VAREX ELSD IIA

(Evaporative Light Scattering Detector For HPLC)

OPTIMIZATION PROCEDURE

(Technical Note # ELSD - 0024)

By

William S. Letter

Varex Corporation
4000 Blackburn Lane
Suite # 150
Burtonsville, MD 20866
(301) 421 - 1616

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Introduction :

To fully utilize your VAREX Evaporative Light Scattering Detector (ELSD) to the best of its ability you should familiarize yourself with these procedures. These procedures are designed to help you optimize the ELSD for your particular application. Sample chromatograms and figures are included from an actual analysis to demonstrate the procedure. The text will make reference to this example and it is suggested that you refer to the figures and tables to help you better understand the relationship that exists between the gas flow and temperature adjustments on the detector.

A set of 3 non-ionic detergents were used to generate the data that are presented in this technical note. The method used to run them is shown below.

Sample I.D. : n-Octyl B-D Glucopyranoside, FW = 292.4
n-Decyl B-D Glucopyranoside, FW = 320.4
n-Dodecyl B-D Glucopyranoside, FW = 348.5
Mobile Phase : 90 % Methanol/ 10 % Water Inj. Vol. : 20 ul
Flow Rate : 1.000 mL / min. Inj. Conc.: 1 ug
Column : C-18, 5u (4.6 x 150 mm)
ELSD IIA Conditions : See Examples.

The figures and graphs presented in this note are illustrations of the suggested optimization method presented. This method may not yield the same results for your unit. The conclusions reached and points made should be the same regardless of the intended application. Please pay particular attention to the helpful hints at the end of the optimization procedures. These points can be helpful, both during the optimization procedure as well as when troubleshooting for a problem.

TABLE I

Solvent (HPLC Grade)	Boiling Point °C	* Suggested Heater Adj. Temp. °C	** Nebulizer Gas Flow (+/- 5mm)
Hexane	69	93	35
Isooctane	99	150	> 60
Chloroform	61	108	35
Methylene Chloride	40	75	35
Tetrahydrofuran	66	95	38
Acetone	56	90	35
Acetonitrile	82	130	40
Isopropanol	82	110	40
Ethanol	78	105	40
Methanol	65	120	40
Water	100	150	> 60

* Note: The exhaust temperature will usually be within 10 degrees of the solvents boiling point.

** Note: These suggested gas flows will vary with different nebulizers. It is important that you determine your own optimum gas flow by following the steps in this technical note. The values shown above are meant to be starting values from which you should have some success.

Also : If you are running a binary mobile phase, calculate the suggested starting temperature by accounting for the different values given above for each of the solvents (e.g. Methanol / Water, 60:40 .... (0.60) (120) + (0.40) (150) = 132 or 132°C starting temperature.).

Temperature Optimization:

Using Table I as a guide, set up the ELSD to the suggested starting temperature and gas flow that is appropriate for your application. Be sure to use clean, dry, inert gas and HPLC grade solvent with the detector. With gas flowing only (No Solvent), allow the detector to equilibrate for 15 minutes. The exhaust temperature will usually be within 15 degrees of the boiling point of your solvent mixture when the detector is warmed up. Once the unit has warmed up, start to pump solvent through the detector at the appropriate flow rate (ie: aqueous 0.1 to 1.0 mL / min. or non-aqueous 0.1 to 2.0 mL / min. are recommended). Monitor the

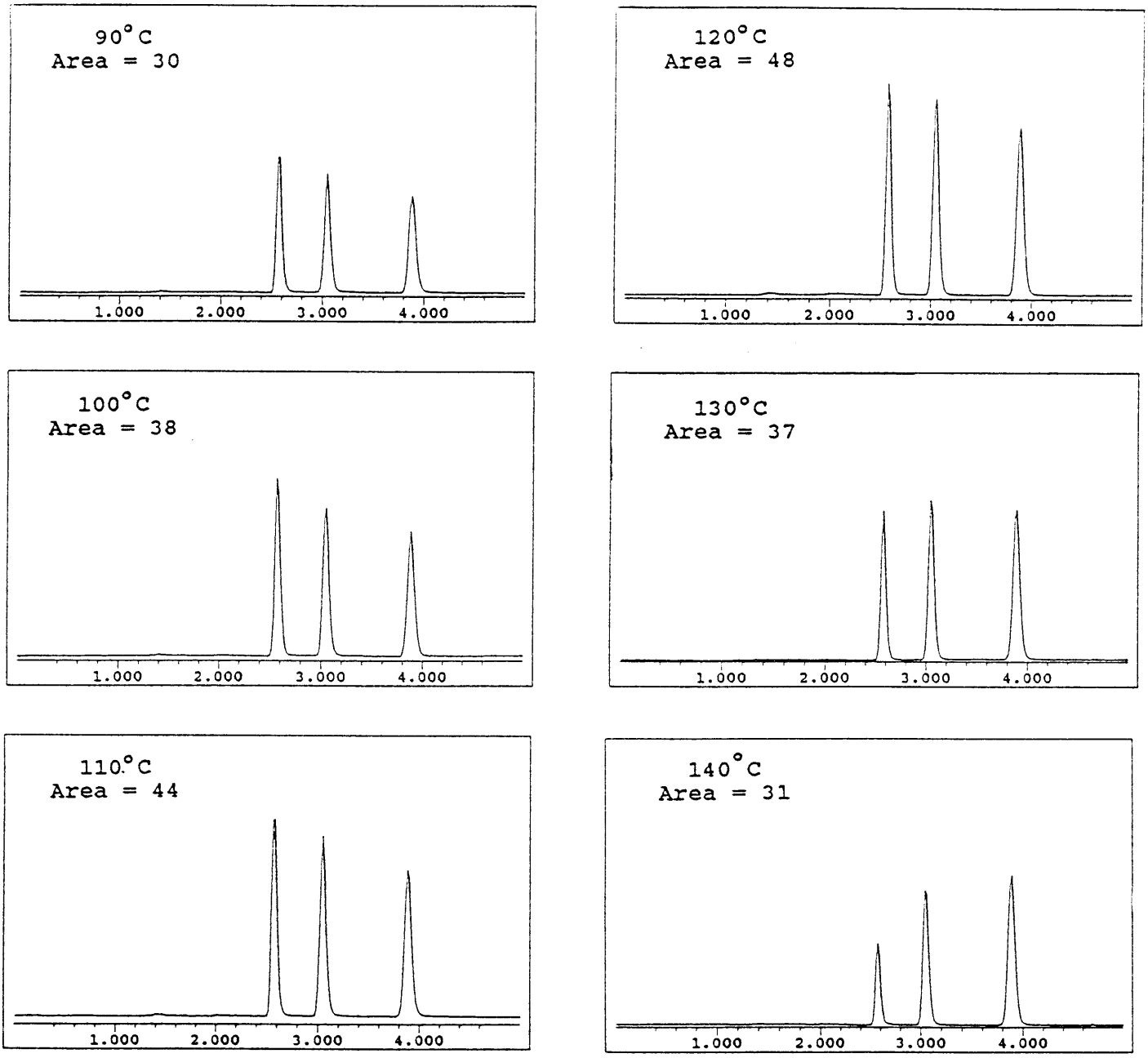


Figure 1. Chromatograms of a 3-Part standard mixture at 6 different heater temperatures selected. The results were obtained with a gas flow of 45 mm for all injections. Note how the heights and areas of the peaks change as the temperature is raised from 90°C to 140°C with a maximum at 120°C. The y-scale is the same on all of the chromatograms for comparison.

signal baseline on a 10 mvFS chart recorder or an integrator set to 8 or 10 mvFS. Within 10 minutes the baseline should stabilize to within < 3 % of the scale. Note and record the present heater

temperature, gas flow (mm) and back pressure (psi) on the detector. Inject an appropriate sample at the concentration you expect to work with into your HPLC System and record the peak area that results. Increase the temperature 5 degrees and allow 5 minutes for the detector to equilibrate. Repeat the procedure 3 or 4 times, each time raising the temperature and recording the peak areas that result. Repeat the procedure again, but this time reduce the temperature from the starting temperature in 5 degree increments and record the areas obtained (Figure 1). Plot the above data (Temperature vs. Peak Area) to determine the optimum operating temperature (Figures 2 & 3). The optimum heater temperature will occur where there is a maximum amount of peak area on the graph.

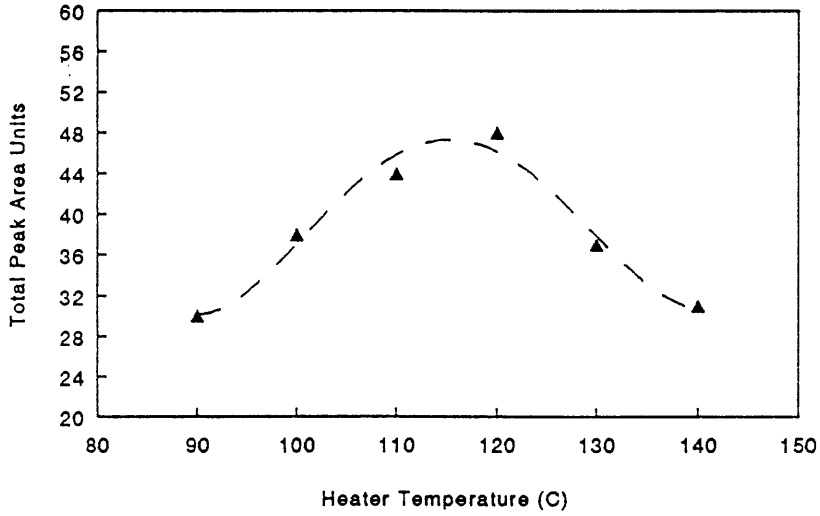


Figure 2. A Graph of the relationship between the heater temperature and the total peak area obtained. The maximum area was achieved between 115 & 120°C heater temperatures.

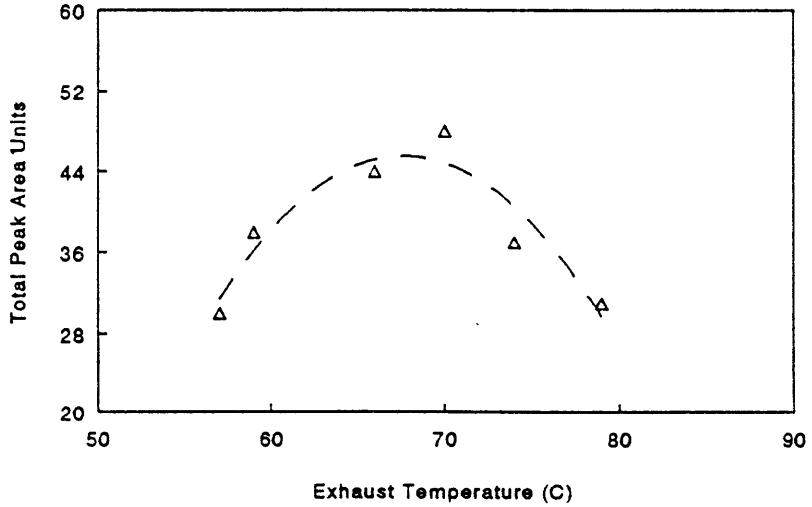


Figure 3. A plot of the detectors exhaust temperature vs. the total peak area demonstrates a maximum peak area between 67 & 70°C. This is very near the boiling point of the binary mobile phase used.

Gas Flow Optimization:

The flowmeter on the ELSD is designed to control the flow of inert gas to the nebulizer at the top of the detector. The flowmeter has 'mm' divisions which can be converted to liters / minute using Figure 4. The flow rate was measured with a mass flow meter using nitrogen gas at ambient temperature.

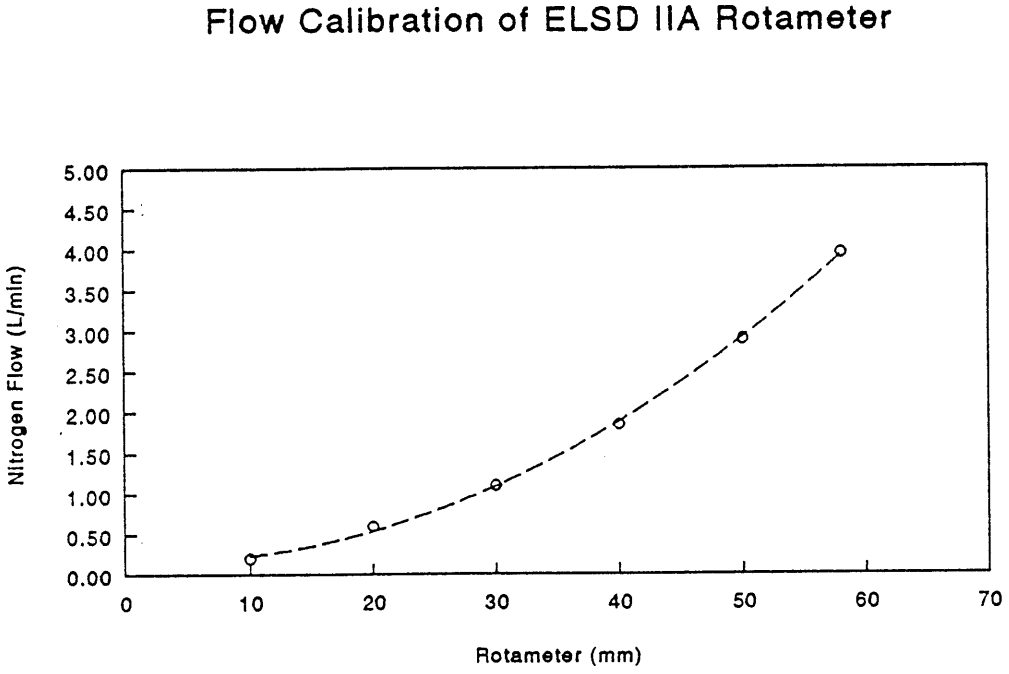
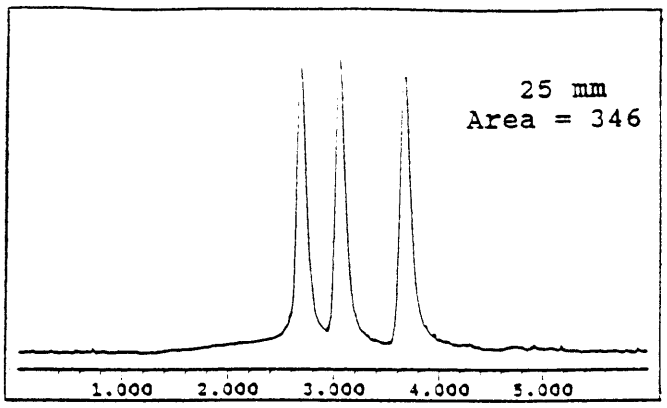
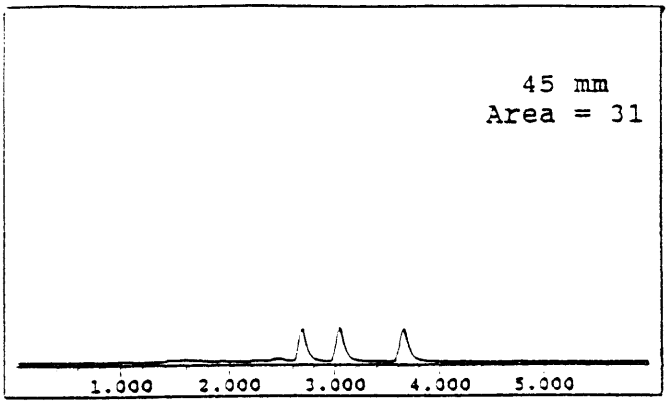
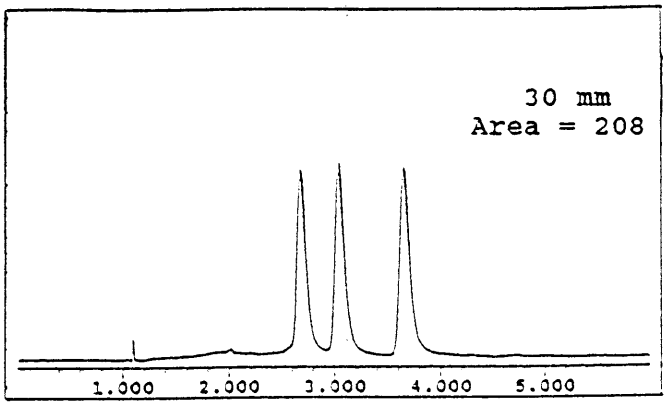


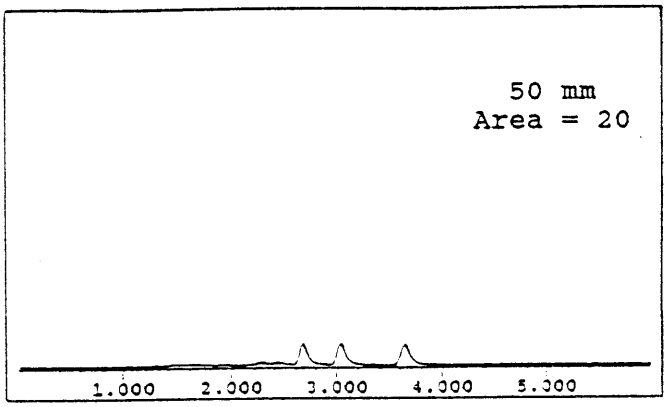
Figure 4. Mass flow calibration of the ELSD flowmeter. The conversion from 'mm' to L / min is shown.

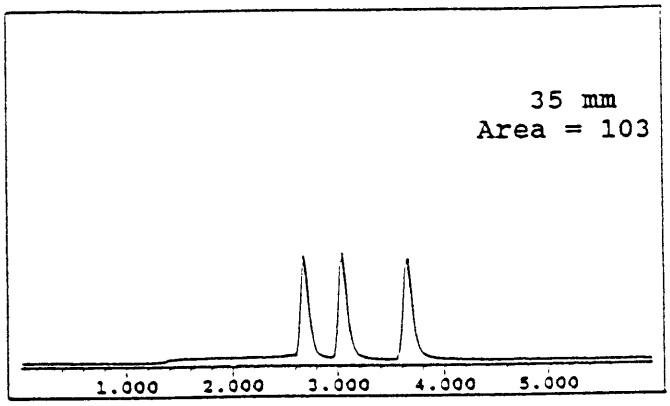
Gas flow optimization can be accomplished in a similar manner as temperature optimization, described above. Set the heater on the detector to the optimized temperature determined earlier using the above procedure. Once the unit is fully equilibrated at this temperature and the solvent has been flowing for at least 10 minutes, inject a sample as before and record both the area obtained and the amount of baseline noise present in the signal. Repeat this process (Note: allow 5 to 10 minutes between gas flow adjustments) several times while changing the gas flow in 5 mm increments up and down until you have a number of chromatograms at different gas flows (Figure 5). Plot the above data (Gas Flow vs. Peak Areas) as shown in Figures 6a & 6b. You should find that the higher the gas flow used the lower the peak area. The maximum signal to noise ratio (S/N ratio) should occur at the inflection of the gas flow vs. area curve (Figure 7). The sensitivity should be highest at this point. Adjust your detector to these optimum parameters to obtain the best results.

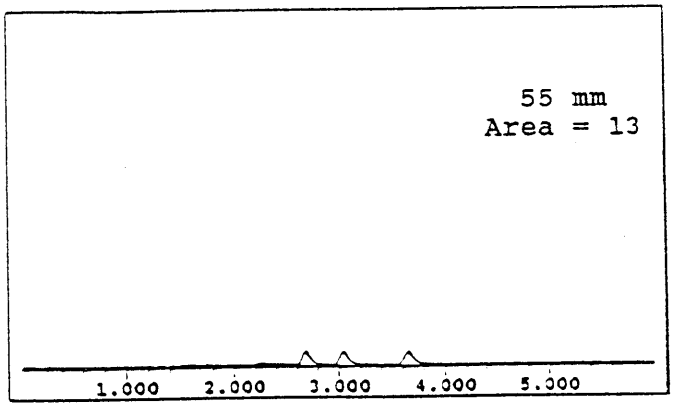












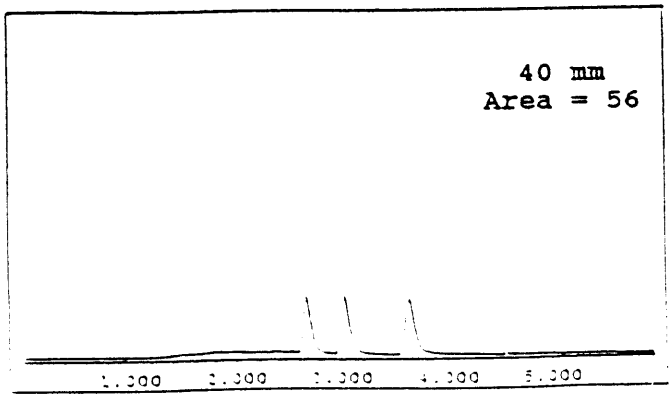


Figure 5. Chromatograms of a 3-Part standard mixture with 7 different gas flow settings. The temperature was set at 120°C.

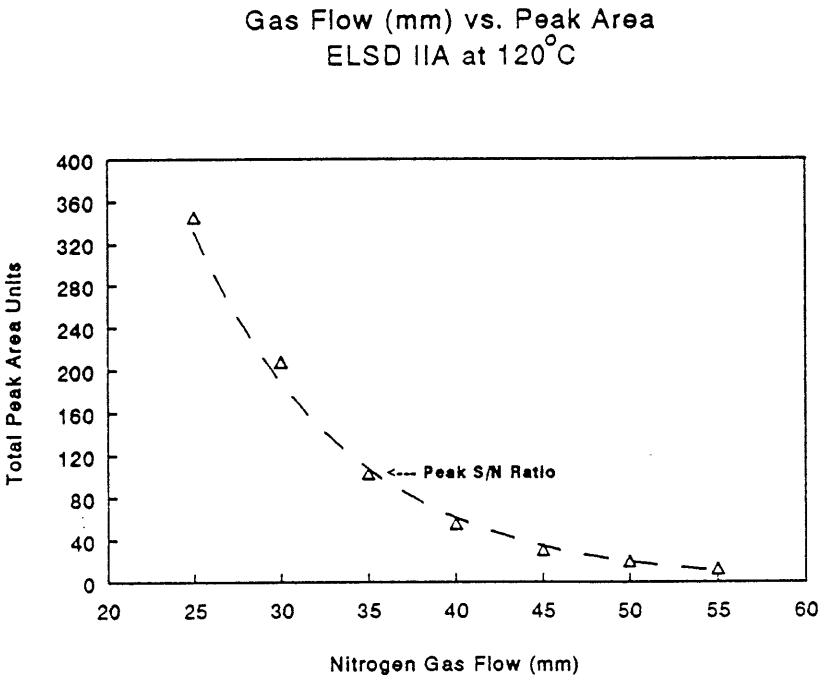


Figure 6a. The effect of varying the gas flow on peak area is shown. Note how a lower gas flow results in higher area counts while a higher flow produces a smaller area count. The optimum signal will be found at the inflection of the curve where the signal to noise ratio is highest.

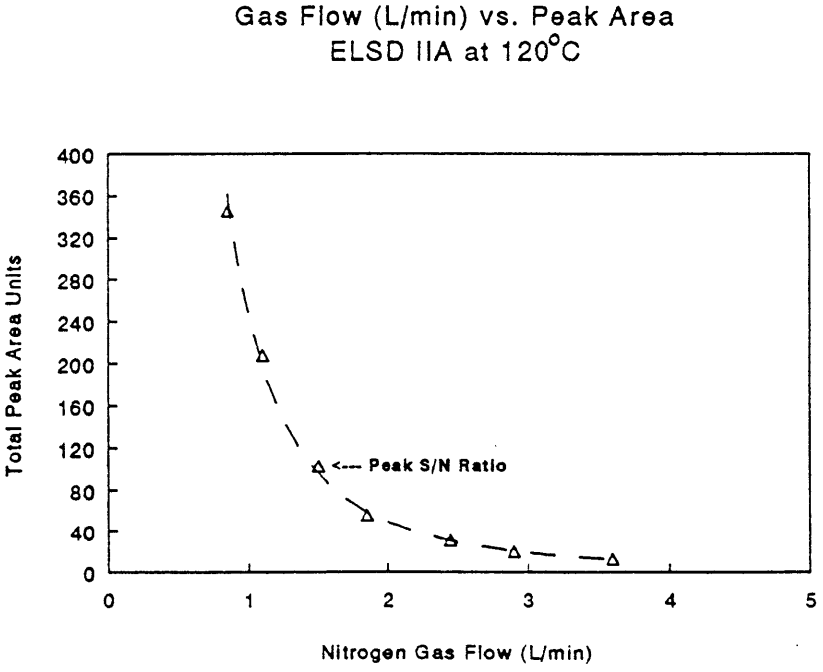


Figure 6b. Gas flow vs. peak area with the x-axis corrected for flow in liters per minute.

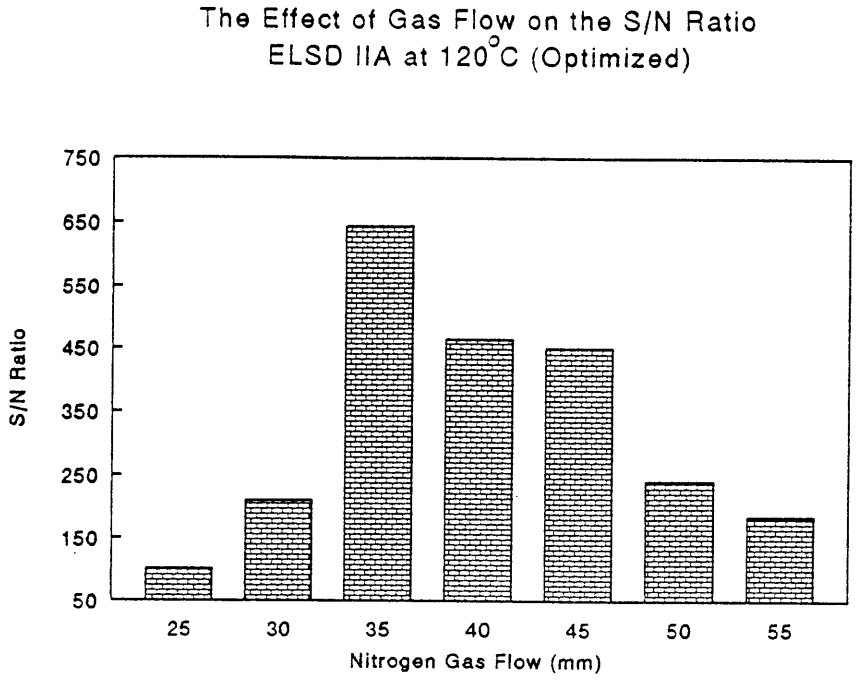


Figure 7. The above bar graph shows how the gas flow actually changes the signal to noise ratio (S/N ratio). The maximum S/N was achieved at a flow setting of 35 mm. This flow setting coupled to the optimum temperature of 120 °C should yield the highest sensitivity under these chromatographic conditions.

Optimization Hints:

Once you have determined the optimum temperature and gas flow for your application keep these hints in mind.

1. Improved sensitivity can usually be achieved by reducing the solvent flow rate to the detector.
2. The use of mini or microbore columns can both save solvent and increase the signal response of most compounds.
3. Filter all HPLC samples prior to injecting onto a column.
4. Make sure your mobile phase is volatile. The lower the boiling point of your mobile phase the better your signal response should be (efficiency of nebulization is increased when more volatile mobile phases are used). The use of many acids and modifiers can increase your noise and possibly damage the detector. Check with VAREX Corporation if in doubt.
5. Insure that your sample is not going to degrade under the high temperature conditions present inside the ELSD. Make sure that its boiling or melting point is above the drift tube heaters temperature.

6. MOST IMPORTANTLY clean your nebulizer, drift tube and vaporizer block regularly to maintain high sensitivity and trouble free operation all the time. Overloading the detector with high concentrations of sticky samples or running with non-volatile modifiers will greatly increase the frequency of required cleanings.

Conclusion:

These procedures should help you optimize the performance of your ELSD and help you achieve the highest sensitivity possible. Understanding the effects of temperature and gas flow should help you set up your detector faster as well as enable you to troubleshoot potential problems that could arise in the future. Careful cleaning on a regular basis will also help to insure that your detector operates at peak performance all the time.