



Agilent Dimension Software for ELSD

User Manual

Notices

© Agilent Technologies, Inc. 2011

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Manual Part Number

G4260-90320

Edition

06/11

Printed in Germany

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn

This product may be used as a component of an in vitro diagnostic system if the system is registered with the appropriate authorities and complies with the relevant regulations. Otherwise, it is intended only for general laboratory use.

Warranty

The material contained in this document is provided “as is,” and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

If software is for use in the performance of a U.S. Government prime contract or subcontract, Software is delivered and licensed as “Commercial computer software” as defined in DFAR 252.227-7014 (June 1995), or as a “commercial item” as defined in FAR 2.101(a) or as “Restricted computer software” as defined in FAR 52.227-19 (June 1987) or any equivalent agency regulation or contract clause. Use, duplication or disclosure of Software is subject to Agilent Technologies’ standard commercial license terms, and non-DOD Departments and Agencies of the U.S. Government will

receive no greater than Restricted Rights as defined in FAR 52.227-19(c)(1-2) (June 1987). U.S. Government users will receive no greater than Limited Rights as defined in FAR 52.227-14 (June 1987) or DFAR 252.227-7015 (b)(2) (November 1995), as applicable in any technical data.

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

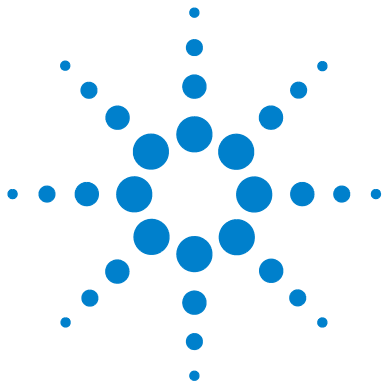
A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

In This Book

This manual contains information on the Agilent Dimension Software for ELSD.

Contents

1	Introduction	5
2	Calibration Procedure	7
	Evaluating Solvent Enhancement	8
	Correcting Solvent Enhancement	9
	Implementing Real-Time Gas Control	11



1 Introduction

A well-known property of aerosol-based detectors is their response dependency on mobile phase composition. For example, when using a reverse-phase gradient the response of analytes eluting at the end of the gradient will be larger than those at the beginning of the gradient. For compounds of equal concentration the response of late eluting compounds can be 2 – 8 x larger than those at the early part of the gradient.

This enhancement in compound response across a solvent gradient is a consequence of the viscosity difference between mobile phase A and B. During a typical reverse-phase gradient, the viscosity decreases as the proportion of organic mobile phase increases. This transition of decreasing solvent viscosity affects the nebulisation process of the ELSD causing an increase in the number of droplets formed, which subsequently leads to an enhancement in the detector's response.

This non-uniform response across a solvent gradient has several implications when quantifying with aerosol-based detectors:

- 1 A slight shift in a compound's retention time between injections will lead to poor reproducibility in area response.
- 2 Irreproducible gradient programs will produce poor accuracy due to variations in solvent composition.

If quantifying unknown compounds (where calibration standards don't exist) using chemically dissimilar calibration standards, then this enhancement effect of the mobile phase has to be considered, otherwise large errors are observed.

The Agilent 38x-ELSD has the ability to compensate for this solvent enhancement by controlling the number of particles entering the detection stage in real-time during the gradient. The detector uses an on-board timetable that can be programmed to control the detector's evaporation gas flow.



1 Introduction

In This Book

This guide outlines how to calibrate & program the Agilent 38x-ELSD, in order to overcome any solvent enhancements effects, to deliver a uniform response when running gradients.



2 Calibration Procedure

Evaluating Solvent Enhancement	8
Correcting Solvent Enhancement	9
Implementing Real-Time Gas Control	11

In order to compensate for the solvent enhancement effect on the Agilent 38x-ELSD, a gas timetable must be developed and programmed into the detector. To achieve the appropriate gas flow timetable for the solvent gradient, the following steps must be taken:

- 1 Inject sample under typical LC gradient conditions.
- 2 Characterise ELSD gradient response for single compound under typical LC conditions.
- 3 Use Agilent 38x-ELSD Dimension software to formulate gas flow program.
- 4 Download Gas program to ELS Detector.
- 5 Inject sample using real-time gas control program.

These procedures are described in more detail below, with real chromatographic examples.



Evaluating Solvent Enhancement

Configure the HPLC system according to the required analytical protocol. An example of the typical hardware configuration is shown in [Figure 1](#) on page 8.

Under the required analytical conditions inject a sample or standard mixture to obtain a reference chromatogram under typical LC conditions. This provides a useful control when comparing with the data obtained under gas flow control.

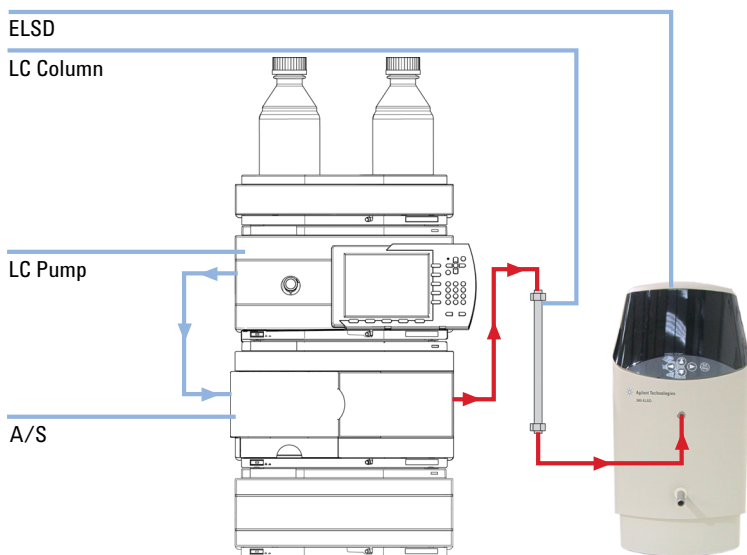


Figure 1 HPLC Configuration under typical operating conditions

Correcting Solvent Enhancement

Having established a response profile for the sample mixture, the contribution of solvent enhancement must be determined by calibrating the solvent gradient using a single-compound. The single compound is injected directly into the ELSD under the same gradient conditions to achieve a response profile.

The HPLC solvent must be re-configured for this test to allow direct injections to be made into the ELSD. The compound that elutes at the mid-point of the gradient should be used to characterize the solvent gradient.

In order to make direct injections into the ELSD, but maintain the same dwell volume for the gradient program, the LC system must be configured as shown in [Figure 2](#) on page 9. The HPLC column must be connected between the HPLC pump and auto-sampler, and the auto-sampler must be connected directly to the ELSD.

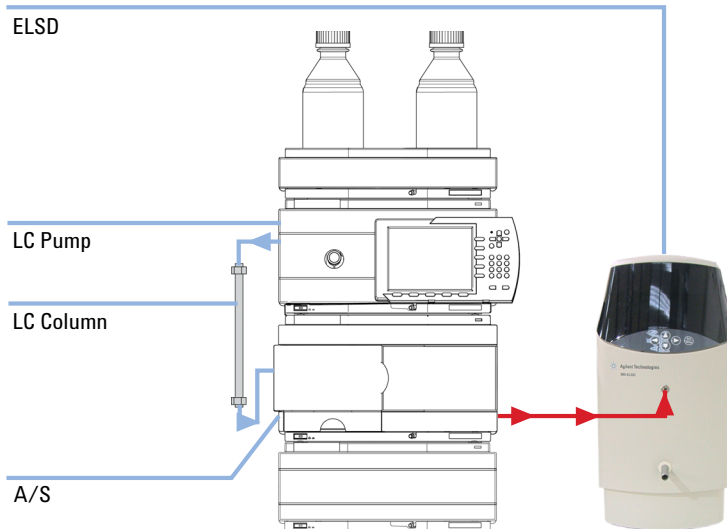


Figure 2 Configuration of HPLC system for direct injection of a single compound

It is recommended that repeat injections be made at 10 % time intervals across the gradient, such that for a 10 min run, injections every 1 min should be

2 Calibration Procedure

Correcting Solvent Enhancement

made. Using these conditions, a chromatogram similar to that shown in [Figure 3](#) on page 10 should be obtained.

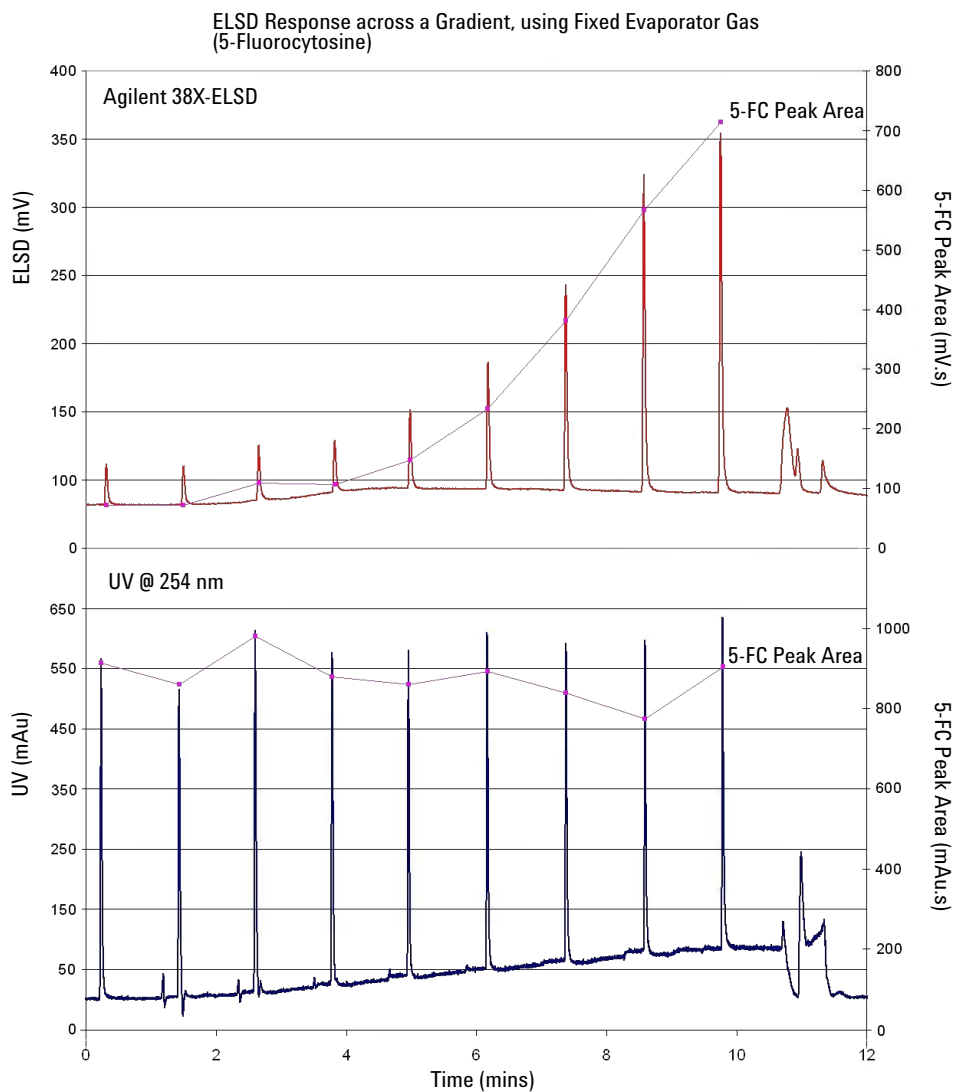


Figure 3 Direct injections of single component across a solvent gradient, with UV data

Implementing Real-Time Gas Control

The Agilent 38x-ELSD Detector can store in memory a series of time-based events, within a single timetable. This timetable allows the operational settings of the ELSD to be changed in real-time during a run.

The timetable can be used to program the gas flow, in order to compensate for the change in ELSD response across a solvent gradient. Alternatively, the timetable can be customized to create adjustable LED intensities across a sample injection for mixtures containing samples with wide concentration ranges.

The single timetable, stored on-board the ELSD, is only customizable using the Agilent 38x-ELSD DIMENSION software, which is a standalone software application.

NB: Once a timetable has been downloaded to the instrument, it cannot be removed. Therefore, if a timetable of events is no longer required, either the trigger cable should be disconnected (to prevent the timetable from being initiated again), or a “blank” timetable (with constant detector settings programmed) should be downloaded to the ELSD instead.

This section describes how to use Dimension software to formulate a real-time gas program.

Creating a real-time program

In order to create or modify the on-board timetable, DIMENSION software must be installed on your PC.

The DIMENSION software package allows you to create a timetable on a PC, which can subsequently be downloaded to the ELSD for later use.

The ELSD can only store a single timetable in memory, so the DIMENSION software can be used to create & save multiple timetables that can be downloaded individually at a later date. For further information on how to use DIMENSION software, please refer to the DIMENSION software on-line help.

Calculating the shape of the gas gradient

From the data obtained in [Figure 3](#) on page 10, whereby the gradient response profile was characterized with a single component, a gas gradient can be defined. Using the peak area data from the single component injections, the values must be normalized against the first injection to determine the change in signal response. These data are shown in [Table 1](#) on page 12.

Table 1 Data for single compound injection

A	B	C	D ¹
Peak Retention Time (mins)	Injection No.	Peak Area (mV.min)	Change in response from injection 1
0.29	1	4.7	1.00
1.44	2	5.1	1.09
2.54	3	6.9	1.47
3.68	4	7.9	1.68
4.77	5	9.8	2.09
5.93	6	10.8	2.30
7.07	7	13.6	2.89
8.22	8	17.4	3.70
9.38	9	19.1	4.06
10.53	10	18.6	3.96

For direct injections of single component across a solvent gradient, with UV data, see [Figure 3](#) on page 10

¹ Change in response from injection 1 (D) = Response at time x (C) / Response at injection 1 (C) e.g. at time 7.07, Change in response from injection 1 = 13.6 / 4.7 = 2.89

By calculating the gradual increase in peak height of the single component, the relative change in ELSD response across the gradient can be assessed. From

the data in [Table 1](#) on page 12, the ELSD response changes by a factor of 4 across the 10 min solvent gradient.

Calculating the required gas flow from the data in [Table 1](#) on page 12 requires an experimental trial and error procedure, but a very close estimate of the gas flow program to start with can be calculated.

This calculation of gas flow value at each 1 min step across the gradient is shown in [Table 2](#) on page 14. By calculating the change in ELSD response at each minute across the gradient normalized against the first injection a value for the necessary increase in gas can be determined.

However, the effect of increasing gas flow does not have a linear relationship on the ELSD response because of the logarithmic response of the ELSD. Therefore to compensate for this non-linear relationship a correction factor must be applied. By dividing the “predicted linear gas increment” by the correction factor, E (i.e. 3), then a more realistic gas increase increment is obtained. From this logarithmic gas increment value the estimated gas flow across the gradient can be made, as highlighted in red within [Table 2](#) on page 14.

2 Calibration Procedure

Implementing Real-Time Gas Control

Table 2 Data for single compound injection

A	B	C	D ¹	E	F ²	G ³
Peak Retention Time (mins)	Single Compound Peak Area (mV.min)	Change in Response vs first inj	Predicted Gas flow increment (if linear)	Correction Factor due to ELSD log response	Predicted Gas Increment (log resp)	Estimated Gas Flow Required
0.29	4.7	1.00	1.00	3	1	1.60
1.44	5.1	1.09	0.09	3	0.03	1.63
2.54	6.9	1.47	0.47	3	0.16	1.76
3.68	7.9	1.68	0.68	3	0.23	1.83
4.77	9.8	2.09	1.09	3	0.36	1.96
5.93	10.8	2.30	1.30	3	0.43	2.03
7.07	13.6	2.89	1.89	3	0.63	2.23
8.22	17.4	3.70	2.70	3	0.90	2.50
9.38	19.1	4.06	3.06	3	1.02	2.62
10.53	18.6	3.96	2.96	3	0.99	2.59

For configuration of HPLC system for direct injection of a single compound, see [Figure 2](#) on page 9

- ¹ Predicted Linear Gas Flow Increment (D) = Change in Response at time x (C) – Change in Response at Injection 1 (C) e.g. at time 1.44, Predicted Linear Gas Flow Increment = 1.09 – 1.00 = 0.09
- ² Predicted Log Gas Flow Increment (F) = Predicted Linear Gas Flow Increment (D) / Correction Factor (E) e.g. at time 2.54, Predicted Log Gas Flow Increment = 0.47 / 3 = 0.16
- ³ Estimated Gas Flow Required (G) = Initial Gas Flow at injection 1 + Predicted Log Gas Flow Increment at time x (D) e.g. at time 4.77, Estimated Gas Flow Required = 1.60 + 0.36 = 1.96

This gas flow program is a step-wise gradient and is only a guide to the required gas flow required to achieve uniformity. The next step is to input these values into Dimension software, which will interpolate between the set values and create a smooth gas transition across the gradient.

Using Dimension software to develop gas program

The start and end values for the gas can be inputted into Dimension to give a smooth gas profile as shown in [Figure 4](#) on page 15. See the Dimension on-line help for further information on using Dimension.

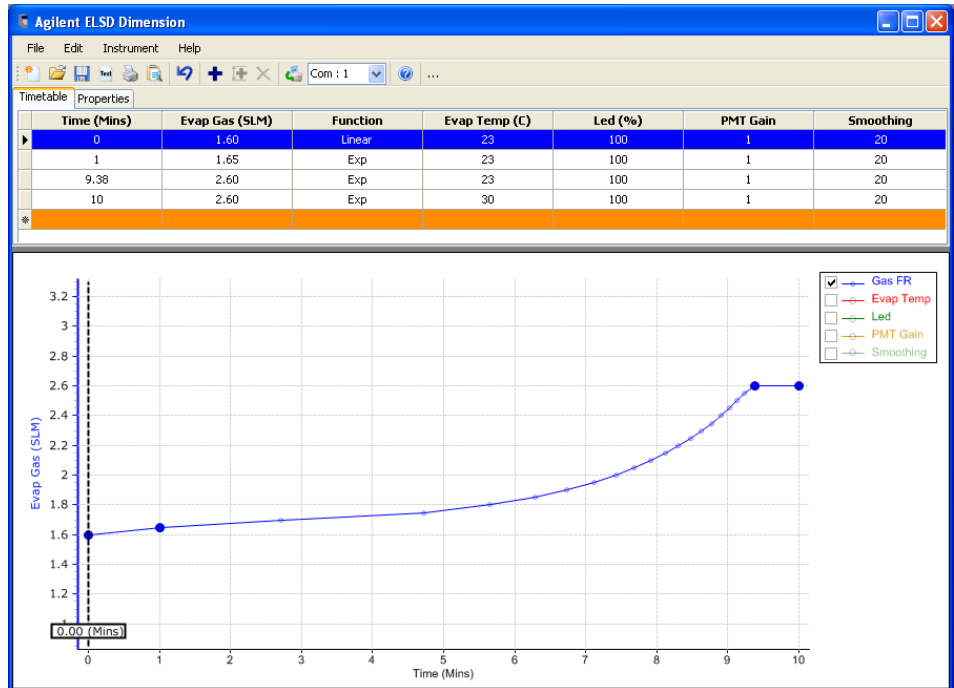


Figure 4 Gas program developed using Dimension software.

Gas program from [Table 2](#) on page 14

Having developed the initial gas profile, the program is downloaded to the ELSD and the direct injections of the single compound are repeated to assess the effect of the gas control. [Figure 4](#) on page 15 shows the result of using the gas flow program in [Figure 4](#) on page 15 compared to the result obtained without gas flow control as shown in [Figure 3](#) on page 10.

2 Calibration Procedure

Implementing Real-Time Gas Control

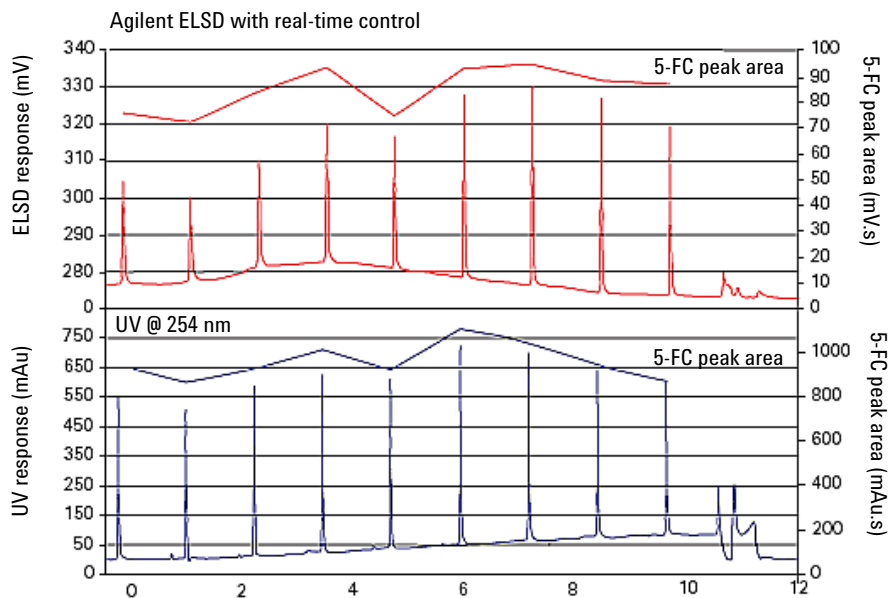


Figure 5 Re-injection of single compound using real-time gas control program.

See [Figure 2](#) on page 9 for comparison

If the initial gas flow program is insufficient to control the changing peak response during the gradient, then Dimension software can be altered accordingly (i.e. by applying a different correction factor) and the new program reloaded onto the ELSD.

Once this iterative process of editing and optimising the gas program has achieved the desired uniformity for the single compound injections, then the HPLC can be re-configured back to its original state as shown in [Figure 1](#) on page 8.

The ELSD stores the gas program in its memory and it is triggered by an input signal from the auto-sampler. To implement this start signal, the ELSD remote signal cable must be connected from the I/O connector on the rear of the unit to the A/S trigger cable.

www.agilent.com

In This Book

The manual describes the following:

- Introduction
- Calibration Procedure
- Evaluating Solvent Enhancement
- Correcting Solvent Enhancement
- Implementing Real-Time Gas Control

© Agilent Technologies 2011

Printed in Germany
06/11



G4260-90320



Agilent Technologies