power cable 1 Autozero cable 1 External event cable 1 Signal cable 1 RS-232 cable (if Software Option activated) 6 mm O.D. gas tubing (2 meters + 1 meter sets) 1 set of replacement fuses

Table 2-2: Components Shipped with the ELSD 80 Low Temperature Evaporative Light-Scattering Detector

Nebulizer	Flow Rate Range	Back Pressure - bar (with water)	Identifying Marks	Part Number
HPLC Nebulizer	100µL/min - 2.5mL/min	4 (1mL/min)	Black Seal 2 Rings White Capillary	**80003**
Combinatorial Chemistry Nebulizer	1.0mL/min - 4.0mL/min	4 (1mL/min)	Red Seal 1 Ring White Capillary	**80008**
Flash Chromatography Nebulizer	100µL/min - 5mL/min	4 (1mL/min)	Black Seal No Ring White Capillary	**80005**

Table 2-3: Nebulizers for the ELSD 80 Low Temperature Evaporative Light-Scattering Detector

SEDERE provides a wide range of accessories available (e.g. Gas Regulator with Filter and Manometer [part number **45100**]) to support the operation of the detector. A complete listing of all spare parts and accessories is included in Appendix 2.

2.2 Lifting and Carrying the Detector

☞ Note: **To ensure safe transport and avoid bodily injury, make certain that the detector is lifted by two persons.**

Once the instrument is unpacked, ensure that no cables or tubing are connected when you carry the instrument. The detector should be lifted by the bottom (e.g. place your hands under the instrument). Two persons are needed to ensure easy transport and avoid bodily injury (Figure 2-1).

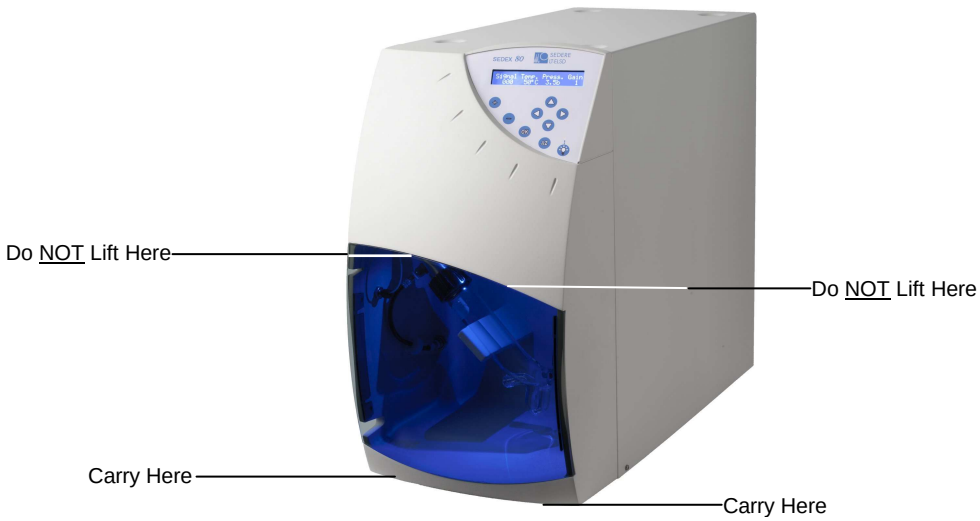


Figure 2-1: Carrying the Detector

2.3 Unpacking the Detector

Carefully inspect all cartons and components against the packing slip to ensure that you have received all items. The nebulizer cell assembly and nebulizer are packed in a separate container for shipping.

If there is any damage to a carton or to any components or if any component appears to be missing, contact both the shipping agent and your local distributor immediately.

If there is any evidence that the main unit has been damaged, do not plug the unit into the power line. Contact your local distributor immediately.

It is recommended that the shipping carton be retained as it can be used if it should become necessary to transport the detector.

2.4 Laboratory Requirements

2.4.1 Power Requirements

The detector is configured for 115V AC/60Hz or 230V AC/50Hz input power (depending on the country to which it is shipped). Ensure that the voltage value indicated on the power connector on the rear panel corresponds to the line voltage in your facility.

The detector requires 115V/1.8A or 230V/1.7A. Check that the power lines can provide sufficient current.

The detector must be connected to a properly grounded three prong plug to ensure proper operation of the instrument. If a two prong outlet is used, make certain that the ground wire is used to ground the instrument. It is recommended that all components of the HPLC system are connected to a common ground.

The detector should not be connected to an electrical line which also serves units with a large power drain or which may be subject to power surges. Such units include refrigerators, ovens, centrifuges and fume hoods.

2.4.2 Gas Requirements

A supply of filtered, oil-free clean gas (e.g. N_2 or air if aqueous mobile phase) is required to operate the detector. Pure gas is not required as gas is only a carrier vector for the solid particles (e.g. air from an air compressor is acceptable if un-reactive with analysis).

Do not use gases that support combustion with combustible solvents!

The gas supply should include a pressure gauge. A filter (0.01µm) and manometer (part number **45100**) is available as an option. Replacement filter cartridges are available as part number **45007**.

2.4.3 Exhaust Venting and Drain Requirements

The black exhaust tube from the detector can be cut and should be directed into a fume hood or exhaust vent. If a vacuum is used, it should be moderate so as to avoid turbulence in the glass cell siphon.

The exhaust and drain should not be allowed to enter the laboratory atmosphere and any appropriate accessory (e.g. solvent filter) should be disposed of in a manner that meets the local regulatory authorities for health and safety requirements.

The vacuum must be moderate to avoid turbulence in the glass cell siphon or liquid spilled into the evaporation tube.

Avoid loops or bends in the black exhaust tubing which could create condensation traps resulting in bad measurement results.

If gas from the hood enters the detector (i.e. a negative pressure exists between the detector and the fume hood), it is possible that foreign material from the hood could contaminate the detector.

Install the drain tubing (it can be cut) in a way to the siphon outlet aligns straight to the waste container –without loops or bends-, so that the waste liquid flows smoothly through the drain tubing.

Fix the drain tubing at the inlet of the waste container so that the end of the drain tubing never dives into the liquid in the container.

☞ Note: **Ensure that the Parafilm™ is removed from the exhaust tube before installing the unit.**

The drain tubing must be directed to an appropriate container regarding to the solvent nature. The user is responsible for decontamination or recycling of any residue, regarding to the local authorities environmental requirements.
Please check your local regulatory authorities for health and safety requirements.

2.4.4 Location of the Detector in the Laboratory

All components of the system (e.g. HPLC pumps, detector) should be located on a sturdy table. The detector should be placed in an area that is free from drafts or significant temperature changes. Do not place it near air conditioning vents, windows, ovens, etc.

When placing the detector in the laboratory, access to the power to disconnect the device (the appliance coupler or the mains plug) must be kept accessible at all time.

☞ Note: **The detector should be placed close to the outlet of the column to minimize extra-column band broadening which would reduce the resolution of the chromatographic separation.**

☞ Note: **As a destructive detector, the ELSD should be the last one in the flow path or can be used with a splitter.**

2.4.5 Environmental Conditions

This instrument has been designed for the following conditions:

- Use inside buildings
- Altitude below 2000 meters
- Ambient temperature from 5°C to 40°C
- Maximum humidity of 80% for temperatures under 31°C, with linear decrease down to 50% at 40°C
- Maximum variations for main power voltage: ±10% from nominal voltage.
- Transitory overvoltage of class II
- Pollution degree: 2

2.5 Installation of the Unit

2.5.1 Gas Supply

The unit is connected to the gas supply via the 6.0mm plastic tubing (supplied) using the fitting on the upper left corner of the supply panel on the back of the detector (Figure 2-2).

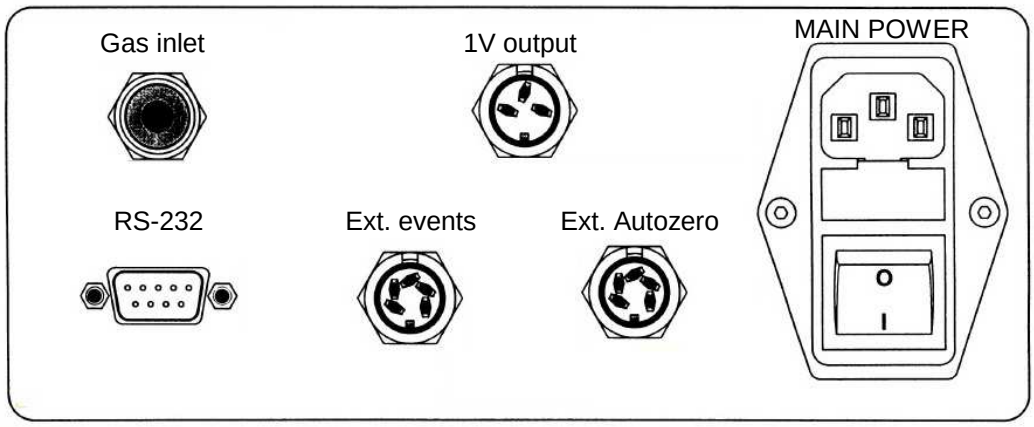


Figure 2-2: Supply Panel

The tubing should be cut and firmly inserted into the fitting as shown in Figure 2-3, after removing Parafilm™ from the detector gas inlet.

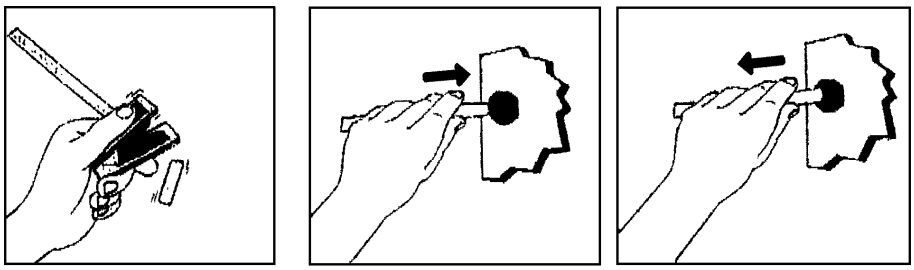
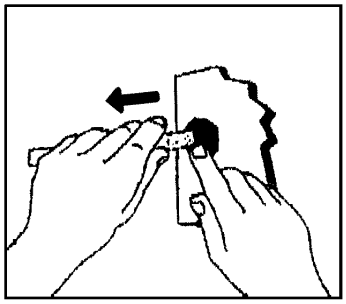


Figure 2-3: Inserting the Gas Inlet Tube

Two pieces of tubing are provided. If you are using the instrument with an external filter, connect the gas source to the filter and then connect the filter to the back of the unit.

Make certain that no tube damage or inappropriate installation could allow a gas leak in laboratory.

To remove the gas inlet tube (if necessary); refer to Figure 2-4.



To remove the tube, dissengage the grab ring teeth by a simple manual pressure on the push sleeve and withdraw the tube from the fitting.

Figure 2-4: Removal of the Gas Inlet Tube

2.5.2 Vent the Exhaust Line to a Fume Hood

The black exhaust line on the back of the unit should be vented to a fume hood. Make certain that the fume hood withdraws gas from the detector (i.e. there should be a positive pressure between the detector and the hood). Verify that no tube damage or inappropriate installation could allow a gas leak in laboratory.

The vacuum must be moderate to avoid turbulence in the glass cell siphon or liquid spilled into the evaporation tube.

Avoid loops or bends in the black exhaust tubing which could create condensation traps resulting in bad measurement results.

If gas from the hood enters the detector (i.e. a negative pressure exists between the detector and the fume hood), it is possible that foreign material from the hood could contaminate the detector.

Install the vent tube so that it cannot become blocked or bent, or restrict the gas flow from the detector to the hood in any way.

Avoid long tube installations in upward direction creating condensation dropping back into the detector.

If an extension tube is required (i.e. the supplied tube is not long enough), a suitable length of ¾"ID of PVC tubing can be fitted over the exhaust tubing.

2.5.3 Electrical Connections

All electrical connections are made via the supply panel (Figure 2-2) on rear panel.

a) Connecting the Recorder/Integrator:

If a recorder or integrator is employed, connect the recorder input to the 1V output terminal on the rear panel of the detector (Figure 2-2) and to the appropriate socket on the recorder/integrator.

b) Connecting the External Autozero:

If the external autozero function is to be employed, plug the cable that is supplied into the *Ext Autozero* socket on the detector (Figure 2-2) and to the appropriate socket on the controlling device (e.g. autosampler, pump, etc.).

Refer to section 4.3.2 to operate external autozero signal.

c) Connecting the External Events Cable

If the external events functions are to be employed, plug the cable that is supplied into the appropriate socket on the back panel of the detector (Figure 2-2) and to the appropriate socket on the controlling device (e.g. autosampler, pump, etc.).

The white cables are contact closure “output” cables that provide the ready/non-ready information to an external device. The detector will be in the “not-ready” mode (the contact will be in closed position) if any one of the following conditions is observed:

- The lamp is off.
- The temperature is not at the indicated setpoint.
- The temperature is at the indicated setpoint but is not stable.
- The pressure is below 3.0bar.

☞ Note: **The controlled device electrical consumption mustn't exceed 20mA under 12V DC.**

The blue cables are contact closure “input” cables that are used to power the unit down (see Section 3.2.2.h) via a signal from an external device to the detector.

d) RS-232 Port (**Only for ELSD 80 Detector with Software RS-232 activated**)

If a personal computer is used with the detector, the detector should be connected to the computer via the RS-232 port using the supplied cable.

☞ Note: **Software drivers for a full LT-ELSD control are available for:**
EZChrom *Elite*™ (SEDERE part number 85090),
ChemStation™ (SEDERE part number 85089),
Xcalibur™ (SEDERE part number 85093),
Clarity™ (The driver is part of Clarity from Version 3.0.2).
This also avoids using an A/D converter by a direct connection to a free RS-232 COM port. Please refer to Appendix 5.

In these cases, only two cables are then required:
• RS-232 cable, to be connected between the detector and the computer RS-232 (avoid USB/RS-232 converter).
• Autozero cable, to be connected for a "Start" information on the controlling device (e.g. autosampler). In this mode, the detector doesn't proceed to an Autozero, it uses it as a signal synchronization for the driver. Please refer to Appendix 5.

Not using the AutoZero connection for a "Start" information will impair the synchronization of the Signal and may not generate the final report and/or impair the retention time reproductibility.

e) Connecting the Power Cord
Place the ON/OFF switch to the OFF position and plug the power cord into the socket on the rear panel of the detector.

Do not turn on the power at this time.

The power cord of this detector contains three wires which must be connected to a grounded line. All components of the chromatographic system should be connected to a common ground. If a two wire outlet is used, make certain that an adapter is used to connect the third wire to ground.

2.5.4 Installing the Nebulizer/Glass Chamber Assembly

Parafilm™ is used to cover various openings inside the compartment, nebulizer and glassware to prevent dust particles from entering the detector during shipment.

☞ Note: **When installing the transparent black front cover, first fix its right side, and then push its left side. When removing the front cover, pull only its left side.**

The installed Nebulizer/Glass Chamber assembly is shown in Figure 2-5.

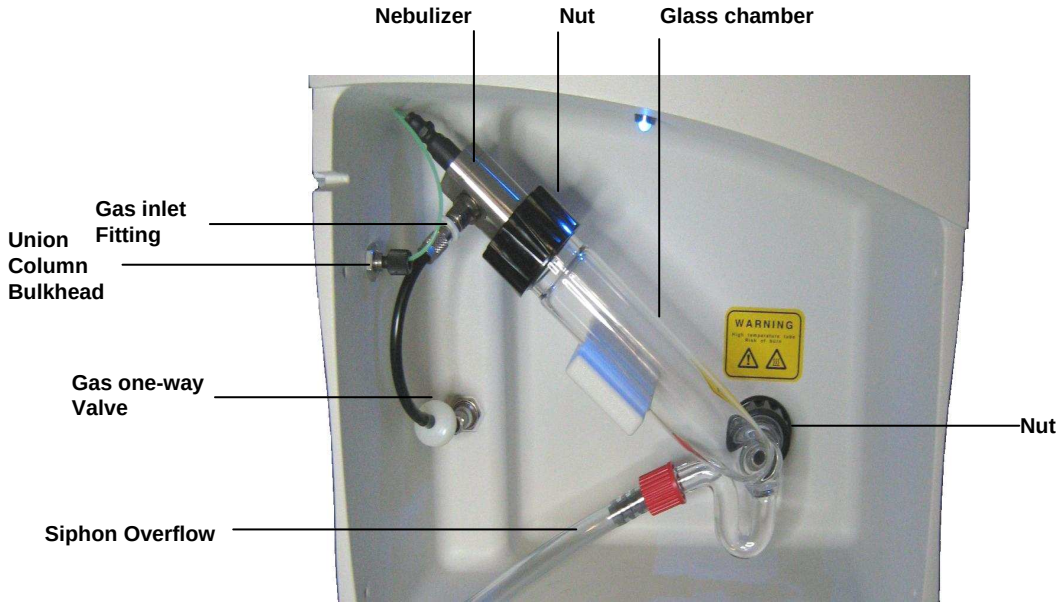


Figure 2-5: Installing the Nebulizer/Glass Chamber Assembly

To install the assembly:

a) Remove the Parafilm™ from all detector openings and from the nebulization cell (these coatings are used to prevent dust particles from entering the instrument during shipment).

b) Position the glass chamber as shown in Figure 2-5 and tighten the black nut at the bottom. The glass chamber should be flush with the back wall as shown in Figure 2-6.

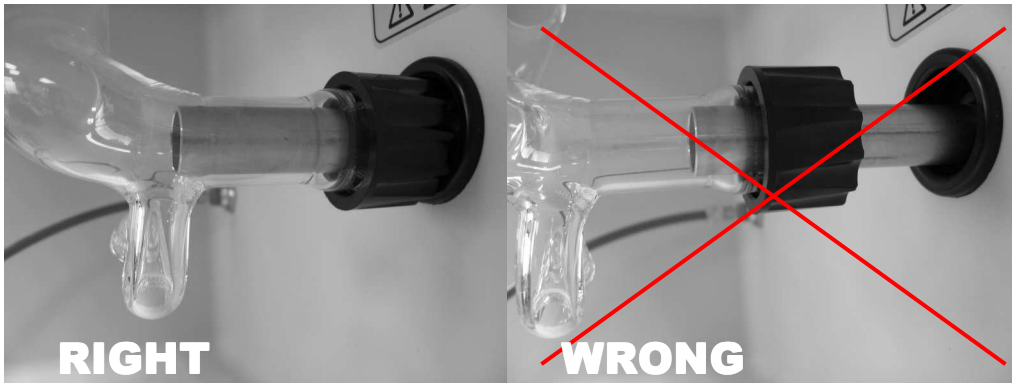


Figure 2-6: Fixing the Tip of Glass Chamber

c) Use the large black nut to position the nebulizer on the glass chamber.

d) Screw the inlet fitting in the bulkhead on the left side of the compartment. Special care must be taken when positioning this fitting. The nebulizer is terminated with a small piece of Teflon tubing with an outer green sleeve. For proper operation, the Teflon tubing must extend less than 2mm past the end of the green sleeve (Figure 2-7).

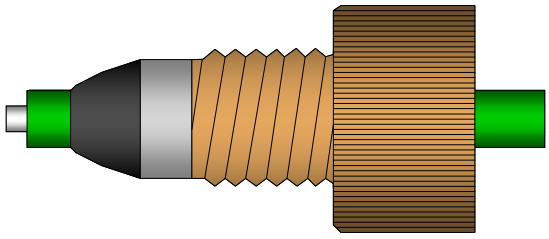


Figure 2-7: Nebulizer Inlet Fitting

Fill the siphon overflow on the nebulizer/glass chamber assembly with the mobile phase that will be used for the separation. If you are using a very volatile solvent (e.g. hexane or CH_2Cl_2), then use water to fill the overflow. The liquid should fill the bent part of the siphon, but should not pool in the bottom of the evaporation tube.

Make sure that no liquid leak could affect the detector performance or create laboratory pollution.

2.5.5 Connecting the Siphon Overflow

Attach a Tygon drain tube assembly to the end of the siphon tube using the tapered hose connector and lead the tube to waste and drain. Locate the tube in such a way that the discarded part of the solvent can flow freely from the siphon and ensure that the end of the tube is not immersed in the collected liquid. Make sure that liquid waste container complies with the solvent nature.

Ensure that no siphon liquid leak could affect detector performances or create laboratory pollution.

Install the drain tubing (it can be cut) in a way to the siphon outlet aligns straight to the waste container -without loops or bends-, so that the waste liquid flows smoothly through the drain tubing.

Fix the drain tubing at the inlet of the waste container so that the end of the drain tubing never dives into the liquid in the container.

A drain tube with a bend or immersing the liquid will create pressure fluctuations in the detector and will result in bad measurement results.

If the solvent that you are using is not compatible with Tygon (e.g. THF), use a piece of Teflon tubing or any material you know compatible with your solvent in its place.

Please check your local regulatory authorities for recycling solvents and health and safety requirements.

2.5.6 Connecting the Nebulization Gas to the Nebulizer

Attach the nebulization gas tube coming out from the front panel to the nebulizer gas inlet fitting located on the nebulizer side. Refer Figure 2-5.

☞ Note: **Make sure you are using the correct black gas tubing orientation, where the white one-way valve is at the lower end (near the gas arrival).**

2.5.7 Connecting the Column

Attach the fitting from the bulkhead to the outlet of the column.

2.5.8 Powering Up the Unit

Place the ON/OFF switch to the OFF position and plug the instrument into the wall socket. Turn on the unit via the ON/OFF switch. The display will present the version number and date it was created for a few seconds (the version number should be recorded as it may be required for service or troubleshooting) and will then present the Software option information (activated or not) and then will present the signal (which should be 0mV or very close to it), the temperature (which should be the ambient temperature), the pressure (which should be zero or very close to it) and the gain. Avoid leaks at all connections and check for leakages when you switch the pump on. Install the black front panel cover, first fix its right side, and then push its left side.

☞ Note: **The liquid level in the siphon must be stable and should be equal at both sides. If the vacuum is too strong, liquid is drawn into the evaporation tube or generate air bubbles from the drain tube and both resulting in bad measurement results.**

Refer to Chapter 3 to prepare the unit for routine operation.

3 Start-up Procedure

3.1 Overview

This chapter describes:

- the role of the controls and the digital display on the control panel
- the start up test procedure
- how to prepare the instrument for operation

3.2 The Control Panel

The Control Panel (Figure 3-1) includes a digital display and a number of buttons that are used to enter data.

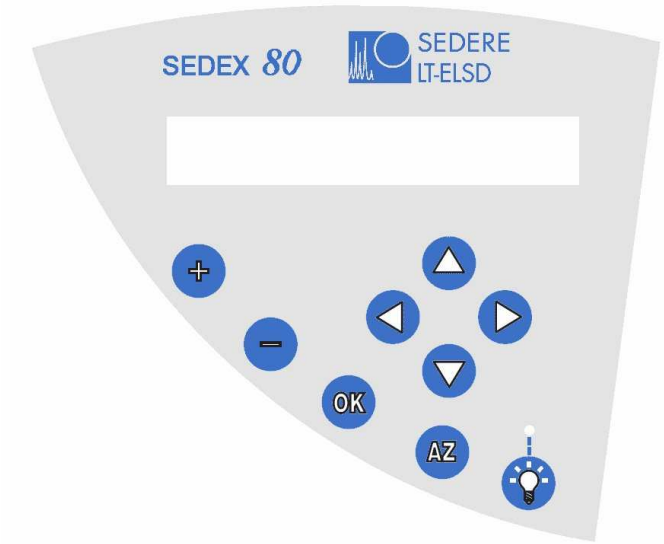


Figure 3-1: The Control Panel

3.2.1 The Digital Display

The digital display presents information about the present status of the detector and is used to enter a variety of parameters. When the detector is powered up, the display will present a greetings message that includes the version number and date that the version was created for a few seconds.

After the detector has completed the initialization procedures, the *Status* screen (Figure 3-2) will be presented. The signal should be close to zero.

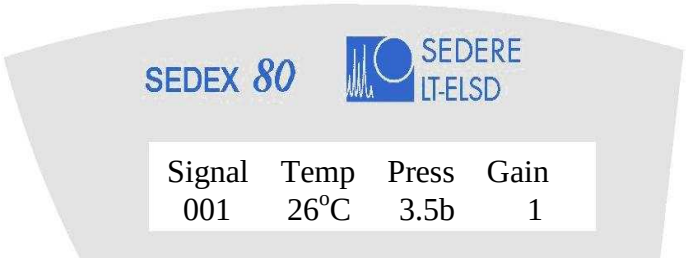


Figure 3-2: The Status Screen

The user interface is provided via a series of screens that are described in Section 3.2.2. Some screens present information about the instrument status and cannot be edited by the user (e.g. the *Status* screen), while other screens (e.g. the *Temperature/Gain* screen, Figure 3-4) are used to enter the desired set points.

The keys on the control panel are used to provide the following functions:

- ⊕ used to increase the present value of a user settable parameter (e.g. the offset) by 1 unit. If you keep the key pressed, the rate of change of the parameter will increase.
- ⊖ used to decrease the present value of a user settable parameter (e.g. the offset) by 1 unit. If you keep the key pressed, the rate of change of the parameter will increase.
- OK validates the value of the parameter that you have edited.
- AZ sets the present signal for the detector to zero.
- is used to power up the LED in the detector. When the LED is lit, the keyboard LED immediately above the button will be illuminated.
- △ ▽ changes the active line on the display to the next (previous) line or the next (previous) screen.
- ◁ ▷ moves the cursor on the display to the next (previous) field.

3.2.2 The User Interface

The *Status* screen (Figure 3-2) is the default screen and is presented after initialization of the detector. In addition, it will be automatically presented again if you have accessed another screen and have not made any keystroke within a few seconds.
Each parameter change must be validated with OK or the change will not be applied.

3.2.2.a The *Status* Screen

The *Status* screen (Figure 3-2) presents the present conditions of the detector. This screen cannot be edited, but the desired offset can be set via the *Offset* screen (Figure 3-3), the temperature and gain can be set via the *Temp/Gain* screen (Figure 3-4) and the pressure units can be selected via the *Noise Filter/Pressure Unit* screen (Figure 3-5).
Temperature value blinks if desired temperature is not reached and stable. Pressure value blinks is gas pressure is lower than 3.0bars.

When the ▽ button is pressed; the *Offset* screen (Figure 3-3), which is used to select the desired offset is displayed.

3.2.2.b The *Offset* Screen

Figure 3-3: The Offset Screen

To increase the offset value, click on the + key. If you press the button quickly, the offset will increase by 1; if you press and hold the button, the value will increase at the rate of 20mV/sec.

Once you have set the desired offset, press the OK button to validate the new value.

When the instrument is Autozeroed, the Autozero operation updates the Offset value to set the Signal to 0mV.

Press the ▽ button to access the *Temp/Gain* screen (Figure 3-4).

3.2.2.c The *Temperature/Gain* Screen

Figure 3-4: The Temp/Gain Screen

The *Temp/Gain* screen is used to set the desired Temperature and Gain. When the screen is accessed, the cursor is on the *Temp* setting. This setting can be changed with the ⊕ and ⊖ buttons and validated by the OK button. The temperature range is 20 to 100°C.

☞ Note: **When the detector is initially powered up or if you change the temperature, the temperature may first overshoot the setpoint slightly and then stabilize at the desired point. This initial overshoot is due to the regulation of the instrument and should not be a concern.**

☞ Note: **To maintain appropriate temperature control, when the lowest temperature is required, it should be set at least 5°C above ambient. Temperature stabilization typical time is 30 minutes. Please, note that the stabilization time for temperature close to the ambient temperature may be higher.**

When you press the ▽ button, the *Gain* field can be edited in the normal manner. The gain range is from 1 to 12, each increment of one unit increases the gain by a factor of 2 (e.g. if you change the gain from 1 to 4, the gain is increased by a factor of 8) and the full range of the gain is 1 to 2048. After you have validated the desired gain setting, press the ▽ button again to display the *Autozero Offset* screen (Figure 3-4b).

3.2.2.d The *Autozero Offset Screen*

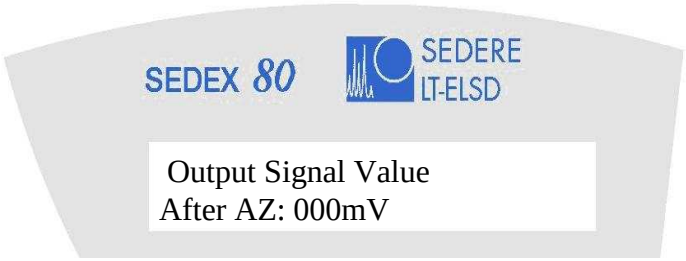


Figure 3-4b: The Autozero Offset Screen

This screen is used to allow the signal to reach the desired value when performing an autozero (by keyboard or external contact closure).

This function can be helpful when the user wishes to have a positive signal value instead of zero, especially with some acquisition systems which have only positive signal acquisition capability.

This setting can be changed with the ⊕ and ⊖ buttons and validated by the OK button.

After you have set the desired autozero offset, press the ▽ button to display the *Noise Filter/Pressure Unit* screen (Figure 3-5).

3.2.2.e The *Noise Filter/Pressure Unit* Screen

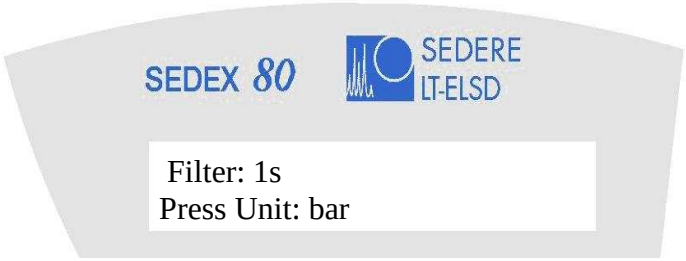


Figure 3-5: The Noise Filter/Pressure Unit Screen

The *Filter/Pressure Unit* screen is used to indicate if digital filtering is desired for the signal data (improves signal-to-noise ratio) and the desired units for the pressure display.

When the screen is presented, the cursor is on the *Filter* field. By pressing ⊕ or ⊖ keys, you change the filtering strength within the following range:

- "NO": no filtering.
- 0.5s: 0.5 second moving average filtering.
- 1s…10s: 1 to 10 seconds moving average filtering.

☞ Note: **For better results, the digital Filter should be used unless the peak(s) of interest are very poorly resolved (e.g. when Rs<1.5).**
Default value is 1s, corresponding to a peak width of approximately 2 seconds at half-height. User manual section 4.5.5 details Filter optimization.

If you have changed the value, press OK to validate it before you press the ▽ button to access the *Press Unit* line. The pressure unit line allows for the selection of KPa, bar or psi for pressure units, the desired selection is made via the ⊕ or ⊖ key, and validated by the OK key.

When you press the ▽ button, the *LED* screen (Figure 3-6) will be displayed.

3.2.2.f The *LED* Screen

Figure 3-6: LED Screen

The *LED* screen is used to turn the light source On/Off and is equivalent to the *Light Source* button on the control panel. Use the ⊕ button followed by the OK button to turn the LED on and the ⊖ button followed by the OK button to turn it off.
The *# hours* field indicates the number of hours that the LED has been in use. The lifetime of the LED is approximately 5000h. When this period has been reached, a message indicating that the maximum usage of the lamp has been exceeded will be presented when the unit is powered up and the orange LED on the keyboard blinks. To reset the field, move the cursor to the *Reset Time Elapsed* field and validate OK by pressing on the key.

☞ Note: **The *Reset Time Elapsed* field should be validated with OK only when you change the lamp.**

When you press the ▽ button, the *Gas Valve* screen (Figure 3-7) will be displayed.

3.2.2.g The *Gas Valve* Screen

Figure 3-7: The Gas Valve Screen

The *Gas Valve* screen is used to open/close the gas valve and to setup a program to close the gas valve after a user selected time period. To use this feature, move the cursor to the time field, indicate the appropriate time, then move the cursor to *Off* and use the ⊕ or ⊖ key to select *On* and press OK.
When you press the ▽ button, the *External Shutdown* screen (Figure 3-8) will be displayed.

3.2.2.h The *Power Down* Screen

The *Power Down Mode* screen (Figure 3-8) is used to indicate which features should be shut down upon receipt of a power down signal from an external source or from the menu.

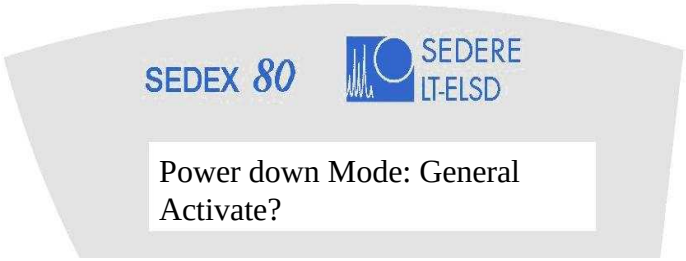


Figure 3-8: The Power Down Screen

The three options provided for external shutdown are summarized in Table 3-1.

Mode	Photomultiplier	Lamp	Heating	Gas flow
General	Off	Off	Off	Off
Standby	Off	Off	On	Off
Cleaning	Off	Off	On	On

Table 3-1: Power Down Options

To select the desired *Power Down* mode, use the ⊕ or ⊖ key to access the desired mode and then press OK to validate the selection.

☞ Note: **It will take a few minutes to attain operating status from G*eneral* power down mode, as the temperature must stabilize.**

Once the Power Down mode has been chosen and validated, the detector can be powered down in two ways:

- **External event cable power down contact closure**: The detector will stay in the selected power down mode while the contact remains closed. It comes back in normal mode when the contact closure is released.
- **Power down screen**: Press the ▽ button to access the power down screen, then press again the ▽ button to place the cursor on the *Power down activate* line. Validate with OK to put the detector in power down mode.

☞ Note: **To leave the power down mode, release the contact closure if power down has been activated by external event or press any key if power down has been activated from the *Power down* screen.**

When the cursor is on the *Power down activate* line, pressing the ▽ button will present the *Total Lifetime Elapsed* screen (Figure 3-9).

3.2.2.i The *Total Lifetime Elapsed* Screen

Figure 3-9: The *Total Lifetime Elapsed* Screen

The *Total Lifetime Elapsed* information screen indicates the usage of the detector and cannot be edited by the user. When you press the ▽ button, the *Serial Number* screen (Figure 3-10) will be displayed.

3.2.2.j The *Serial Number* Screen

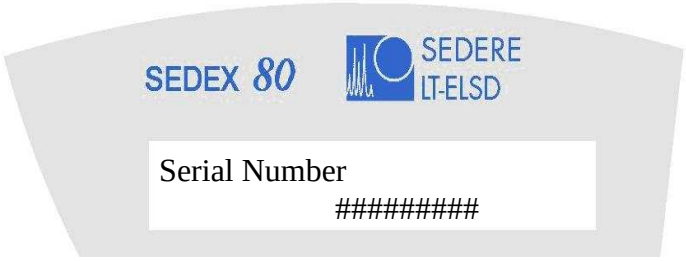


Figure 3-10: The Serial Number Screen

The *Serial Number* screen cannot be edited by the user. The last character indicates the detector hardware revision. When you ▽ press the button, the *Firmware* screen (Figure 3-11) will be displayed.

3.2.2.k The *Firmware* Screen

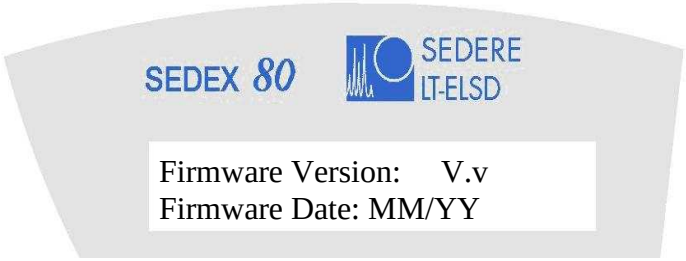


Figure 3-11: The Firmware Screen

This information screen presents the firmware version and date, where MM is the month, and YY the year. The *Firmware* screen cannot be edited by the user.

When you press the ▽ button, the *Factory Menu Code* screen (Figure 3-12) will be presented.

3.2.2.l The *Factory Menu Code* Screen

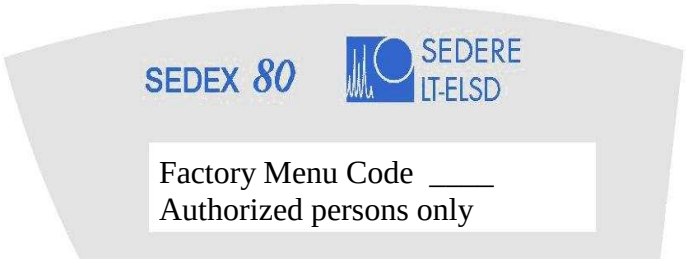


Figure 3-12: The Factory Menu Code Screen

The *Factory Menu Code* screen is used by the service engineer to access a variety of service features and is not designed to be employed by the user.

3.3 Initial Test Procedure

3.3.1 Preliminary Activities

This section presents a protocol that can be used to ensure that the detector is working in the proper way. A detailed standard operating procedure (I.Q./O.Q./P.Q.) is presented as Appendix 3.

☞ Note: **When the instrument is set-up, the procedures indicated below should be performed to determine the specific characteristics of your unit. We suggest that you save the results in a permanent location, as they can be very useful when you are performing troubleshooting activities.**

☞ Note: **Before starting the tests for a new instrument or after storage, flush the detector with water at a flow of 1mL/min at least 15 minutes.**

The following activities should be performed:

a) Power up the instrument. When the detector is shipped from factory, the gain is set to 1 and the offset to 0mV. The *Signal* screen should indicate 000 (or a very small signal).

b) Access the *Temperature/Gain* screen, set the temperature to 50^{o}C and press (OK). View the *Status* screen and verify that the temperature is rising to the setpoint on the *Status* screen. Temperature regulation is stable when the Temperature value stops blinking.

☞ Note: **When the detector is initially powered up or if you change the temperature, the temperature may first overshoot the setpoint slightly and then stabilize at the desired point. This initial overshoot is due to the regulation of the instrument and should not be a concern.**

c) Provide gas to the detector and adjust the pressure to 3.5bar (51psi). If the pressure is less than 3.0bar (44psi), the pressure value blinks, indicating that the detector is not ready.

☞ Note: **Make certain that the pressure of gas supplied to the detector is less that 4.5bar (67psi). If the pressure increases above 4.5bar, the pressure sensor may be damaged. This damage is not covered by the warranty.**

If you have an external gas gauge, make sure that the external reading and the reading on the *Status* screen are in good agreement.

d) Press the AZ button. The signal should be close to zero and remain constant.

e) Set the noise filtering to **1s** (Refer to Section 3.2.2.e).

3.3.2 Electronic Noise Test

To determine the electronic noise:

a) Do not switch the light source on. Do not switch the HPLC pump on (no solvent flow).

b) Make sure that the siphon is filled and the bulkhead is blocked with Parafilm™ to avoid a Venturi effect.

c) Set gas pressure to 3.5bar and temperature to 50°C. Wait for stable temperature.

d) Set gain 12 and monitor the signal for a period of 5 min. The variation in the signal should be less than +/- 2mV (there may be some spiking of the signal).

e) Record the level and autozero the detector again.

3.3.3 Background Noise (Stray Light) Test

To determine the background noise:

a) Do not turn on the HPLC pump (no solvent flow).

b) Make sure that the siphon is filled and the bulkhead is blocked with Parafilm™ to avoid a Venturi effect.

c) Set gas pressure to 3.5bar and temperature to 50°C.

d) Switch on the light source.

e) Change the Gain to 1.

f) Set the offset to 0mV.

g) Set the offset after Autozero to 0mV (Refer to Section 3.2.2.d).

h) Autozero the detector.

i) Change the Gain to 12.

j) Wait 15 minutes for stabilization and record the signal level. The expected level is typically 100mV to 150mV. The exact value will vary slightly and small deviations should not be a cause for concern. The values for your unit are provided on the test report supplied with the detector.

3.3.4 Solvent Noise Test

To determine the solvent noise:

a) Ensure that the gas is flowing at 3.5bar (51psi), the temperature is set to 50°C and stable and the pump is switched off.

b) Switch on the light source and set the gain to 12 and monitor the signal. Do not autozero the detector. The signal may be negative.

c) Bypass the column and connect the detector to the mobile phase delivery system and pump the solvent that you expect to use for your analyses through it at a flow rate of 1mL/min.

d) Monitor the baseline for a few minutes.

- If water is used as the solvent, the signal should be less than 10mV. Higher values could be observed if non-HPLC grade water (with a higher non-volatile residue) is used.
- If an organic solvent is used, the signal should be less than 200mV.
- For mixed aqueous/organic solvents, the expected signal is approximately linear with respect to the concentration of organic phase in the solvent (e.g. a water/organic solvent (50:50) mixture should provide a signal of approximately less than 100mV).

☞ Note: **The purity of the solvent is critical for a low background noise. The sensitivity is inversely proportional to the solvent noise.**

☞ Note: **In most cases, distilled water and HPLC grade solvents are satisfactory. When you are comparing solvents from different sources, the most critical parameter is the *Residue After Evaporation*; this parameter should be less than 1ppm to maximize the sensitivity of the detector.**

If the instrument fails the Solvent Noise test, it is most likely due to an impurity in the solvent rather than a fault with the instrument. If changing the solvent source does not solve the problem, it may be necessary to decontaminate the instrument as described in Section 5.4.2 or clean the nebulizer as described in Section 5.3.5.

When filtering the solvent, verify that it does not extract any contaminant from the filter.

The mobile phase should not contain non-volatile solvent modifiers. Volatile solvent modifiers (e.g. CHOOH, CH_3COOH, CF_3COOH, NH_4 Formate, NH_4, Acetate, $(C_2H_5)_3N$)…) can be used, but they may increase the noise level at high gain settings. In addition, the solvent should not contain preservatives, (e.g. Tetrahydrofuran may contain BHT as a stabilizer).

3.3.5 Column Noise Test

☞ Note: **When strongly retained compounds are slowly eluted from the column, excessive noise will be observed.**

To determine the column noise:

a) Turn off the pump and connect the column.

b) Restart the pump and allow the mobile phase to flow through the system. It is suggested that you flush the column with a strong solvent for a few minutes before attaching it to the detector. The flow rate to be used is dependent on to the column ID and is indicated in the following table.

Column ID (mm)	Flow Rate (µL/min)
4.6	1000
2.1	208
1.0	47
0.8	30
0.32	4.8

Table 3-3: Flow Rate versus Column Diameter Indication

c) Set the gain to 12 and monitor the baseline for a few minutes. A suitable column will provide a baseline that is 20-50mV above the solvent baseline.

☞ Note: **If the mobile phase contains acidic modifiers (e.g. CF_3COOH), disconnect the detector and wash the HPLC system for 12h before starting to analyze unknown samples. This wash should be performed after the column noise test is completed, but need not be performed after each analysis.**

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4 Operating the Detector

4.1 Overview

This chapter describes the operations that should be performed on a routine basis when you want to collect chromatographic data using the ELSD 80 Low Temperature Evaporative Light-Scattering Detector. In this discussion, we assume that you have demonstrated that the instrument is operating in an acceptable manner (see Chapter 3) and that the general chromatographic conditions for the separation have been determined.

4.2 Preparing the Detector for Operation

To prepare the detector for operation:

a) Power up the detector by pressing the switch on the rear panel.

b) Open the gas distribution valve and set the pressure to 3.5bar (51 Psi). The pressure is indicated on the *Status* screen.

c) Ensure that the overflow siphon for the nebulization chamber contains sufficient liquid. If necessary, pump few mL of solvent through the instrument to fill the siphon.

d) Select the desired temperature. The temperature is set on the *Temp/Gain* screen, which is accessed by pressing the ▽ button two times when the *Status* screen is displayed.

e) Start the mobile phase flow through the instrument and allow the overall system to operate for at least 15 minutes to ensure that all components are equilibrated and a stable baseline is obtained.

☞ Note: **The Solvent Noise test (Section 3.3.4) and the Column Noise test (Section 3.3.5) should be performed to verify that the detector is functioning in a proper manner.**

☞ Note: **The liquid level in the siphon must be stable and should be equal at both sides.**

4.3 Auto-zeroing the Detector

4.3.1 Manual Auto-zeroing of the Detector

To auto-zero the detector:

a) Set the Gain to the desired value. The gain is set on the *Temp/Gain* screen, which is accessed by pressing the ▽ button two times when the *Signal* screen is displayed.

b) Press the AZ button. The detector will be automatically auto-zeroed at this point.

c) If the signal is to be offset, set the offset at this time. The *Offset* screen is accessed by pressing the ▽ button when the *Status* screen is displayed.

☞ Note: **The offset must be selected after the detector is auto-zeroed, as the Auto-zero operation sets the signal to 0.**

☞ Note: **If you change the gain selection, make sure that the detector is auto-zeroed again before taking any measurement.**

4.3.2 External Auto-zeroing of the Detector

If desired, the auto-zero command can be initiated by an external device such as the HPLC system controller. To employ this feature, a cable from the external device is plugged into the Ext. Autozero socket on the rear panel (Section 2.5.3).

To auto-zero the detector, a contact closure signal or a TTL signal is used to short circuit the contacts. The signal should be at least 1 sec long, with a maximum current of 20mA at 5V.

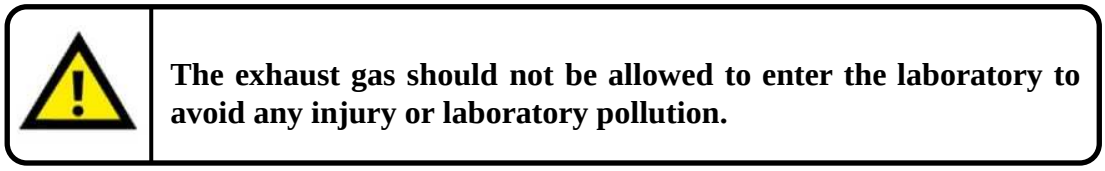
If a TTL signal is used please make sure to use the correct polarity identified on the cable.

4.4 Routine Operation of the Detector

In general, operation of an HPLC system with Evaporative Light-Scattering Detection is similar to operation of the system with other detectors.

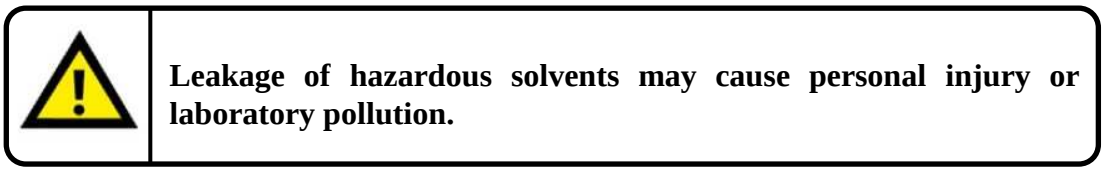
During operation of the detector, the following points should be considered:

a) Make certain that the exhaust from the detector is led into a fume hood or other device and make sure that there is a continuous flow of gas through the detector (i.e. no constriction). If a vacuum is used, ensure that the vacuum effect will not disturb the detector (Section 2.5.2).



b) Ensure that the siphon is filled with liquid at all times. The overflow from the siphon should be collected in a suitable container.

c) Make sure that all flow connections are properly tight. In case of any leak, switch off the pump immediately and remove the liquid.



d) Never exceed a gas pressure of greater than 4.5bar (67psi).

e) Avoid the use of solvent or compounds that could corrode the detector. The mobile phase is in contact with Glass and Teflon tubing and the evaporation tube is made of Stainless Steel.

4.5 Optimizing Performance

4.5.1 Selecting the Optimum Temperature

There are two factors that should be taken into account when selecting the optimum temperature for the detector:

- Increasing temperature will optimize the evaporation of the mobile phase.
- Decreasing temperature will minimize the decomposition of thermally labile compounds and the volatilization of semi-volatile compounds.

A very reasonable start is to set the temperature to 60°C if an aqueous mobile phase is used and 40°C if an organic mobile phase is used (these temperatures are suggested for a flow rate of 1mL/min). At higher flow rates, more elevated temperatures may be required to minimize the noise.

☞ Note: **If the mobile phase used is not easily volatile, such as DMSO or DMF, temperature should be increased to allow correct evaporation process.**

The temperature can be readily adjusted during the method optimization process.

If you suspect that the compound of interest is thermally labile or semi-volatile, a lower temperature could be used to improve the sensitivity by reducing the thermal decomposition or evaporation. For a given flow rate and solvent, there is, however, a point at which the noise in the chromatogram is dramatically increased because not all of the mobile phase is vaporized.

As an example, consider the analysis of caffeine with evaporation temperatures of 30°C and 60°C (Figure 4-1) [the conditions for the separation are - Column: ODS Kromasil™ (5µm, 30 x 2.1mm), Sample: 4µL (10mg/L) Caffeine]. Eluent: Water, 0.2mL/min, temperature as indicated). It is clear that the use of a low temperature provides significantly better sensitivity for volatile and thermally sensitive compounds.

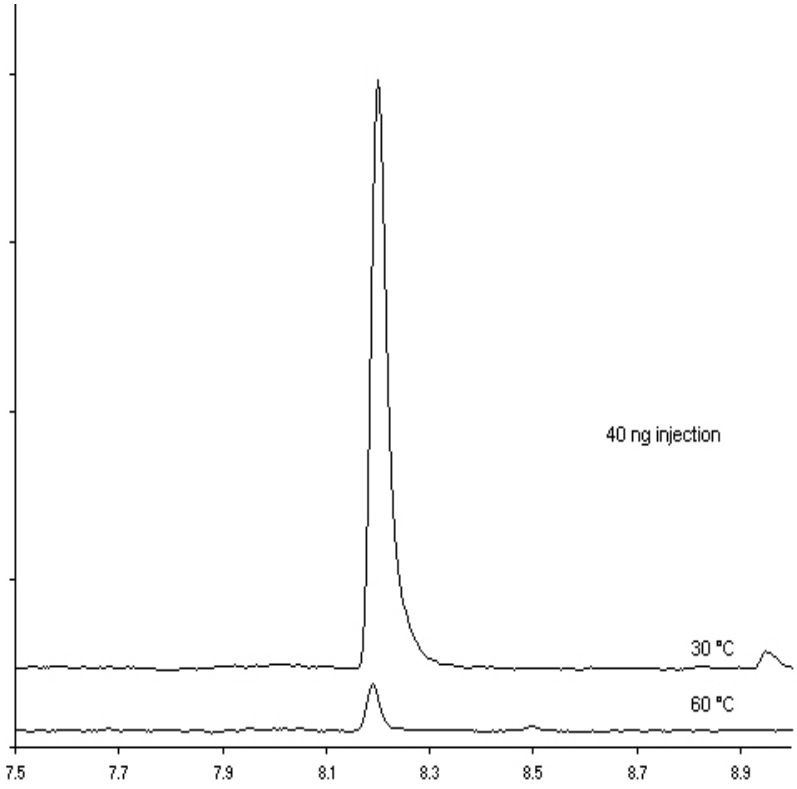


Figure 4-1: Chromatogram of Caffeine at Various Temperatures

The minimum temperature that can be used is dependent on the flow rate and the nature of the mobile phase.

4.5.2 Optimizing the Mobile Phase

Particulate matter in the mobile phase will increase the background noise.

The purity of the solvent is a critical issue in the noise. When filtering the solvent, verify that it does not extract any contaminant from the filter.

The purity of the solvent is critical for a low background noise. The sensitivity is inversely proportional to the solvent noise.

In most cases, distilled water and HPLC grade solvents are satisfactory. When you are comparing solvents, the most critical parameter is the *Residue After Evaporation*; this parameter should be less than 1ppm to maximize the sensitivity of the detector.

As an example, consider the analysis of a sample in a pure water mobile phase and a polluted water mobile phase. It is clear that the use of an insufficient quality solvent can dramatically decrease your S/N ratio (Sensitivity).

Figure 4-2: Chromatogram with Various Solvent Quality

The mobile phase should not contain non-volatile solvent modifiers. Volatile solvent modifiers (e.g. CHOOH, CH_3COOH, CF_3COOH, NH_4 Formate, NH_4. Acetate, $(C_2H_5)_3N$…) can be used, but they may increase the noise level at high gain settings. In addition, the solvent should not contain preservatives, (e.g. Tetrahydrofuran may contain BHT as a stabilizer).

The wetted parts of the detector are made from Teflon, Stainless Steel, and Glass. Make sure that the solvents do not react with these materials.

Note: **Depending on the mobile phase nature and flow rate, the suggested gas pressure 3.5bar (51psi) may have to be adjusted in order to optimize the background noise and so Signal-to-Noise ratio.**

4.5.3 Sample Pretreatment

If the sample contains any particulate matter, it should be filtered through a 0.2µm or 0.45µm filter before injection.

4.5.4 Column Treatment

The chromatographic column typically contains microparticles which are used to separate the compounds of interest. Under certain conditions, the column packing will undergo chemical and/or mechanical breakdown, this may lead to the introduction of particulate matter in the detector, which may lead to an increase in the noise.

☞ Note: **When strongly retained compounds are slowly eluted from the column, excessive noise will be observed.**

The breakdown of the column packing is dependent on a variety of factors including the particle size, type of column packing, the manufacturer of the column and the nature of the mobile phase (high pH may degrade silica based columns).

When you install a new column, we suggest that you pump the mobile phase through it for few minutes before connecting it to the detector. This will flush out the microparticles that remained in the column after its manufacture. After installing a new column, we also suggest that you perform the Column Noise test (Section 3.3.5) to obtain the baseline signal value corresponding to this column.

4.5.5 Optimizing the Noise Filter

The Digital Filter (see section 3.2.2.e) allows maximizing Signal-to-Noise ratio by filtering the noise. The filter strength should be optimized according to the peak shape, and more specifically to the peak width.

The following table proposes some Filter settings depending on peak width:

Peak Width at 50% (Second)	Proposed Filter (Second)
<1	0
2	1
4	2
6	4
8	6
>10	8 and higher

Table 4-1 Digital Filter versus Peak Width Indication

These suggested values can be optimized depending on your specific chromatography, by decreasing Filter if peaks are poorly resolved (e.g. when Rs<1.5), or increasing Filter when optimizing Signal-to-Noise ratio.

Example: Comparison of digital filters using the SOP test (injection of 5ppm caffeine at gain 12). Peak width at half-height is 2.5S.

	Filter 0s	Filter 1s	Filter 2s
Signal Height	124 mV	122mV	110mV
Noise (ASTM)	3.2mV	1.1mV	0.7mV
Peak Width (at 50% height)	2.5 second	2.5 second	2.8 second
S/N	37	**110**	**157**

Table 4-2 Sensitivity improvement depending on Filter

Signal-to-Noise ratio is multiplied by 3 when selecting Filter 1s without any peak broadening effect. If Signal-to-Noise ratio is more important than resolution, a Filter 2s or higher can be set to improve sensitivity even better.

4.6 Powering Down and Shutting Down the Detector

If desired, some or all functions of the instrument can be powered down at the end of an automated series of analyses. These power down features are described in detail in Section 3.2.2.h.

To shut down the instrument:

a) Turn off the pump.
b) Allow the nebulization gas to flow through the detector for few minutes (30min is recommended) to drain the evaporation tube and detection chamber.
c) Turn off the power to the detector (if desired).

If you are using a mobile phase which contains salts, acids or bases, pump few mL of water or methanol through the system before switching off the detector to prevent any deposition of substances and possible corrosion of the instrument.

If ELSD is used as a second detector and is not being used for some time, it is recommended to remove it from the liquid chromatography flow path in order to avoid any clogging of the nebulizer or deposition of substances inside the detector.

Closing gas valve while the pump is still running may result in serious nebulizer damage.

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5 Maintenance and Troubleshooting

5.1 Overview

This chapter describes:

- The maintenance procedures that should be performed by the operator on a routine basis (Section 5.2).
- Troubleshooting activities that should be useful in determining the cause of erratic or erroneous results (Section 5.3).
- Cleaning and decontamination procedure that should be performed to maintain instrument performance (Section 5.4).
- Light source exchange (Section 5.5)

5.2 Maintenance

The ELSD 80 Low Temperature Evaporative Light-Scattering detector is designed to require a minimum of maintenance activities. If preventive maintenance activities are followed, the detector should provide high sensitivity measurements without intervention by the operator.

The following general recommendations are proposed:

- Maintain the detector in a clean laboratory environment.

- If the instrument is not going to be used for a period of time, flush out any mobile phase that contains acids, bases or salts to prevent the deposition of foreign matter on components or corrosion of the instrument.

- Only use clean gas (particle-free and oil residue-free).

Closing gas valve while the pump is still running may result in serious nebulizer damage.

If ELSD is used as a second detector and is not being used for some time, it is recommended to remove it from the liquid chromatography flow path in order to avoid any clogging of the nebulizer or deposition of substances inside the detector.

For an efficient Preventive Maintenance:

After each session and before shutting down the HPLC system, the ELSD should be cleaned in order to ensure good performances.

Preventive maintenance consists in cleaning the detector before shutting down after the last analyses:

a) Let the mobile phase or solvent flow to flush particles which could remain in the detector.

b) Eventually increase temperature in order to dissolve possible deposit.

c) Stop the mobile phase flowing but let the gas flow at least 30min to dry to avoid particles deposit.

d) Stop the gas flow.

e) Shut down the detector.

The time required for each step depends on the application, solvents, type and concentration of the samples and should be determined accordingly.

☞ Note: **It is not necessary to access inside the instrument in routine operation. If the suggestions provided in this chapter do not remedy the problem, contact your local distributor.**

☞ Note: **The L.E.D. used as the Light Source has a long but finite lifetime (~ 5000 hours) and should be replaced periodically by a skilled technician. When this period has been reached, a message indicating that the maximum usage of the lamp has been exceeded will be presented when the unit is powered up and the orange L.E.D. on keyboard will blink.**

5.3 Troubleshooting

5.3.1 General Troubleshooting Information

The SEDEX Model 80 Low Temperature Evaporative Light-Scattering Detector is designed to be incorporated into a Liquid Chromatography system. It is important to note that the detector response reflects the overall performance of the system, and a "problem" that is seen on the detector output may not necessarily be a "detector problem". In almost all cases, there is one and only one cause for a problem. As an example of this point, if the user observes a noisy baseline, it is possible that the problem is due to:

- The pump (e.g. a defective check valve).
- The mobile phase (e.g. improper degassing or high residue after evaporation).
- The column (e.g. elution of strongly retained components).
- The nebulizer (e.g. lack of maintenance)
- The detector (e.g. an electronic problem).

It is very unlikely that two problems occur at the same time. The role of the troubleshooting activities is to determine the cause of the problem. In the following, we assume that the operator has already determined that other components of the system are operating in an appropriate way.

Do not disassemble the nebulizer. Disassembling the nebulizer will destroy it and this will void the warranty.

☞ Note: **The control panel and instrument electronics do not contain any replaceable components. If the suggestions provided in this chapter do not remedy the problem, contact your local distributor.**

If the digital display does not illuminate when the detector is powered up, turn the unit off and inspect the main fuses. If necessary, replace the fuses with some of the same rating as the original ones for all 115V and 230V units. The fuses are located inside the main power module on the rear panel (Figure 2-2). A set of replacement fuses is delivered in the starting kit.

If the fuses are not blown or if the replacement fuses blow up again, contact your local distributor.

5.3.2 Initial Troubleshooting Activities

a) Make sure that the instrument and all components of the detector are grounded to a true ground.

b) Check to ensure that the liquid level in the siphon is appropriate, and there is no liquid pooling close to the evaporation tube inlet.

c) Check that the gas pressure is sufficient and stable. The selected pressure for most applications is 3.5bar (51psi) and gas consumption is 3L/min for HPLC nebulizer and 4L/min for the CC nebulizer. Pressure above 4.5bar (67psi) can damage the pressure sensor. The gas filter should be clean and in place. Only use gas free of oil residue.

d) Ensure that the flow rate of the pump is constant and check that there are no leaks in the chromatography system.

5.3.3 Perform the Noise Tests

Repeat the tests described in Section 3.3 and compare the observed data to the results that were obtained when the unit was initially installed. These tests can be very valuable to isolate the problem.

As an example of this point, if the Electronic Noise test (Section 3.3.2), Background Noise test (Section 3.3.3) and Solvent Noise test (Section 3.3.4) provide results that are similar to that obtained when the unit was initially installed, but the Column Noise test (Section 3.3.5) provides a significantly different value than what was obtained at installation, it is likely that the problem is in the column (e.g. highly retained compounds are being eluted).

5.3.4 Specific Detector Troubleshooting

a) The mist from the nebulizer should be homogeneous. If it is not homogeneous, the nebulizer, the needle or the Teflon tube may be partially obstructed. To remove the obstruction, pump a solvent that can dissolve the foreign material. As an alternative, the nebulizer can be placed in an ultrasonic bath to dissolve the foreign material. Instructions about cleaning of the nebulizer are presented in Section 5.3.5.

Do not disassemble the nebulizer. Disassembling the nebulizer will destroy it and this will void the warranty.

b) If the sensitivity of the detector is low, ensure that there are no leaks in the system. Make sure you are using a fresh sample and consider running the test using a backpressure loop instead of a column. Alternatively, the L.E.D. may need to be replaced or the nebulizer could be obstructed.

   If the noise test did not show that the problem could be caused by the application or the system, a decrease in the sensitivity is often caused by the nebulizer (main cause). Clean the nebulizer as described in Section 5.3.5. If the sensitivity does not return to normal, the nebulizer might need to be replaced. Please note that the root cause might also be in different module, i.e volumes injected by the autosampler might be too low or dead volumes in capillary connections may cause peak broadening.

c) If the detector signal is saturated or if there is a decrease in the dynamic range of the system, it is possible that a residue is passing through the detector cell: this will lead to an intense signal due to a significant amount of light-scattering. This residue may be a result of the elution of strongly retained materials from the column, or may come from the solvent. To determine the cause of the problem, bypass the column and observe the signal intensity:

   - If the signal returns to normal, strongly retained materials are eluting from the column. Flush the column with a strong solvent to elute all material.

   - If the signal does not return to normal, the solvent contains a too high residue material, after evaporation and is not suitable for use with the detector.

d) If the noise of the detector without solvent is high or if ghost peaks occur, it is possible that foreign material is present in the drift tube. In this situation, increase the temperature to 100^{o}C and pump solvent at the rate of 2mL/min, using a gas pressure of 3.5 bar (51 psi).

5.3.5 Nebulizer Cleaning and Replacement Procedures

If the mist of the nebulizer is not homogeneous, the nebulizer, the needle or the Teflon tube may be obstructed. To remove the obstruction, pump a solvent that can dissolve the foreign material. As an alternative, the nebulizer can be placed in an ultrasonic bath to dissolve the foreign material.

Handle the nebulizer carefully and do not disassemble the rear part of the nebulizer, which is protected by the colored thermal seal. Improper handling of the nebulizer will destroy it and this will void the warranty.

The nebulizer rear part is a strategic setting which mustn't be dismounted for any reason. In case the user has removed it, the only solution is to perform a nebulizer replacement.

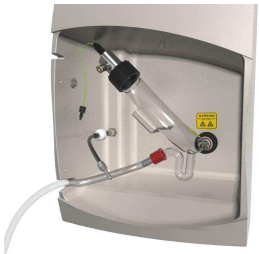
If the nebulizer cannot be repaired by cleaning by pumping solvent through it or with an ultrasonic bath, it requires a replacement.

In case of the nebulizer doesn’t produce a spray and the liquid drawn directly to the siphon even if the pressure display is 3.5bars, make sure you are using the correct black gas tube orientation fitting for the nebulizer, where the white one-way valve is at the lower end (near the gas arrival) on the front panel. The installed Nebulizer/Glass Chamber assembly is shown in Figure 2-5.

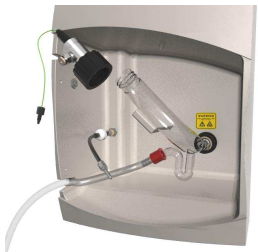
To remove the nebulizer from the instrument:

a) Switch off the pump and the ELSD detector.

b) Remove the black front panel cover. Pull its left side.

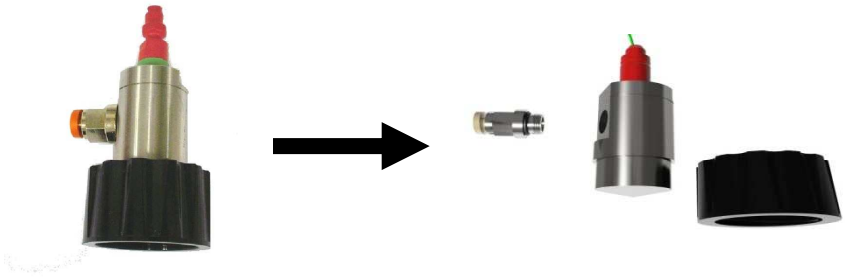


c) Disconnect the nebulizer liquid inlet from the column and,
Disconnect the gas inlet from the nebulizer by pushing on the white inlet (refer to Figure 2-4 for detail).



d) Remove the nebulizer from the glass cell by unscrewing the black plastic nut with the right hand whilst maintaining the nebulizer with the left hand. Take care not to pull or twist the nebulizer capillary. The black nut which maintains the nebulizer on the glass cell and its seal should be removed from the nebulizer.

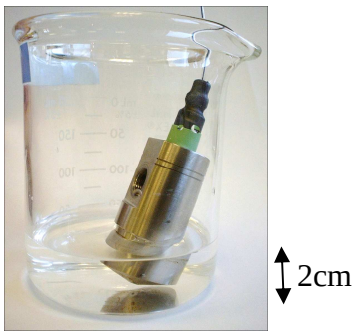
e) Remove the gas inlet quick fitting and the black plastic nut to avoid damaging the seals with the cleaning solvent.



To clean the nebulizer:

a) Fill an ultrasonic bath with water. Fill a beaker (50 or 100mL) with approximately 2cm of an appropriate solvent. The solvent is dependent on the nature of the material that is present in the nebulizer. In most cases, ethanol is a satisfactory solvent.

b) Place the nebulizer vertically in the beaker 2cm solvent bath. The nebulizer outlet should be placed at the bottom of the bath and the nebulizer inlet liquid tubing should be pointing up. Take care to ensure that the rear part of the nebulizer is not in contact with the solvent.



c) Clean the nebulizer for approximately 30 minutes with the solvent, and then replace the solvent with water and clean for an additional 30 minutes.

To re-install the nebulizer or replace it by a new one or another nebulizer model

a) For re-installing the nebulizer after a nebulizer cleaning, re-install the gas inlet quick fitting and the black plastic nut with its seal.

b) Reverse the order of previous steps (nebulizer removing). In case the black gas tubing has been removed, make sure you are using the correct orientation, where the white one-way valve is at the lower end (near the gas arrival).

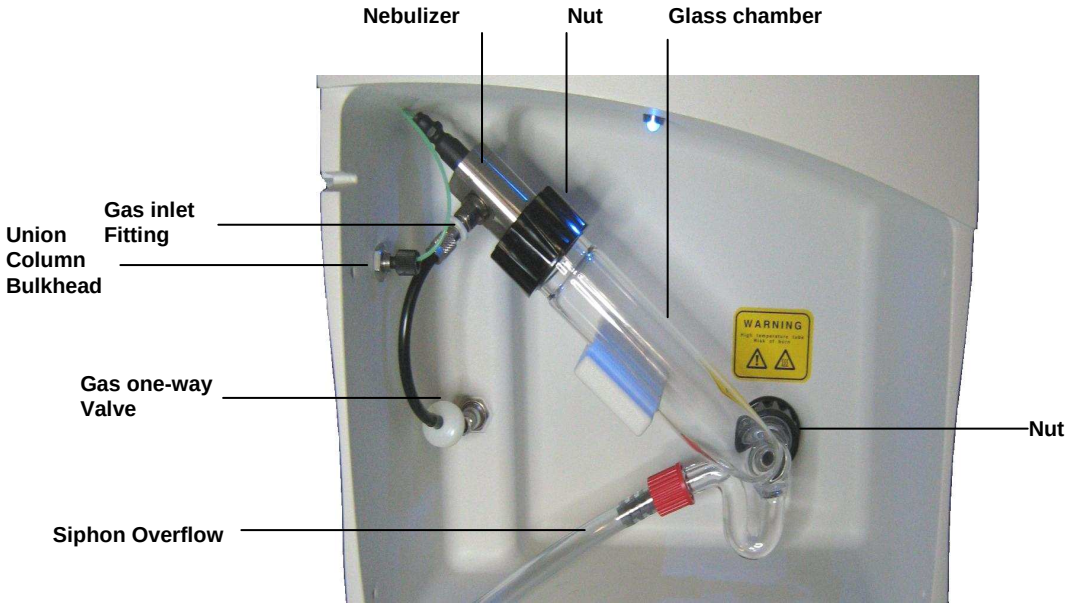
c) Make sure there is no liquid or gas leak at all connections and check for possible leakage that could affect the detector performance or create laboratory pollution when you turn on the pump.

d) Install the black front panel cover, first fix its right side, and then push its left side.

e) Test the nebulizer to ensure that it is working properly.

☞ Note: **If the Nebulizer cleaning procedure does not solve the problem, contact your local distributor for a nebulizer replacement.**

In case the black gas tubing has been removed, make sure you are using the correct orientation, where the white one-way valve is at the lower end (near the gas arrival). Avoid leaks at all connections and check for possible leakage when you turn the pump on again.



5.3.6 Gas Flow Problems

The gas flow is indicated on the *Status* screen and is controlled by a valve inside the detector housing. If the gas flow is stopped and the menu command does not work, it may be possible to perform a manual bypass.

To bypass the internal gas valve:

a) Disconnect the detector from the mains.
b) Remove the cover from the detector.
c) Turn the red button on the gas flow valve on the rear panel to bypass the valve (Figure 5-1).
d) Replace the cover and power up the instrument.

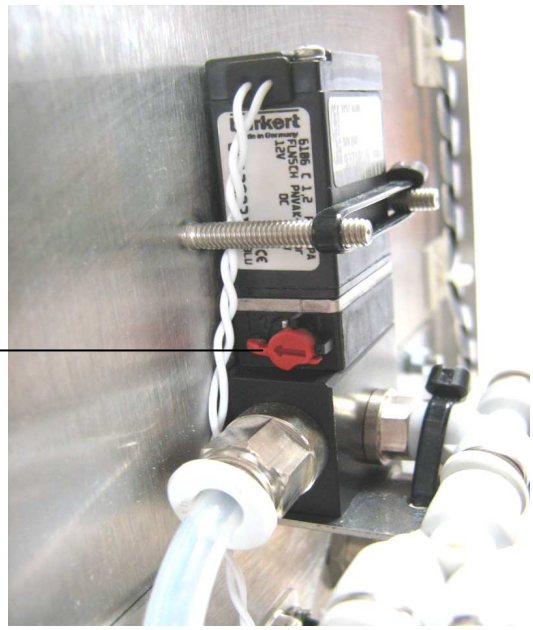


Figure 5-1: Gas Flow bypass Valve

5.4 Cleaning and Decontamination

5.4.1 Instrument Cleaning

1. Switch the instrument off.

2. Disconnect all connection cable (power cable, signal cable, autozero cable, RS-232 cable if any, instrument gas input and nebulizer tubing).

3. Allow the detector to cool down.

4. Clean the outside of the detector with a non-abrasive cloth. If necessary, a liquid such as soapy water or ethanol can be used to remove stains or foreign material.

5.4.2 Instrument Decontamination

a) Set the evaporation temperature to 100°C and the gas pressure to 3.5bar (51psi).

Pump the appropriate solvent through the system at the rate of 1mL/min. The solvent will be determined by the nature of the samples that were previously analyzed by the detector. If you do not know the nature of the sample, ethanol is a good choice. Do not use solvents that can potentially corrode the instrument. Maintain the flow and temperature during 3 hours at least.

b) Clean the outside of the detector with a non-abrasive cloth. If necessary, a liquid such as soapy water or ethanol can be used to remove stains or foreign material.

5.5 Light source exchange

The L.E.D. used as Light Source has a long but finite lifetime (~ 5000 hours) and should be replaced periodically. A decreasing L.E.D. light intensity will cause decreasing signal heights over time. When this period of 5000 hours has been reached, a message indicating that the maximum usage of the lamp has been exceeded will be displayed when the unit is powered up and the orange L.E.D. on keyboard will blink.

The Light Source exchange part number is:

- 80007 for Sedex 80 Hardware from A to C
- 80023 for Sedex 80 Hardware from D

Hardware revision can be checked as the last letter of the detector Serial Number (refer to 3.2.2.k).

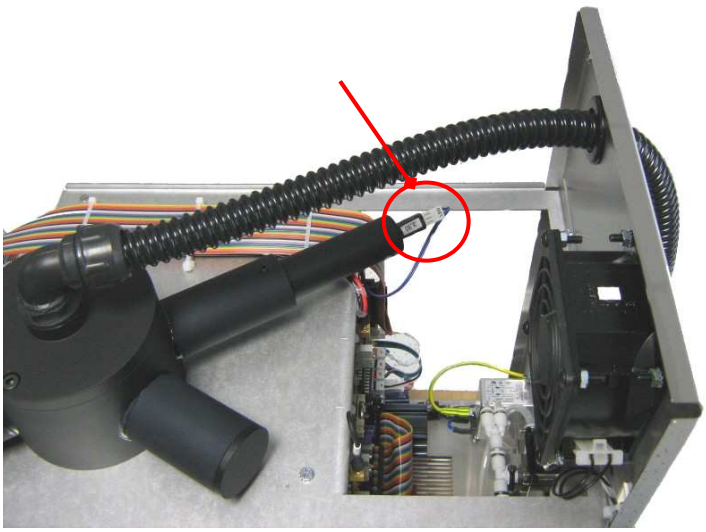
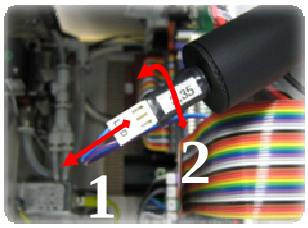


Figure 5-3: The Light Source

a) Switch the detector on and switch off the lamp by pressing the button.

b) Switch the detector off and remove the detector cover.

c) Disconnect the light source connector (step 1) and unscrew the light source cap (step 2). Note: For reinstallation, the white connector cannot be placed in the wrong way (mechanical protection).



d) Screw the new light source cap. Do not plug in the light source connector now.

e) Switch on the detector and switch on the lamp by pressing the button. Do not plug in the light source connector now.

f) Check the voltage at VLED test point on ALIM004v2 Board (Figure 5-4). Do not plug in the light source connector now.

The ground should be one of the fixing screws that attach the board to the detector.

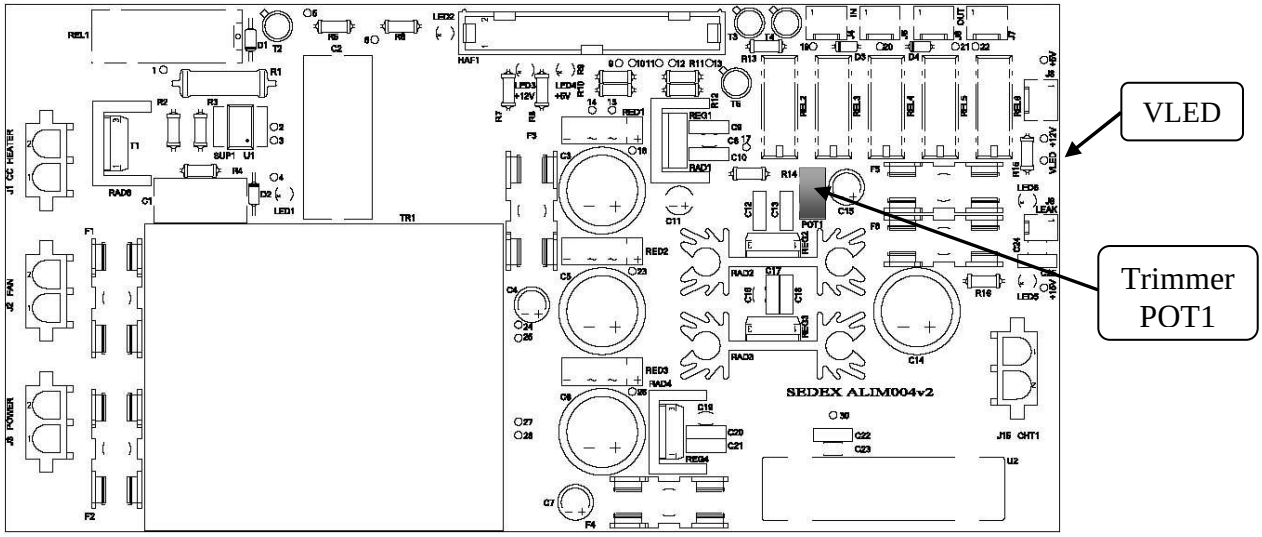


Figure 5-4: The ALIM004V2 Board Lamp Setting

g) The voltage at VLED must be set to the value that is provided with the new L.E.D. with a precision of 2 digits (example: + 3.40V). If the voltage is incorrect, adjust with trimmer POT1. Do not plug in the light source connector now.

h) Switch off the light source by pressing the button and plug in the white light source connector.

i) Switch on the light source by pressing the button.

j) Wait for 5 minutes and check the VLED voltage. If necessary, adjust the voltage with trimmer POT1.

☞ Note: **If the potential is not set accurately, the lifetime of the L.E.D. will be adversely affected.**

k) In the *LED menu* (Section 3.2.2.f), access the "Reset time elapsed" line, and press OK to reset the elapsed time counter for the L.E.D.

Appendix 1: Specifications

Detection	High Sensitivity Photomultiplier
Light Source	Selected High Efficiency Blue L.E.D.
Temperature Range	Ambient to 100^{o}C
Gas Flow Control	Manual and computer controlled (power down) nebulization gas flow and patented auxiliary gas flow
Gas Consumption	Less than 3L/min for HPLC Nebulizer, less than 4L/min for CC Nebulizer and less than 5L/min for Flash Chromatography Nebulizer
Eluent Flow Rate	HPLC Nebulizer: 100µL/min to 2.5mL/min. CC Nebulizer: 1 to 4mL/min. Flash Chromatography Nebulizer: 100µL/min to 5mL/min.
Instrument Control	Microprocessor with stand alone manual keypad or Windows based PC control (Windows 9x, NT, 2000 or XP) for Models with RS-232 activated
Operating Parameters Control	Liquid Crystal Digital Panel
Software/Driver (option)	Full Windows Compatibility (Windows 9x, NT, 2000 or XP). Universal interface with full control of ELSD parameters and data collecting
Signal Drift	Less than 2mV/30min
Signal Output	0-1V (Analog) RS-232 (Digital) when activated
Inputs	Remote Autozero (Contact Closure and TTL) Remote Powerdown Mode (Contact Closure and TTL)
Power Down Mode	General Standby Cleaning
Zero Control	Manual Auto Zero and Remote Auto Zero

Interface	RS-232 I/O Serial Output with RS-232 activated Model
Power	115 V/60 Hz, 1.8 A or 230V/50 Hz, 1.7 A
Dimensions	250 mm (10”) W x 450 mm (18”) H x 550mm (22”) D
Weight	18.5 kg (40 lb.)

Appendix 2: Spare Parts List

Part	Part Number
Nebulizer [1]	see below
Glass Nebulization Chamber [2]	85009-10
Light Source (L.E.D.)	
Hardware revision can be checked as the last letter of the detector serial number (refer to 3.2.2.k)	
For SEDEX 80LT Hardware A to C	80007
For SEDEX 80LT from Hardware D	80023
Black Plastic Nut for Nebulization Chamber (13 mm Diameter) [3]	45700-13
Black Plastic Nut for Nebulization Chamber (30 mm Diameter) [9]	45700-22
Gas Regulator with Filter and Manometer	45100
Pneumatic tube (diameter 4 mm) for Nebulizer (includes stainless steel fitting) [6]	85016
Drain Assembly (includes fitting P.N. 45108) [4]	45200
Drain Assembly Fitting [5]	45108
Tube Fitting (6 mm diameter) for Gas Regulator	45018
Wall Mounting Fitting (4 mm diameter) [7]	55014
Bulkhead Fitting [8]	85002
Peek Nut 1/16” with Ferrule [10]	85010
Cartridge for Gas Regulator	45007
Power Cable	45600
Autozero Cable	55500
Signal Cable	55400
RS 232 Cable	75092
External Event Cable	754006
Main Power Fuse	45300-3.15T

Nebulizer	Flow Rate Range	Identifying Marks	Part Number
HPLC Nebulizer	100µL/min - 2.5mL/min	Black Seal 2 Rings White Capillary	80003
Combinatorial Chemistry Nebulizer	1.0mL/min - 4.0mL/min	Red Seal 1 Ring White Capillary	80008
Flash Chromatography Nebulizer	100µL/min – 5mL/min	Black Seal No Ring White Capillary	80005

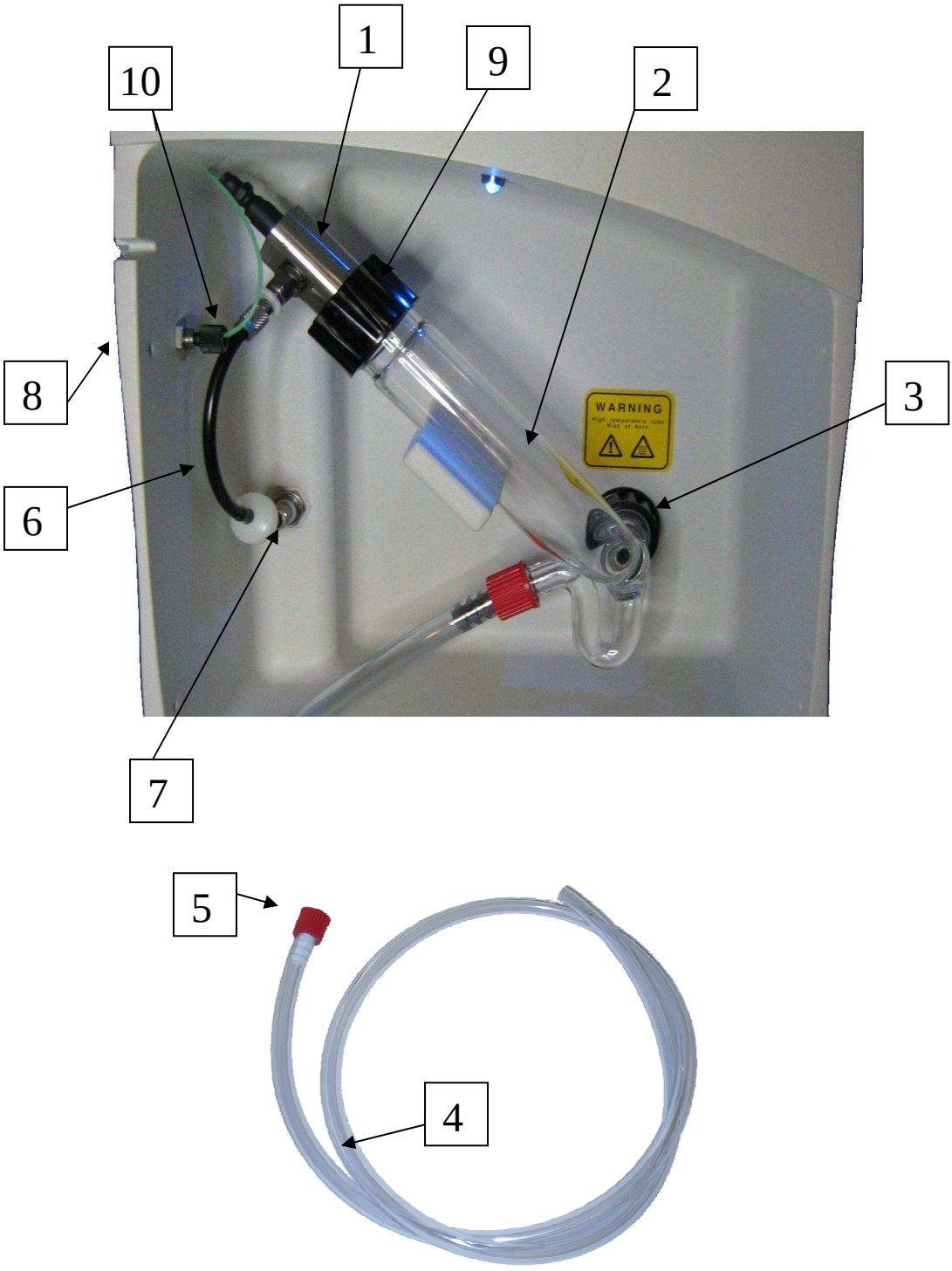


Figure A2-1: Identification of Spare Parts

Appendix 3: Standard Operating Procedure and I.Q./O.Q./P.Q.

A3.1 Overview

The Standard Operating Procedure **(SOP)** is provided to perform the Installation Qualification **(I.Q.)**, Operational Qualification **(O.Q.)** and Performance Qualification **(P.Q.)** to validate the instrument at your site. The original test report is shipped with each instrument to certify that the instrument passed the final test.

Installation of the unit is described in Chapter 2.

An *Installation Qualification* checklist is presented in Section A3-2.

The procedure for the *Operational Qualification* is described on the worksheet presented in Section A3.3.

The procedure for the *Performance Qualification* is described on the worksheet presented in Section A3.4.

The overall *Detector Performance report* is presented in Section A3.5.

☞ Note: **The present document should be filled with blue ink, except customer authorization for black ink.**

☞ Note: **Before starting S.O.P for new instruments or after storage, the following operations must be performed:**
- Set gas pressure to 3.5bars.
- Set the temperature to 50°C and wait for stabilization.
- Flush detector with 1mL/min of water at least 15 minutes.

Issue 1 : March 2007	Performed by S. Bertoli	approved by Ph. Raynaud
Issue 2 : December 2007	Performed by S. Bertoli	approved by Ph. Raynaud
Issue 3 : February 2008	Performed by S. Bertoli	approved by Ph. Raynaud
Issue 4 : February 2011	Performed by A. Ignasiak	approved by E. Verette

A3.2 The Installation Qualification (I.Q.) Checklist

Model Number: ELSD 80LT Instrument Serial Number: _____

Location of the Detector: _____

a) Has the instrument been delivered as ordered (e.g. according to the U.R.S. or purchase order)? YES [ ] NO [ ]

b) Has the instrument been checked and verified as undamaged? YES [ ] NO [ ]

c) Has the required documentation been supplied? Is it of correct issue and appropriately identified by Model Number, Serial Number and Date? YES [ ] NO [ ]

d) Have details of all services and utilities required to operate the instrument been provided? YES [ ] NO [ ]

e) Have methods and instructions for user maintenance been provided along with contact points for service and spare parts? YES [ ] NO [ ]

f) Is the selected environment suitable for the instrument (i.e. is adequate room provided for installation, operation and servicing)? Have appropriate solvent services and utilities (electricity, nitrogen gas, ventilation, solvent waste recovery, etc.) been provided? YES [ ] NO [ ]

g) Has health, safety and environmental information relating to the operation of this instrument been provided? YES [ ] NO [ ]

The manufacturer's procedure for the proper **Installation Qualification** of this instrument was completed by the following certified person:

Name _____

Title/Affiliation _____

Date _____

Signature _____

A3.3 Operational Qualification (O.Q) Protocol

This procedure checks the proper operation of the detector with respect to stability of the electronic boards, the energy of the light source and the sensitivity of the photomultiplier tube (PMT).

Model Number: ELSD 80LT Instrument Serial Number: _____

Location of the Detector: _____

☞ Note: **This procedure is designed to certify the initial conditions of the ELSD and requires the original test report. This report is located in the Operator's Manual immediately after this section (and before the reference chromatograms).**

a) Turn on the detector

b) Seal the solvent inlet connection (1/16" male fitting) with a plug connection or a piece of Parafilm™ and fill the glass siphon with water.

c) Apply air or nitrogen pressure of 3.5 bar (51 psi), monitored by a regulator and checked with a pressure gauge.

The display should read 3.5bar +/- 0.1 bar (51 psi)

PRESSURE VALUE _____ PASS [ ] FAIL [ ]

d) Set the temperature to 50°C and wait until the temperature stabilizes (30 minutes).

The display should read 50 °C +/- 1 °C.

SETTING TEMPERATURE _____ PASS [ ] FAIL [ ]
DISPLAY TEMPERATURE _____

e) Set FILTER "1s" in the *Noise Filter/Pressure Unit* Screen. (Refer to Section 3.2.2.e).

f) Set the gain to 12.

g) Measure the noise over ten 1 min segments. For each segment, measure the difference between the lowest noise peak and the highest noise peak.

Segment	+ Dev.	- Dev.	Difference.	Segment	+ Dev.	- Dev.	Difference.
1				6			
2				7			
3				8			
4				9			
5				10			

Minimum Value: _____ Maximum Value: _____ Mean Value: _____

The mean noise value should be less than 2mV. PASS [ ] FAIL [ ].

h) Set the Gain to 1 and set the temperature to 50°C (wait until the temperature stabilizes), allow nitrogen to flow through the instrument at a pressure of 3.5bar (51 psi), set "Offset After Autozero" to zero if needed as described in section 3.2.2.d. and autozero the detector. Raise the Gain to 12, monitor the signal for 5 seconds and enter the value below.

Observed Value _____mV
Reference Value _____mV (see Test Report)

The observed stray light readings should be in the range of 100 – 150mV.

PASS [ ] FAIL [ ]

i) This test is performed in the same conditions as for the mean noise value determination. Position the recorder near 10mV by adjusting the offset and allow the recorder collecting data for 30 minutes.

Initial Signal Level ____mV Final Signal Level ____mV 30Min Drift ___mV

The baseline drift should be less than 2.0mV. PASS [ ] FAIL [ ]

☞ Note: **If any part of the diagnostics fails, please refer to your local distributor for service.**

The manufacturer's procedure for the proper **Operational Qualification** of this instrument was completed by the following certified person:

Name _____

Title/Affiliation _____

Date _____

Signature _____

A3.4 The Performance Qualification (P.Q) Protocol

Model Number: ELSD 80LT Instrument Serial Number: _____

Location of the Detector: _____

☞ Note: **Before this procedure is performed, it is necessary to complete the Installation Qualification (Part I) and the Operational Qualification (Part II). The test report presented below or your own appropriate reference test should be used.**

1) Turn on the detector and set the conditions as follows:

Temperature	: 40°C
Gain	: 12
Noise filtering	: 1s
Gas pressure	: 3.5bar (51psi) air or nitrogen
Siphon	: Filled with water
Inlet tube **(*)**	: Connected to column (4.6mm I.D. x 30mm) C_{18}, 5µm or 1/16'' tubing, 0.005'' I.D. x 200cm
Solute	: Caffeine as specified on the test report.
Flow rate	: 1mL/min
Mobile phase	: 100% HPLC water

(*) Remove the plug connector (or Parafilm™) from the solvent inlet connector before connecting the siphon to the column. Note that when using a loop, it must be placed between the pump and the autosampler.

2) Autozero the detector.

3) Make three (3) 20µL injections of 5ppm caffeine standard (for a full 20µL loop injection, deliver 100µL of the standard each time to ensure that the loop is filled with the standard). The average peak height (mV) should be:

- At least 50mV* under test report conditions (column test).
- At least 70mV* under test report conditions (loop test)
* For CC version, please deduct 20mV from the above values
* For FLASH version, please deduct 45mV from the above values

- Nebulizer type (see certificate): HPLC [ ] CC [ ] FLASH [ ]
- Inlet connection type: COLUMN [ ] LOOP [ ]

Peak height 1 _____ mV
Peak height 2 _____ mV
Peak height 3 _____ mV
Average Peak height _____ mV

PASS [ ] FAIL [ ]

4) Set gain 6 then autozero the detector.

5) Make six (6) 20µL injections of a 250ppm caffeine standard at gain 6. The repeatability should be:

3.0% RSD maximum.

Peak Area 1: _____
Peak Area 2: _____
Peak Area 3: _____
Peak Area 4: _____
Peak Area 5: _____
Peak Area 6: _____

Avg. Peak Area: _____

Round Standard Deviation (RSD): _____
% Round Standard Deviation (RSD): _____%

PASS [ ] FAIL [ ]

The manufacturer's procedure for the proper **Performance Qualification** of this instrument was completed by the following certified person:

Name _____

Title/Affiliation _____

Date _____

Signature _____

A3.5 Overall Detector Performance

After the Installation Qualification (I.Q.), Operational Qualification (O.Q.) and Performance Qualification (P.Q.) procedures have been completed, the **Overall Detector Performance** document should be completed to verify the completion of all tests.

Model Number: ELSD 80LT Instrument Serial Number: _____

Location of the Detector: _____

Part I - Installation Qualification (I.Q.) Date _____ PASS [ ] FAIL [ ]

Part II - Operational Qualification (O.Q.) Date _____ PASS [ ] FAIL [ ]

Part III - Performance Qualification (P.Q.) Date _____ PASS [ ] FAIL [ ]

The above instrument was certified by the following certified manufacturer's representative:

Name _____

Title/Affiliation _____

Date _____

Signature _____

The above instrument was certified within presence of the following customer's representative:

Customer name _____

Customer title/Affiliation _____

Customer Signature _____

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Appendix 4: Applications

What follow represent several typical applications that can been performed with ELSD 80 Low Temperature Evaporative Light-Scattering Detector.
To obtain more details about an application, please contact your local distributor.

Page	***Application***	***Chemical Family***	***Application number***
A4-3	Fructose Glucose Lactose Sucrose Raffinose Stachyose	Carbohydrates	75
A4-3	Maltodextrins DP1 to DP17	Polysaccharides	35
A4-4	Alkyl Glucosinolates	Carbohydrates Derivatives	73
A4-4	Vitamins A, D_2, E_1, K_1 β-carotene Fatty acids 18:3, 18:2, 16,0, 18:0	Vitamins	101
A4-5	Ginseng Extract Saponins	Saponins	60
A4-5	Cerebroside Hydroxylated Cerebroside Phosphatidyl Ethanolamine Phosphatidyl Inositol Phosphatidyl Serine Phosphatidyl Choline Phosphatidyl Acid Sphingomyeline Lysophosphatidyl Choline	Phospholipids	5
A4-6	Trioleine α-Tocopherol Oleic Acid β-Tocopherol γ-Tocopherol δ-Tocopherol Diolein Cholesterol	Oleins, Tocopherols Cholesterol	116
A4-6	Polyoxyethylene Alcohols ($C_{12}OE_9$)	Polyoxyethylene Alcohols	18

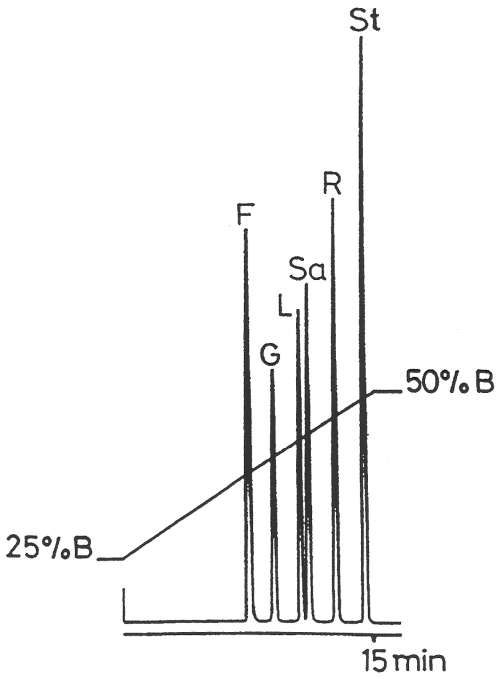
A4-7	PEG 400	Polyethylene Glycols	23
A4-7	21 Amino Acids sample	Amino Acids	114
A4-8	D & L Amino Acids (Trp, Val, Asn)	Amino Acids	140
A4-8	Di, Tri & Tetrapeptides	Peptides	142
A4-9	Inorganic Anions	Inorganic Anions	109
A4-9	Tergitol type B Octyl B glucoside Sodium Dodecyl sulfate N Lauryl sarcosine Zwittergent 3 14 Triton X 100 Lubrol PEG 10 Tridecyl ether	Detergents	61
A4-10	Low Temperature Operation: Ethylene Glycol Quantitation	Ethylene Glycol	141
A4-11	Low Temperature Operation: Thermosensitive Urea	Urea	94

Table A4-1: Applications Table

Carbohydrates

HPLC
Gradient

F: Fructose
G: Glucose
L: Lactose
S: Sucrose
R: Raffinose
St: Stachyose



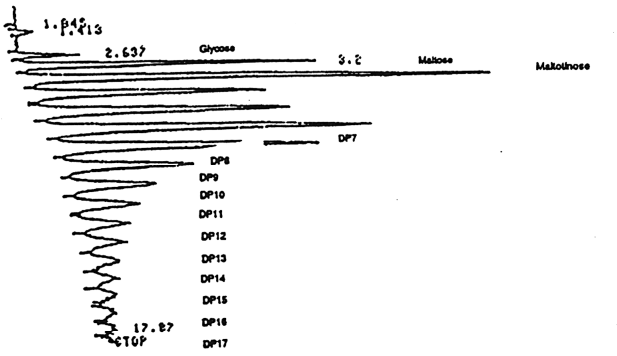
Gradient: Acetonitrile, 25 % to 50 % in 15 min

Analyzed by: LCBA 45000 Orleans FRANCE

Maltodextrines

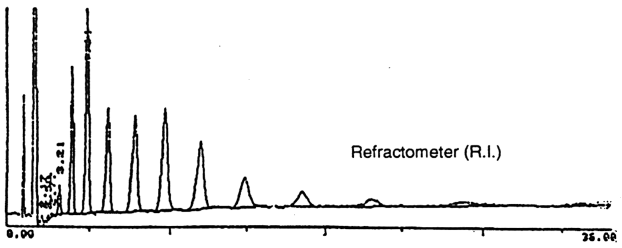
HPLC
Gradient

Evaporative Light Scattering Detector can detect concentrations of 1000 ppm of DP 1 to DP 17



60% to 80% Acetonitrile in 20 minutes

Refractive Index detector can detect concentrations of 50,000 of DP 1 to DP 10

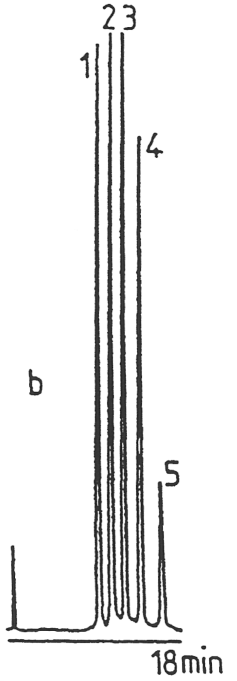


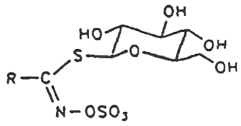
Alkyl Glucosinolates (R-GI)

HPLC
Gradient

Peaks

1. C_{10} GI
2. C_{11} GI
3. C_{12} GI
4. C_{13} GI
5. C_{14} GI





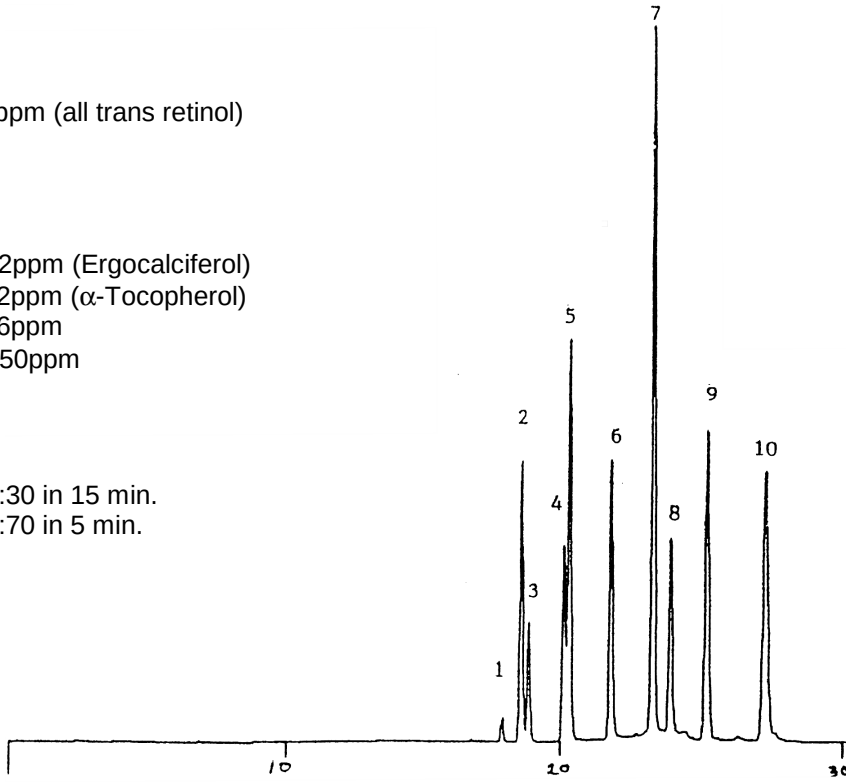
Gradient:
Methanol, 40 to 75% in 10 min
Hold at 75% Methanol for 8 min.

Vitamins and Fatty Acids Standard

HPLC
Gradient

1 = 18:3, 14ppm
2 = Vitamin A, 80ppm (all trans retinol)
3 = 18:2, 42ppm
4 = 18:1, 56ppm
5 = 16:0, 98ppm
6 = 18:0, 70 ppm
7 = Vitamin D_2, 92ppm (Ergocalciferol)
8 = Vitamin E_1, 42ppm (α-Tocopherol)
9 = Vitamin K_1, 76ppm
10 = β-Carotene, 50ppm

Gradient:
85:10:5 to 0:70:30 in 15 min.
0:70:30 to 0:30:70 in 5 min.



Analyzed by: Richard Scientific, Novato CA 94949 USA

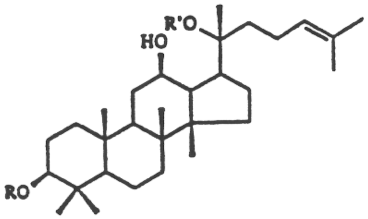
Ginseng Saponins

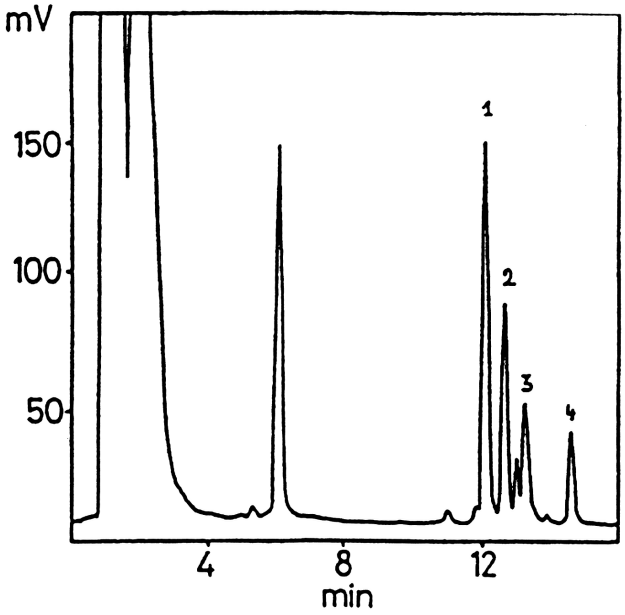
HPLC
Gradient

R = D GLU	D GLU	
R = D GLU	D GLU	1
= L Ara	D GLU	2 Furanose
= L Ara	D GLU	3 Pyranose
= D GLU		4

Gradient:
20-40% Acetonitrile in 15 min

Detection limit (S/N = 3) = 120ng





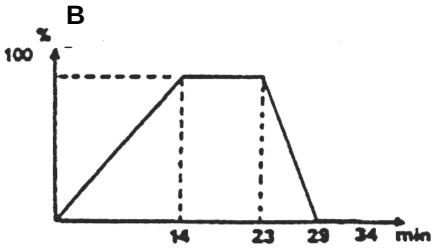
Analyzed by: J. Becart - Parfums Christian Dior

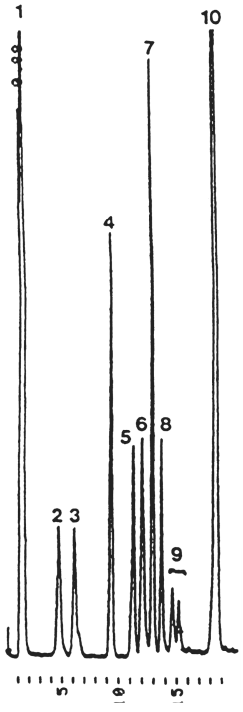
Phospholipids

HPLC
Gradient

1. Apolar lipids
2. CR : cerebroside – fatty acid not hydroxylated
3. CROH: cerebroside – fatty acid hydroxylated
4. PE : phosphatidyl ethanolamine
5. PI : phosphatidyl inositol
6. PS : phosphatidyl serine
7. PC : phosphatidyl choline
8. PA : phosphatidyl acid
9. Sph : sphingomyeline
10. LPC: lysophosphatidyl choline

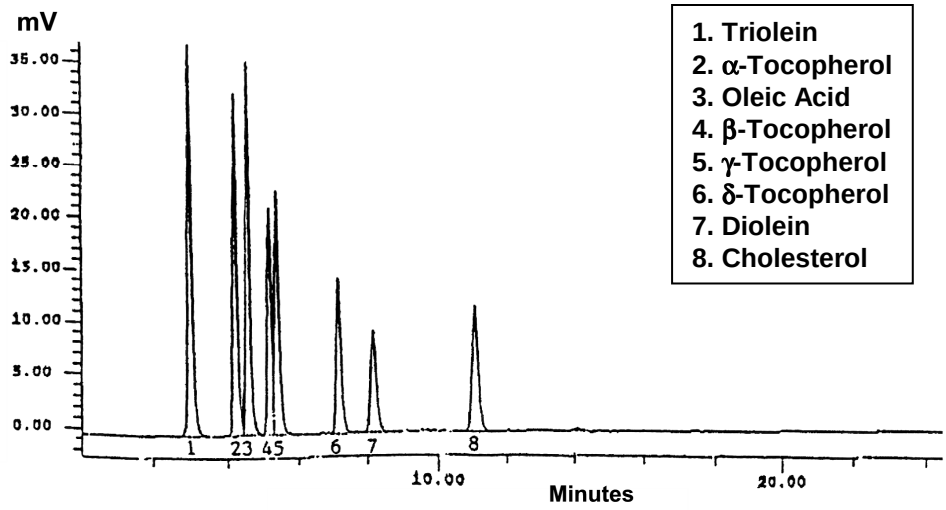
Gradient Profile





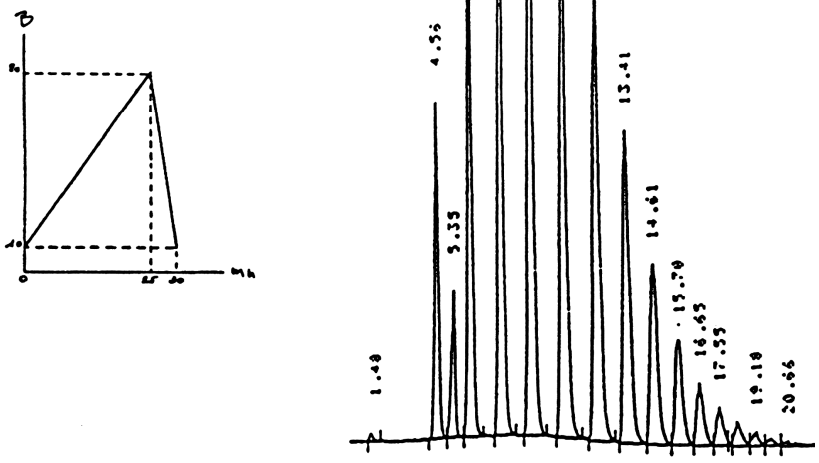
Analyzed by: Mr. Becart, Parfums C. Dior [J. High Res. Chrom. 13, (1990) 126]

Oleins, Tocopherols, Cholesterols HPLC



Analyzed by: Courtesy of Bunge Foods, Bradley, IL

Polyoxyethylene Alcohols HPLC
($C_{12}OE_9$) Gradient

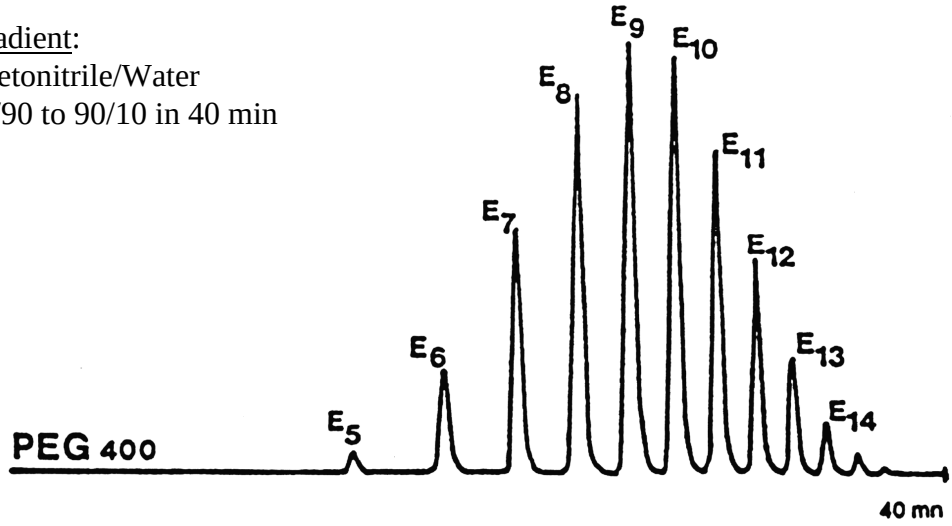


Analyzed by: Mr. Becart, Parfums C.Dior

Polyethylene Glycol PEG 400

HPLC
Gradient

Gradient:
Acetonitrile/Water
10/90 to 90/10 in 40 min

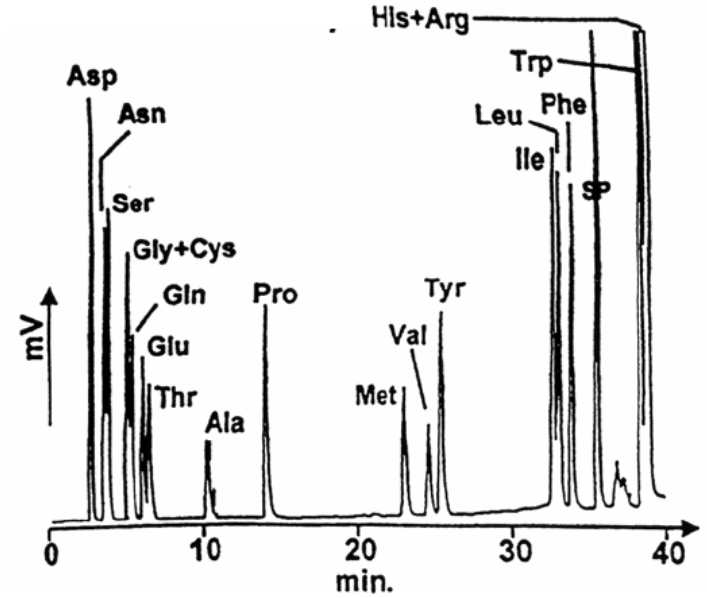


Analyzed by: LCBA 45100 Orleans FRANCE

Underivatized Amino Acids

HPLC
Gradient

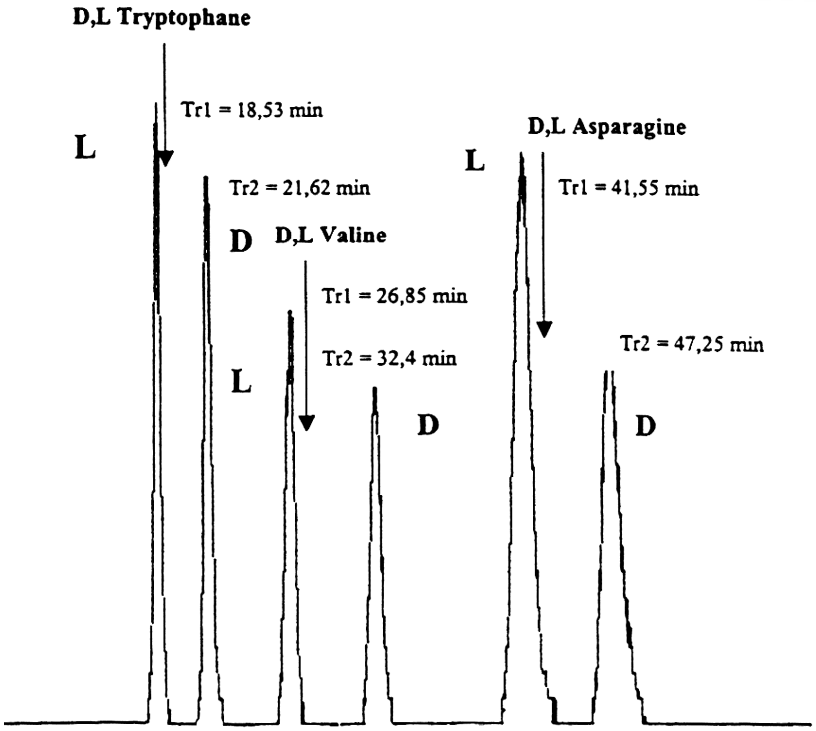
Gradient:
H_2O (+volatile ion pairing agent)
0% to 40% organic solvent in 40 min.



Analyzed by: Mr Dreux et al. - UNPUBLISHED RESULTS - SP: system peak

D and L Amino Acids HPLC

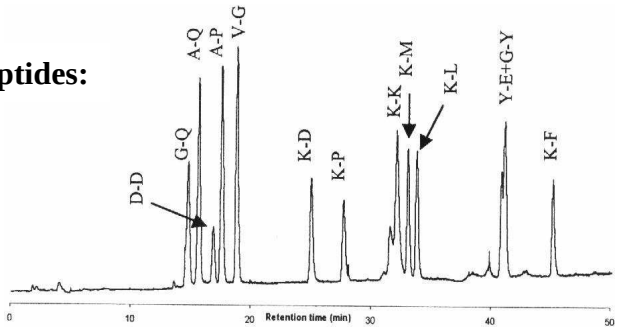
Direct Enantiomeric separation



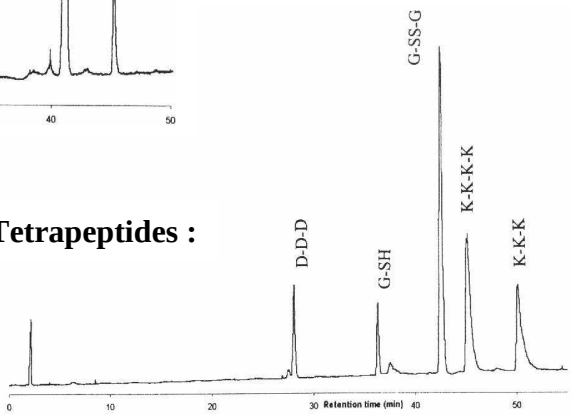
Analyzed by: CEA SACLAY – Service des molécules marquées – 91191 GIF/YVETTE CEDEX – FRANCE

Underivatized Small Peptides HPLC

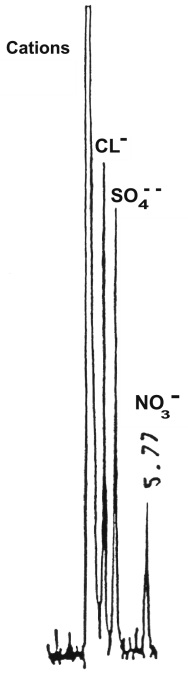
Dipeptides:



Tri & Tetrapeptides :

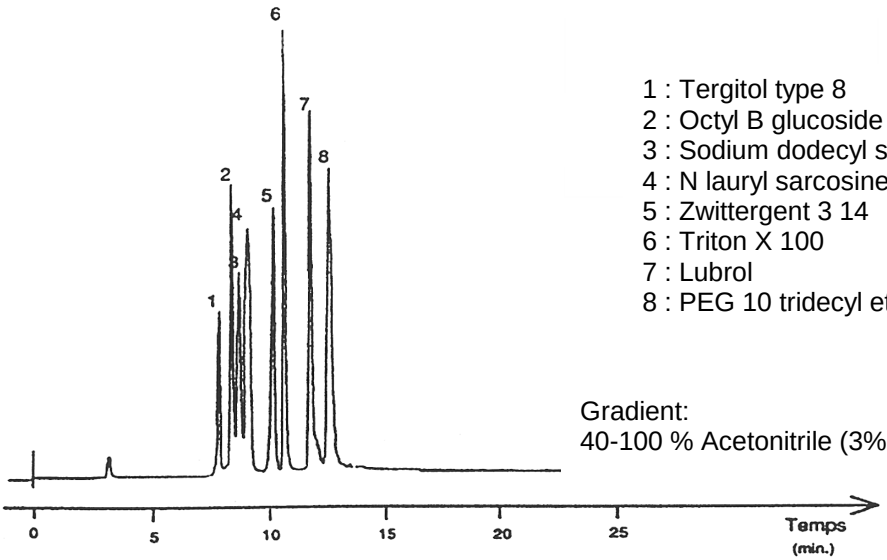


Inorganic Anions HPLC



Analyzed by: LCBA 45100 Orléans FRANCE

Detergent HPLC Gradient

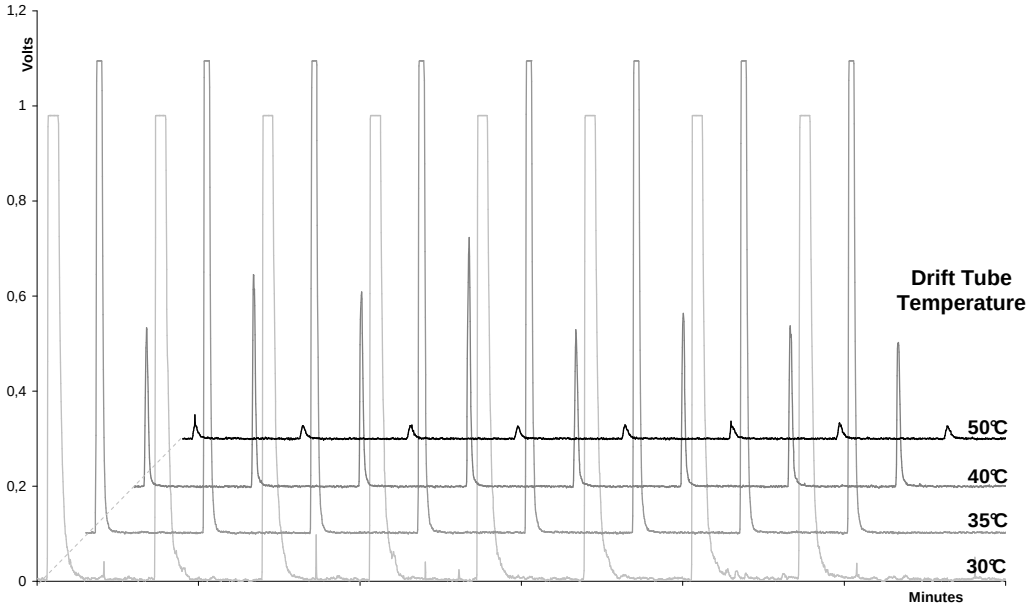


1 : Tergitol type 8
2 : Octyl B glucoside
3 : Sodium dodecyl sulfate
4 : N lauryl sarcosine
5 : Zwittergent 3 14
6 : Triton X 100
7 : Lubrol
8 : PEG 10 tridecyl ether

Gradient:
40-100 % Acetonitrile (3% min)

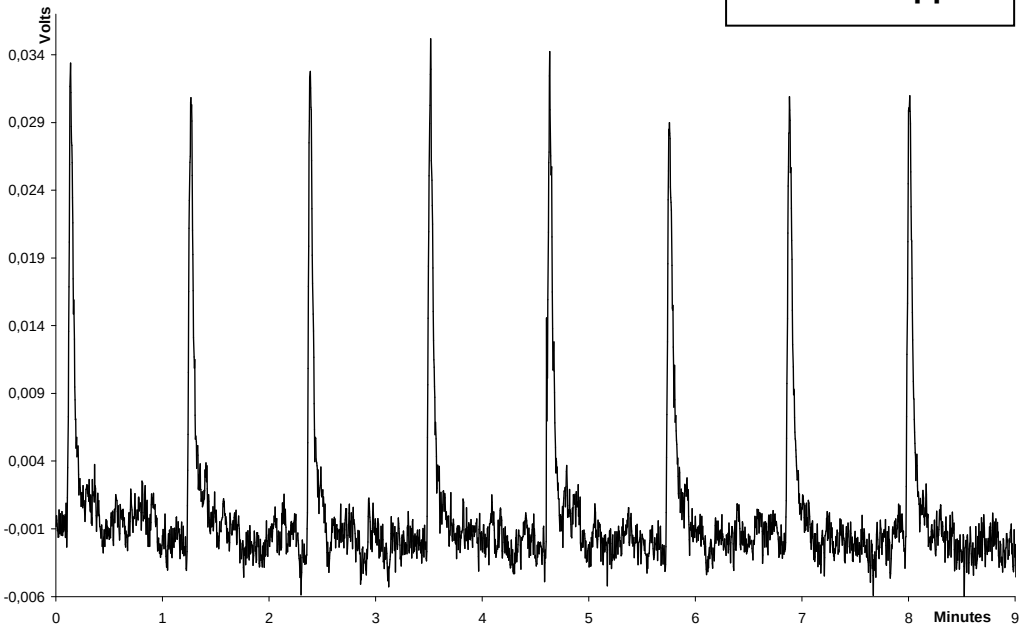
Analyzed by: Pasteur Merieux- R & D Laboratory 69280 Marcy L'Etoile FRANCE

Low Temperature Operation: Ethylene Glycol HPLC



LOQ = **2.16ppm**

LOD = **0.65ppm**



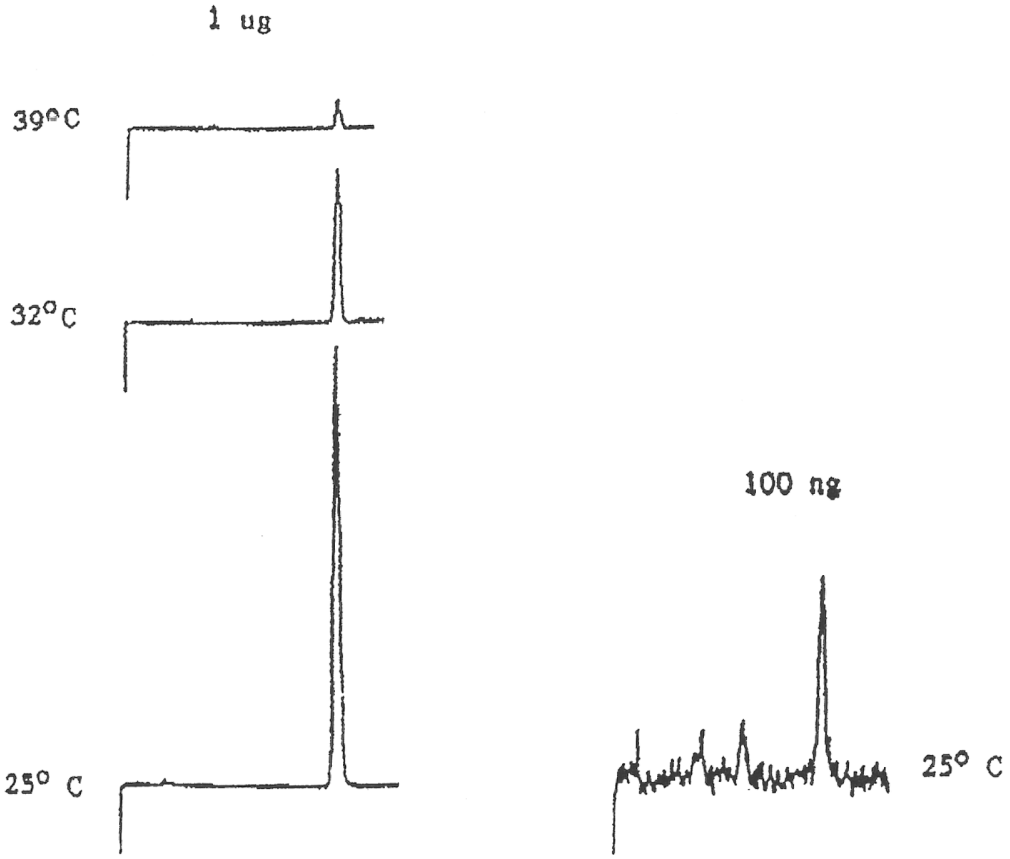
10 µl injections (2.5 mg/L), mobile phase 100% water

Analyzed by: R. Pennanec, SEDERE Orléans

Low Temperature Operation: Urea HPLC

Effect of Temperature on Detector Signal

As the temperature of the detector increases, urea decomposes and its contribution to the observed signal decreases.



Analyzed by: Richard Scientific, Novato CA 94949 U

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Appendix 5: Drivers for SEDEX Model 80LT

Drivers in option can be only proposed for ELSD 80LT Detector with RS-232 activated to get a direct single-point control and digital data acquisition and to eliminate the need for A/D converter. It also makes this detector fully integrated in many HPLC equipments.

The RS-232 cable connection from the detector to the PC requires a straight/direct (non-cross) serial cable bundled with the detector. If your PC has multiple COM ports, make sure you have selected the correct one.

 Note: **By experience, some RS-232 ports added on computers by USB modules may present some communication troubles: complete or partial miss of communication. For laptop, PCMCIA cards are recommended as no problem has been experienced so far.**

The ELSD 80LT Detector is normally controlled by the keyboard on the front panel, but once it receives correct orders from the RS-232 link, the keyboard is deactivated and then the ELSD 80LT Detector can be only controlled by the PC. It avoids orders conflicts between a keyboard operator request and a PC request. This is called "distant mode" and is activated once a valid command is received. To exit "distant mode" exists the driver's program.

The Autozero cable (refer to Section 2.5.3) must be connected to a "Start" information on the controlling device (e.g. autosampler…). In this mode, the "Start" information is used as a signal synchronization for the driver. Not using it will impair the synchronization of the signal and could not generate the final report and/or impair the retention time reproducibility.

Driver for EZChrom *Elite*™ [SEDERE Part number **85090**, from Version 3.1.4]

Driver for ChemStation™ [SEDERE Part number **85089**, From Version B.02.01.SR1 (259)]

Driver for Xcalibur™ [SEDERE Part number **85093**, From Version V1.4]

Clarity™ [The driver is part of Clarity from Version 3.0.2]

For Installation and Operation with these Drivers, you can directly refer to their respective Operator's Manuals.

To obtain more information about Drivers, please contact your local distributor.

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