

Technical Note

Use of Computer Spreadsheet Template to Evaluate Drifting Gas-Liquid Chromatograph or High-Pressure Liquid Chromatograph Detector Responses of Pesticides

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Abstract

The analyses of pesticide residue samples by gas-liquid or high-pressure liquid chromatography often produces a slow, continual drift in detector response resulting from the accumulation of poorly separated, co-extracted sample materials that are injected onto the column. In order to compensate for drifting detector responses, a Lotus 1-2-3® spreadsheet template has been designed to make time-dependent adjustments in the standard regression lines used for calculating pesticide concentrations. The template also provides a permanent record for maintaining an audit trail of the sample analysis.

Introduction

Accurate determination of trace levels of pesticides are made more difficult as a result of drifting detector responses (gas-liquid chromatography [GLC] or high-pressure liquid chromatography [HPLC]). Although GLC detectors such as electron capture (ECD), nitrogen-phosphorus (NPD), or the ultraviolet (UV) HPLC detector tend to be quite electronically stable, responses from these detectors often appear to drift with time because of the accumulation of residual, poorly chromatographed co-extracted sample materials that are injected onto the column. These residual materials not only influence column performance, but may also affect detector performance as they slowly bleed off the column.

In environmental quality mass balance studies requiring both accuracy and precision, it is a common practice to autoinject both pesticide samples and external standards in duplicate or even in triplicate. Because observed detector responses seldom are linear or have zero intercepts, it is necessary to use two or even three standards in the expected range of the unknown samples, both preceding and following each sample group. Analysis times (assuming a 10-min cycle) for each sample group (standards [2-3], sample set [6-8], standards [2-3]) can require 3-6 h, and entire runs may last 30 h or more.

It has been an ongoing challenge to accurately evaluate pesticide sample concentrations when detector responses continually change throughout a sample group analysis. To our knowledge, there is no commercially available data handling software that can properly assess several levels of standards (using multiple injections) before and after a sample set, and for which the response factors can be reset (not just averaged or updated) for each sample group. It is straightforward to calculate regression lines for the "Pre-" and "Post-" set of standards in each sample set using a programmable calculator. However, regression line slopes and intercepts of the Pre- and Post- standard sets often shift during an analysis, making it inappropriate to simply average the Pre- and Post- standard responses to establish an "averaged" regression line.

We will illustrate a Lotus 1-2-3® spreadsheet template for a MS-DOS microcomputer that will greatly simplify data reduction for drifting detector responses from any analytical detector, as well as providing a permanent record for maintaining an audit trail of the sample analysis.

Results and Discussion

Because GLC detector responses (and to a lesser extent HPLC detector responses) often tend to slowly increase or decrease in sensitivity over extended periods during analytical runs, it was practical to use this as a basis for a time-dependent adjustment in the standard regression line used for calculating pesticide concentrations. In fact, the detector drift was sample-dependent, but could be treated as time-dependent if uniform injection cycles were used. Thus, the technique is more useful for autosampler applications because of uniform injection cycle times. We have developed a macro-driven Lotus 1-2-3 spreadsheet template that automatically generates a linear regression line for both the Pre- (Set 1) and Post- (Set 2) standard sets in each sample group (Table I). All sample integrator counts (rinse-blank injections included) are entered sequentially in the input area of the template and both sets of standard regression lines are linearly blended to calculate the sample concentrations.

The influence of the first set of standards decreases linearly throughout the sample set, while the second standard set linearly increases. The first sample is evaluated only by the Pre- standard set while the last sample is evaluated only by the Post-standard set. These regression lines usually have different slopes and intercepts (Table I, Figure 1). We used a simple fractional formula, which divided the total response difference between the Pre- and Post- sets into "*n*" equal portions (*n* = number of sample injections between pre- and post- standard sets), which then was applied in a step-wise, accumulative manner from the first sample in the sequence to the last. For demonstration purposes, three vials of one atrazine sample (in methanol-water, 50:50) were alternated with three vials of a second atrazine sample (in water) (Table I). Four output concentration columns are displayed for comparison purposes: (i) Blended = both standard sets linearly blended; (ii) Set 1 = only Pre- standard set used; (iii) Set 2 = only Post- standard set used; (iv) Avg. = average of Pre- and Post-standard sets.

The effect of calculation method upon reported sample concentration values is shown in Figure 2. Replicate injections (1,3,5 or 2,4,6) in the "blended line" remain relatively constant compared to those in the "Set 1 line" or the "Set 2 line". The concentrations shown in the "Avg." column of Table I show that simply averaging Pre- and Post-standards, as would be done with a calculator, produces much less consistent results than these produced using the "Blended" standards.

Table I. Lotus 1-2-3 Spreadsheet Template for Evaluating Drifting GLC Responses During Pesticide Analyses*

SAMPCALC.WK1 Press: <ALT> M for MENU

Compound: atrazine	Standard Entry Area			
	Set No. 1		Set No. 2	
Analysis I.D. : atrazine water samples	Conc.	Counts	Conc.	Counts
	0.356	116146	0.356	130769
Sample Date : 23-Aug-91	0.593	198838	0.593	219124
Analysis Date : 27-Aug-91	1.186	403576	1.186	439969
Data File Name : 23Aug				
Process Date : 03-Sep-91				
Process Time : 09:12 AM				

REGRESSION CONSTANTS			Dilution Information/Comments
Set #1	Set #2		
Intercept : -6788	-1815		Conc. Units :
Slope : 346100	372509		
R Squared : 0.9999	0.9999		

COUNT EVALUATION								
Inj. No.	SAMPLE I.D.	SAMPLE COUNTS	Blended	Set #1	Set #2	Avg.	Dilute Factor	Orig. Conc.
1	atrazine - solvent	220074	0.655	0.655	0.596	0.626	1.0	0.655
2	atrazine - water	216051	0.632	0.644	0.585	0.615	1.0	0.632
3	atrazine - solvent	228041	0.654	0.678	0.617	0.648	1.0	0.654
4	atrazine - water	224563	0.632	0.668	0.608	0.638	1.0	0.632
5	atrazine - solvent	237295	0.655	0.705	0.642	0.674	1.0	0.655
6	atrazine - water	230692	0.624	0.686	0.624	0.655	1.0	0.624

* Three sample vials each of atrazine-solvent and atrazine-water were alternatively injected.

For optimum accuracy and reliability of the calculated concentrations, it is unwise to evaluate sample counts that occur much outside of the count range of the standards sets. To guard against accidental occurrence of this, we have included minimum and maximum limits in the "Count Evaluation" formulae, which display a "Range" label if sample counts fall outside the user-set limits (Table II, Lo/Hi Allow. Std. Count). For most practical applications, the calibration curves don't radically change just outside their upper and lower limits, so we have included a "Percent Count Deviation Tolerance" (set at 2.0% in Table II) that extends the Lower and Upper Evaluation Limits by a user-set percentage of the minimum or maximum count, respectively. This allows the user flexibility in including some sample counts in an analysis set that falls only a few counts beyond the selected cut-off limits. To change the "Count Tolerance" setting, the user enters a new value, and recalculates the spreadsheet.

Before we introduced the "Out of Range" feature, it was a relatively frequent occurrence to accidentally include sample counts outside the standards range which should have been evaluated by another set of standards. When sample counts fall below the lowest available standard, it is possible to enter zeros for both standard concentrations and counts (both sets), thereby forcing a regression through the origin. However, it is recommended that the sample set should be reanalyzed using a lower set of standards.

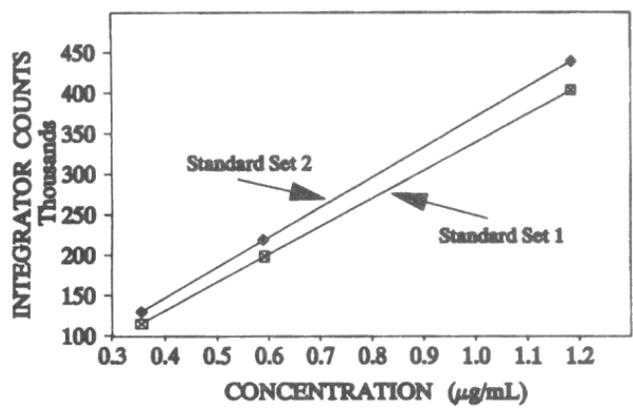


Figure 1. Linear regression lines for Pre- and Post-standard sets, which exhibit different slopes and intercepts.

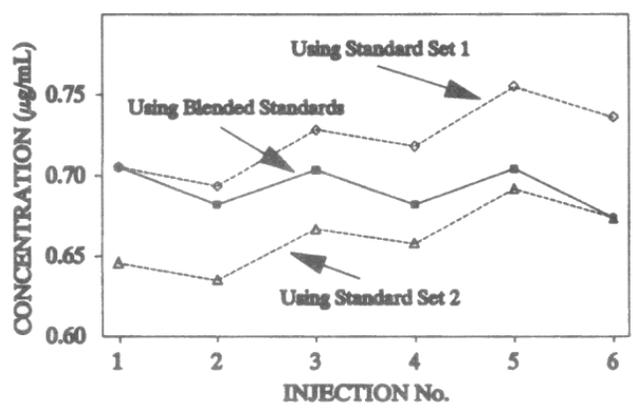


Figure 2. Evaluation of repeated injections of the sample by three methods: (i) standard set 1; (ii) blended standards, and (iii) standard set 2.

All operations performed by the spreadsheet template are selected from macro-driven menus, so that complete familiarity with the spreadsheet is not necessary. There are five main Menu Selections: (i) DataRegress = best-fit linear regression calibration lines are derived for each set of standards, and the calculated sample concentrations are displayed (Table I); (ii) Calib.curve = regression points with respective best-fit regression lines for both sets of standards are graphed for comparison purposes (show detector drift and slope differences) (Figure 1); (iii) Sample- Conc = calculated sample concentrations for three calculation methods (Blended, PreSet 1, PostSet 2) are graphed for each injection to show effect of different calculation procedures (Figure 2); (iv) Replicate = This option allows the user to select output concentration values (Orig. Concentration) to be reported individually or to be averaged if duplicate injections were used (Table II); and (v) Print = template can be printed out for a permanent record and includes all of Table I.

Other Features and Limitations

Sample identification. There are entry areas for the Analysis I.D., Sampling Date, Analysis Date, and Data Handling File Name. Additionally, the spreadsheet automatically includes its own Process Date and Time stamp, which is useful in distinguishing different output versions of the same sample set reprocessed on the same date (for cases where samples have been re-analyzed and only have minor changes). We have found the thorough sample analysis description to be useful in maintaining an accurate sample audit trail through the entire analysis procedure, and the printout can be stored with the raw data files.

Table II. Spreadsheet Template Showing: (i) Rejection of Sample Counts for Being Outside Count Deviation Tolerance Limits; (ii) Use of Dilution Factor; (iii) Averaged Sample Concentrations for Duplicate Injections

Compound: atrazine		Standard Entry Area					
		Set No. 1		Set No. 2			
Analysis I.D.:	Soil Cores	Conc.	Counts	Conc.	Counts		
Set 1							
Sample Date:	20-Aug-91	0.606	102423	0.606	118325		
Analysis Date:	28-Aug-91	0.606	102520	0.606	118129		
Data File Name :	28Aug	1.011	154452	1.011	185144		
		1.011	153025	1.011	186153		
Process Date :	04-Sep-91	1.500	233122	1.500	271510		
Process Time :	02:25 PM	1.500	233544	1.500	272089		
REGRESSION CONSTANTS							
		Set #1	Set #2	Dilution Information/Comments			
Intercept :	10438	13174	Dilution Factor: 25 mL → 50 mL (2X)				
Slope :	147005	172004					
R Squared :	0.9951	0.9997					
Count Dev. Tolerance (%) :	2.0						
Lo/Hi Allow. Std. Count :	100375	277531					
No. of Sample Injections :	12	COUNT EVALUATION					
Inj. No.	SAMPLE I.D.	SAMPLE COUNTS	Concentration.....			Dilute Factor	Orig. Conc.
			Blended	Set #1	Set #2		
1	Core 1-1	98452	Range	Range	Range		
2	Core 1-2	99121	Range	Range	Range	2.0	xxxx
3	Core 2-1	115688	0.694	0.716	0.596		
4	Core 2-2	116897	0.691	0.724	0.603	2.0	1.385
5	Core 3-1	94123	Range	Range	Range		
6	Core 3-2	96654	Range	Range	Range	2.0	xxxx
7	Core 4-1	185985	1.091	1.194	1.005		
8	Core 4-2	188964	1.092	1.214	1.022	2.0	2.183
9	Core 5-1	265213	1.538	1.733	1.465		
10	Core 5-2	268564	1.534	1.756	1.485	2.0	3.072
11	Core 6-1	278952	Range	Range	Range		
12	Core 6-2	281321	Range	Range	Range	2.0	xxxx

Maximum samples. Although the spreadsheet template can accommodate a maximum of 30 injections between the two sets of standards, it would be wise to rerun standards more frequently. Because of the manner in which the spreadsheet evaluates the sample injection count (@COUNT) to determine the blending factor, every valid injection entry must be accompanied with a numerical sample count entry (including zero, and no label prefixes [i.e., ' ^, or"]) entered below the last valid sample count entry). The template uses the Sample Count column to calculate the injection count.

Regression limitations. Lotus 1-2-3 does not allow data regression to be performed using only two data points (i.e., two standards). In cases where only single injections of only two levels of standards are used, this limitation can be circumvented by entering both standard data points twice. From a computational standpoint, this procedure introduces no errors into the final calculated concentrations. This procedure is not necessary where either three levels of standards or duplicate injections of two levels of standards are used. It is recommended that the same number and levels of standards be used in both sets (Pre- and Post-), to maintain consistency in evaluation methods.

Dilution factor. A dilution factor other than unity can be entered in the Count Evaluation Section, if desired. When duplicate injections are used, the dilution factor appears beside the second injection (Table II). This column of the template is unprotected and can be customized as required by the user.

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