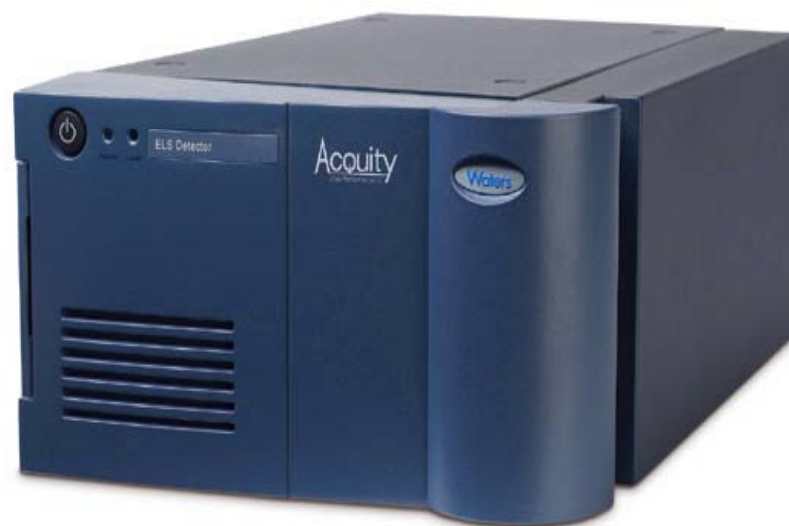


ACQUITY ELS Detector Training



ELS Detector Training

- Theory of light scattering detection
 - Light scattering detection is a “mass” detection method. It has some similarities to refractive index detection in that it can be used for multiple types of assays. In refractive index detection the measurement is made of the difference between the refractive index of the mobile phase and sample. In light scattering detection the mobile phase must be more volatile so it can be removed by vaporization and only then are the less volatile compounds detected as their particles scatter the light in the chamber and direct it to a photo multiplier tube. Unlike refractive index you are not measuring the mobile phase which allows light scattering detection to be used for assays which require gradients.

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— Why use evaporative light scattering detection?

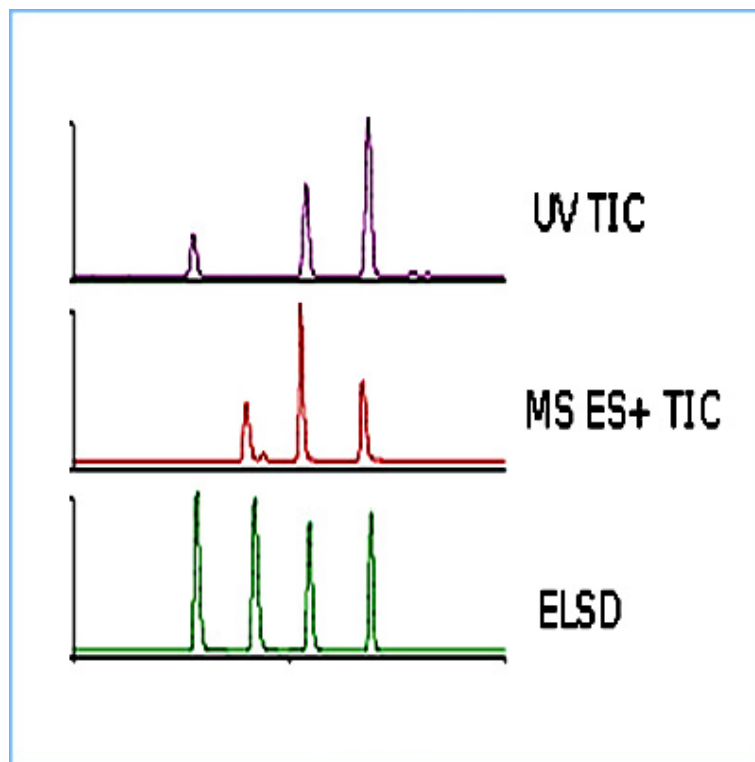
Advantages

- o --Detects compounds without UV/VIS chromophores
- o --Gives an approximation of amount of material
- o --Compatible with MS mobile phases, can be used with a wide range of modifiers and solvents with high UV background
- o --Less prone than refractive index detection to environment and compositional changes. Can run gradients to speed up separations

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Why use the ELSD?

The ELSD is used because not all compounds are seen by all other detectors.



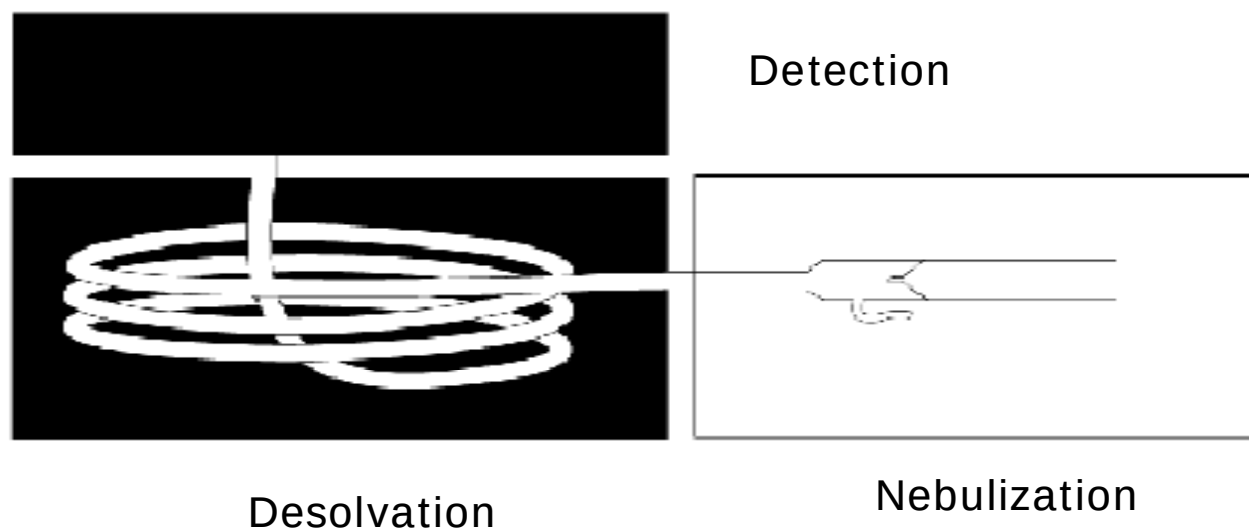
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- Disadvantages

- Compounds that are volatile or have a low boiling point will not be detected.
- Destructive (must be the last detector in the flow path)
- Need to supply gas and venting to the detector
- Fundamentally non-linear

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- How Does It Work?
- 3 Basic Operations



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- Nebulization
 - The nebulizer is tailored to the flow rate (high or low flow)
 - Adjustable gas flow
 - The effluent from the column is passed through a capillary steel needle surrounded by a gas to produce an aerosol of droplets
 - The nebulizer can be heated or cooled depending on the application.
 - Larger particles that do not enter the drift tube are diverted to waste(90 to 95%). The u-bend in the drain tube prevents pooling inside the nebulizer tube.

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- Nebulization

- High gas flow produces small droplets which requires less heat to vaporize off the solvent to leave the non-volatile sample.
- Low gas flow produces larger droplets which require more heat to evaporate off the solvent to leave the non-volatile sample. If the solvent is not evaporated away it can cause noise.

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■ Desolvation/ Drift Tube

- Droplets which are driven by the carrier gas introduced at the nebulizer, diffuse into the drift tube.
- In the drift tube particles are generated . The non-volatile portion of the eluent remains after the volatile mobile phase is evaporated.
- The drift tube is heated to facilitate solvent removal, without sample depletion. It is recommended that the drift tube temperature is set to a minimum of 5 degrees C higher than the nebulizer temperature if heating the nebulizer.

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- Detection



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■ Detection

- The scattering chamber is the equivalent of the flow cell in other detectors.
- Particles are detected by scattering a light beam.
- The scattering chamber is also heated to avoid condensation.
- Waters has used our expertise in the design of optics benches to improve detection sensitivity. The design utilizes a mirror and light absorbing material to insulate detection of un-scattered source light.

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- ELSD Considerations

- Contributors to non-linearity

- Droplet size distributions produced by the nebulizer
 - Particle losses on the walls of the drift tube
 - Particle beam profile entering the scattering chamber
 - Particle flow patterns in the scattering chamber(affected by venting strategy)
 - Contribution of stray light vs Rayleigh background
 - Effect of changes in backpressure due to compositional changes such as you see in gradient LC

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- ELSD Considerations cont..

- The volatility of the mobile phase

- Non volatile HPLC eluents cannot be used , such as Phosphate buffers, Phosphoric acid, Sodium buffers, and Ion-pairing reagents

- Particulate contamination

- Use high quality HPLC solvents, use in line solvent filters and be careful of column shedding

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- Features
- Front mounted ,user replaceable ,long-life(tungsten halogen) lamp.
- High sensitivity PMT calibrated at the factory.
- Sophisticated autozero implementation.
- Contact closure to provide stop flow output if a temperature runaway, gas flow interruption, or lamp failure arises.
- User Diagnostics
 - Lamp history
 - Gain Optimization
 - Stand-alone programmability allowing users to build and store up to 10 methods with as many as 50 timed events.

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- Features continued

- a specific nebulizer with a right angle tubing connection for use with the Acquity ELS only.
- Heated nebulizer. The user can set a power level to achieve pre-heating in the nebulizer before the droplets enter the drift tube.
- Nebulizer cooling to provide better equilibration when running fast gradients
- Sheath flow
 - An additional stream of carrier gas added at the entrance of the scatter chamber to provide a more concentric particle profile into the light path.

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- Start-up considerations
 - Site Requirements (site prep document PN 715000809)
 - The detectors location should be in close proximity to a fume hood or trunk line for evacuation of the vapors produced by the nebulization of the mobile phase.
 - The detector must be placed at a sufficient height so that the waste and vapors can escape the detector in a downward direction.
 - Exhaust tubing from the rear of the detector to the vapor trap should not be shortened.
 - Nitrogen supply should be located within five to six feet of the detector.

ELS Detector Training

- Start-up considerations,
 - The ELS may be stacked on other detectors , but must be the last detector in the system as the light scattering technique is destructive.
 - If the pumping system being used has a stop flow input it must be connected to the ELS stop flow output to stop the flow in the event there is , loss of gas pressure, heater runaway, or lamp failure.
 - It is important to fill the siphon tube on the drift tube assembly to prevent the escape of carrier gas. This can be accomplished by filling it with a syringe.

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- Start-up considerations

- Ensure that the system to which the detector will be connected can produce a pulse free flow at a rate compatible with the nebulizer to be used.
- When selecting a waste container for the drift tube waste line remember that only 5 to 10 percent of the eluent being flowed into the detector is nebulized and the rest exits the detector via the siphon tube. The drift tube waste line must be suspended above the level of the waste to allow for proper drainage.

ELS Detector Training

- Start-up considerations

- The preferred gas supply is a nitrogen generator providing a constant supply between 65 and 90 PSI. Parts are provided in the start-up kit to allow tying into the nitrogen supply for a Mass Spec if there is one on the system. Tanks of gas should not be used due to insufficient capacity.
- Compressed air can possibly be utilized, but consideration must be given to the combustibility of the mobile phase being used.
- Filtration of the gas must be considered to prevent unwanted particulates or contamination such as oil from being introduced into the eluent stream.

ELS Detector Training

- Start-up considerations

- All eluents used on the ELS Detectors should be of the highest quality available. Filtration of solvents is important as any particulates introduced will be seen as sample or noise.
- Water quality is also very important. Lab water systems should have a particulate filter that is suitable and changed on a routine basis. If there is doubt about the quality of the customers water source then bottled HPLC grade water should be used.
- The use of in line filters is also recommended to prevent particulates.

ELS Detector Training

- Start-up considerations
 - When a new column is installed on a system consisting of an ELS detector, it is highly recommended that the column not be connected to the detector until at least 5 column volumes of solvent have been flushed through the column.
 - Recommendations for an appropriate solvent and what the column volume is for different columns can usually be found in the care and use manuals supplied by the column manufacturer.
 - All Waters ELS Detectors utilize ethernet for communication to either Empower or MassLynx. Always verify the need for ethernet crossover cables and contact closure cables with the workstation or network being used.

ELS Detector Training

- Waters Acquity ELS Detector
- Released November of 2005
- Substantially shorter and narrower drift tube than the 2420 to be compatible with Acquity system flow rates.
- Combined nebulizer heater/cooler to facilitate faster equilibration during fast gradients.
- Data rate capability increased to 80HZ (Although long term testing in-house shows no additional benefit to be gained above 20HZ)

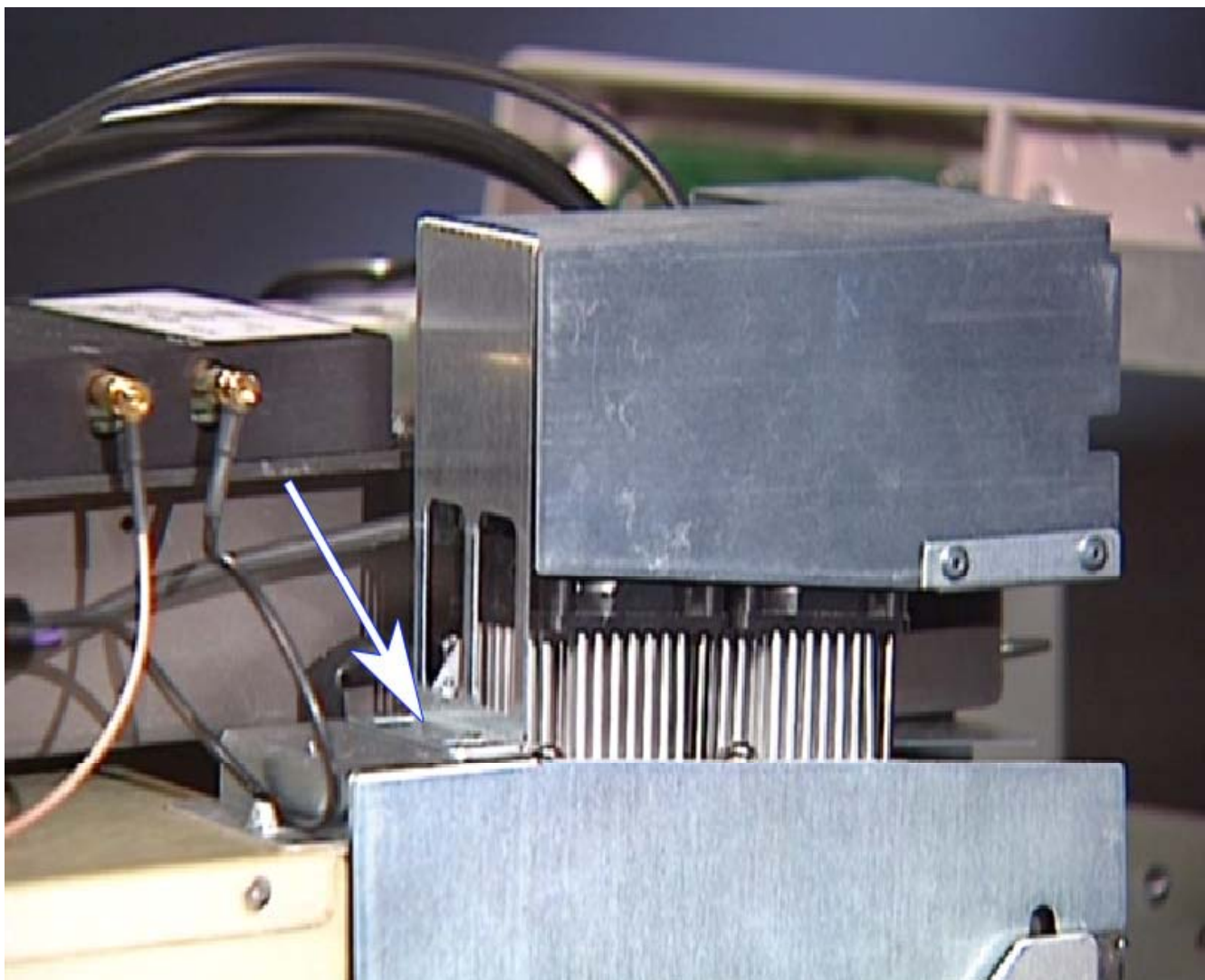
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- Waters ELS Detectors are in a class of detectors that are referred to as a low temperature evaporative light scattering detectors.
- Low temperature detectors of this type do not accept the entire flow rate produced by the system. The Waters ELS Detectors only introduce 5 to 10 percent of the actual flow into the drift tube for evaporation and ultimately detection.
- The majority of the flow introduced into the detector collects into the siphon drain tube and is sent to waste. For this reason the setup of the waste is critical as it is directly related to how much sample actually makes it to the scatter chamber.

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- Also because of the low percentage of material which reaches the scatter chamber, the exhaust set up is very critical as well. Any restrictions on the exhaust or excess vacuum can be responsible for poor reproducibility.

ELS Detector Training



ELS Detector Training

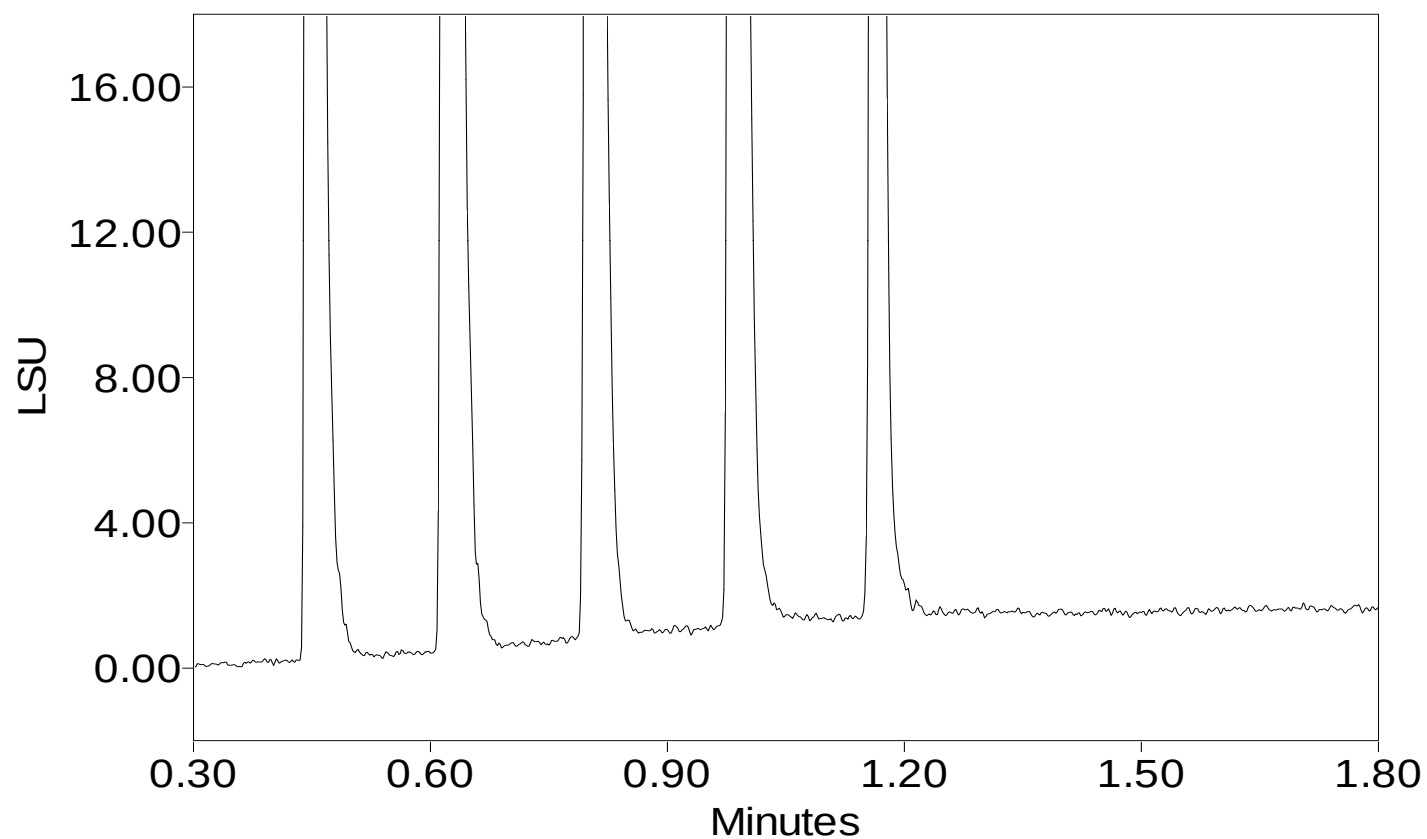
- The block is surrounded by insulation to maintain a stable temperature.
- The nebulizer heater/cooler is a Peltier device which is mounted to a block clamped to the nebulizer portion of the drift tube.
- The fans and Peltier chips are encased in a shroud to direct airflow and allow maximum efficiency of the device.
- The assembly can be replaced. The part number for the replacement assembly is 700002807

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- The drift tube coil has been reduced to a narrower bore tube of a shortened length to reduce band broadening.
- The drift tube assembly can only be replaced by replacing the desolvation oven assembly.
- The nebulizer heater cooler and clamp must be moved from the old desolvation oven assembly to the new one if replacement is required.

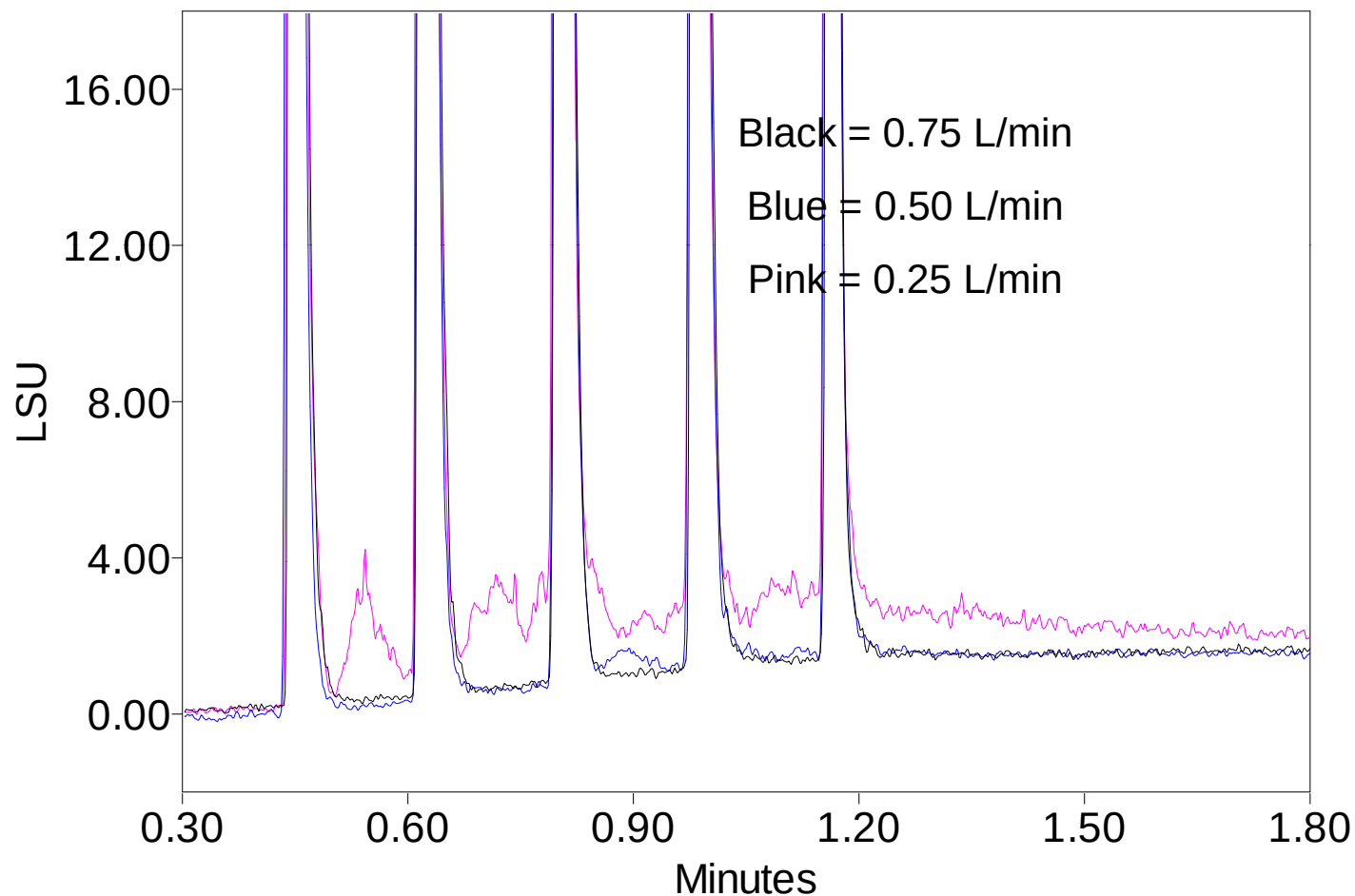
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- Sheath Gas Flow Rates of:
0.75 L/min at 40 psi



ELS Detector Training

- Sheath Gas Flow Rates:
0.75 versus 0.50 and 0.25 L/min at 40 psi



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- The sheath flow needle valve is part of the pneumatics assembly. It is adjusted by the vendor who assembles the pneumatics for Waters.
- No adjustment of the needle valve should be attempted in the field.
- If you suspect an issue of peak tailing that might be due to insufficient sheath flow, replace the pneumatics.

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Issues

ELS Detector Training

- Normalization firmware defect
- At approximately the beginning of the second quarter of 2008 it was discovered that there was a firmware defect in all of the ELS Detectors.
- The defect is that each time the detector is powered on there is a small increase to the gain applied by the firmware.
- It was designed to keep the gain at the proper level to account for lamp and optics degradation.
- Unfortunately the increase in gain multiplies each time the detector is powered on so that a customer that turns off the detector daily will see a large increase in sensitivity over time.

ELS Detector Training

- This would not be bad if it were not for the fact that installing a new lamp or running the normalization routine will reset the gain to the actual level where it should be and then this change is perceived as a loss of sensitivity by the customer.
- The defect will be corrected in the next ICS release and the fix will be for all versions of the ELS Detector. Engineers should load the new firmware and ICS.
- Until the new firmware is released, you should try to avoid running the normalization diagnostic as it may have an adverse effect .

ELS Detector Training

- Nebulizers
- A new Nebulizer design has been introduced to improve the manufacturing process.
- While the original design of the nebulizer was adjusted in the front, the new design is adjusted from the rear.
- The new design no longer requires the use of a liquid thread locker which made the adjustment of the original design very difficult.

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- A new pre-formed siphon waste tube has been designed to route the waste out the left side of the detector and exit at the rear.
- A clamp provided in the startup kit mounts to the cover to make sure the tubing remains at the correct angle.
- This design is to satisfy the requests of customers who desire to route all the system waste to the rear of the system.

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