Software Suitability Verification of ChromGraph™ Software

I. Introduction

ChromGraph software is a data-collection and analysis program written and copyrighted by Bioanalytical Systems, Inc. ChromGraph takes analog output from gas or liquid chromatography detectors, digitizes the data, and produces a report consisting of the heights or areas of component peaks. These areas may optionally be compared to internal and external standards to produce a report containing predicted amounts of the components.

This document contains instructions for validating the performance of ChromGraph. The general approach will be to input a well defined series of simulated chromatographic signals, using a commercially obtained and validated Interface Validation Module (IVM). The results produced by ChromGraph will be compared to either 1) expected results, or 2) results calculated by independent programs, as appropriate.

Note that these are advanced procedures that should only be performed by someone well-versed in the operation of both ChromGraph and Microsoft Excel™.

II. Equipment Details

Item	Serial Number or Version
BAS ChromGraph Software	Version: 2.5
BAS epsilon™ amperometric detector	Serial #: 164
PE Nelson 500 IVM	Serial #: 2290050040
Computer	Dell Dimension L-700cx
Operating System	Windows XP 5.1.2600 SP-2 Build 2600
Microsoft Excel Software	Version: 9.0.6926 SP-3

III. Connections

1. Connect the red output lead from the PE Nelson signal generator (Model 500 IVM) to the 'EXT 1' input of the epsilon.
2. Connect the black output lead from the PE Nelson signal generator to the 'GND 1' input of the epsilon.
3. Connect the two 'start in' wires from the PE Nelson signal generator to the Start Out and GND terminals of the epsilon.
4. Connect the RS-232 port on the epsilon to the Com1 or Com2 port on the computer, using a standard RS-232 cable.

IV. Warmup

Both the epsilon detector and the PE Nelson signal generator must warm up for 45 minutes before testing can begin.

V. ChromGraph Control Methods

The following methods will be used for performance validation:

	Trial1	**Trial2**
Data Name	one	two
Run Length	7.0 min	7.0 min
Data Rate	150 ppm	150 ppm
External Channels	1	1
Ext1 input volts	1.0 V	1.0 V
Ext1 filter	1 Hz	1 Hz
Trigger Type	Automatic	Automatic

VI. IVM Settings

	Trial1	Trial2
Test number	3	4
Output Voltage	1.0 V	1.0 V

VII. Data-Collection Procedure

Perform ten replicates each for the two trials described in parts V and VI.
Save the replicates as one1 ... one10, two1 ... two10.

VIII. Precision Tests

Use a Report method with the following parameters to process Trial1:

Prebunch:	none
Prefilter:	none
Smooth Width:	9
Initial slope thresh:	10
Minimum peak area:	1000
Perpendicular drop	off

Determine the % RSD (100*stdev/mean) for the heights and areas:

	one1		one2	
Peak	**Heights**	**Areas**	**Heights**	**Areas**
1	810412	6107130	810505	6108561
2	810486	6107716	810717	6111367
3	810539	6109407	810839	6117376
4	810492	6107719	810727	6115273
5	810549	6109607	810795	6113934
6	810537	6108286	810510	6108278
7	810479	6107532	810519	6108260
8	810522	6108533	810507	6107951
9	810489	6107674	810550	6109476
10	810504	6107873	810460	6107613

Peak	one3 Heights	Areas	one4 Heights	Areas
1	810497	6108034	810480	6107782
2	810362	6109355	810561	6109216
3	810448	6107656	810514	6107920
4	810497	6108477	810545	6108117
5	810461	6107256	810533	6108154
6	810482	6107839	810508	6108039
7	810491	6107948	810634	6111360
8	810445	6107822	810520	6108242
9	810502	6108496	810514	6107432
10	810473	6107793	810539	6108708

Peak	one5 Heights	Areas	one6 Heights	Areas
1	810625	6111647	810551	6109036
2	810462	6107564	810497	6107592
3	810500	6108807	810546	6108926
4	810465	6107416	810457	6107806
5	810479	6108072	810516	6107375
6	810460	6107627	810515	6107795
7	810474	6107794	810499	6107504
8	810528	6109675	810534	6108602
9	810464	6107414	810489	6107737
10	810545	6109871	810522	6108291

Peak	one7 Heights	Areas	one8 Heights	Areas
1	810202	6109450	810484	6108326
2	810527	6108048	810468	6107753
3	810546	6108482	810495	6108701
4	810559	6108516	810474	6107981
5	810533	6108069	810458	6107728
6	810523	6107729	810465	6108566
7	810519	6107623	810414	6108002
8	810561	6108785	810468	6108372
9	810582	6109096	810453	6107956
10	810526	6108035	810440	6107612

Peak	one9 Heights	one9 Areas	one10 Heights	one10 Areas
1	810539	6109167	810488	6107748
2	810425	6107124	810523	6109077
3	810507	6107796	810479	6108253
4	810530	6108736	810557	6109607
5	810465	6107874	810494	6107529
6	810532	6108993	810504	6107731
7	810512	6108030	810494	6107196
8	810497	6107445	810520	6107845
9	810514	6107958	810512	6107871
10	810551	6109138	810525	6107988

Summary Table for Precision Tests:

	% RSD Heights	% RSD Areas
one1	0.005	0.013
one2	0.017	0.058
one3	0.005	0.010
one4	0.005	0.018
one5	0.007	0.023
one6	0.003	0.010
one7	0.013	0.010
one8	0.003	0.006
one9	0.005	0.012
one10	0.003	0.012

RSDs must all be ≤ 0.05% for height, ≤ 0.1% for area **Pass:** √
Fail:

IX. Accuracy Tests

Use the same method as in Part VIII to process Trial2. Using Excel, regress the peak heights obtained with ChromGraph (actual) against the expected peak heights given in the IVM specifications. Calculate % Accuracy (Gain Error) by the following formula:

% Accuracy = 100*abs(m-1)

Where m = slope of regression.

	two1		two2	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	810466	811214	810465
2	405600	405273	405600	405287
3	202807	202619	202807	202603
4	101396	101659	101396	101406
5	50706	50692	50706	50683
6	25345	25326	25345	25327
7	12680	12671	12680	12677

	two3		two4	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	810524	811214	810516
2	405600	405259	405600	405266
3	202807	202631	202807	202659
4	101396	101336	101396	101260
5	50706	50685	50706	50680
6	25345	25326	25345	25371
7	12680	12667	12680	12727

	two5		two6	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	810507	811214	810843
2	405600	405223	405600	405278
3	202807	202601	202807	202637
4	101396	101328	101396	101323
5	50706	50685	50706	50679

6	25345	25352	25345	25336
7	12680	12681	12680	12670

	two7		two8	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	810662	811214	810545
2	405600	405262	405600	405286
3	202807	202630	202807	202639
4	101396	101386	101396	101317
5	50706	50683	50706	50669
6	25345	25339	25345	25346
7	12680	12662	12680	12723

	two9		two10	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	810472	811214	810510
2	405600	405384	405600	405455
3	202807	202578	202807	202633
4	101396	101380	101396	101338
5	50706	50679	50706	50690
6	25345	25422	25345	25360
7	12680	12682	12680	12670

Summary Table for Accuracy Tests

Run	**Slope**	**% Accuracy Error**
1	0.999	0.101
2	0.999	0.094
3	0.999	0.086
4	0.999	0.089
5	0.999	0.091
6	1.000	0.049
7	0.999	0.072
8	0.999	0.085
9	0.999	0.093
10	0.999	0.083

Accuracy errors must all be ≤ 0.5%.

Pass: √
Fail:

X. Linearity Tests

Using the observed and expected peak heights from part IX, determine the deviation from linearity as follows:

% Linearity Error = 100 * average(abs(Y-Yo)/Yo)

Where Y = peak height calculated by ChromGraph, Yo = expected peak height.

Note: these are automatically determined when part IX is completed.

	Linearity Errors				
Peak	**two1**	**two2**	**two3**	**two4**	**two5**
1	0.00092	0.00092	0.00085	0.00086	0.00087
2	0.00081	0.00077	0.00084	0.00082	0.00093
3	0.00093	0.00101	0.00087	0.00073	0.00102
4	0.00259	0.00010	0.00059	0.00134	0.00067
5	0.00028	0.00045	0.00041	0.00051	0.00041
6	0.00075	0.00071	0.00075	0.00103	0.00028
7	0.00071	0.00024	0.00103	0.00371	0.00008

	Linearity Errors				
Peak	**two6**	**two7**	**two8**	**two9**	**two10**
1	0.00046	0.00068	0.00082	0.00091	0.00087
2	0.00077	0.00083	0.00077	0.00053	0.00036
3	0.00084	0.00087	0.00083	0.00113	0.00086
4	0.00072	0.00010	0.00078	0.00016	0.00057
5	0.00053	0.00045	0.00073	0.00053	0.00032
6	0.00036	0.00024	0.00004	0.00304	0.00059
7	0.00079	0.00142	0.00339	0.00016	0.00079

Summary Table for Linearity Tests

Run	% Linearity Error
two1	0.100
two2	0.060
two3	0.076
two4	0.129
two5	0.061
two6	0.064
two7	0.066
two8	0.105
two9	0.092
two10	0.062

Linearity Errors must all be ≤ 1.0%. **Pass:** √
Fail:

XI. Calculated Amount (by Area) Test

Using Trial2 run two1, create a one-level standards file in ChromGraph Report, using area. Use the expected values for Trial2 in part IX for the 'amount' entries for each peak. Process the other Trial2 runs against this standards file and enter the calculated amounts below. Calculation Error is determined by the following formula:

Calculation Error = abs(Y-Yo)/Yo

Where Y = amount calculated by ChromGraph, Yo = expected amount (IVM spec's)

		two2		two3	
Peak	**expected**	**actual**	**error**	**actual**	**error**
1	811214	811206	0.00001	811293	0.00010
2	405600	405641	0.00010	405539	0.00015
3	202807	202746	0.00030	202754	0.00026
4	101396	101704	0.00304	101469	0.00072
5	50706	50647	0.00116	50659	0.00093
6	25345	25389	0.00174	25361	0.00063
7	12680	12715	0.00276	12693	0.00103

		two4		two5	
Peak	expected	actual	error	actual	error
1	811214	811199	0.00002	811330	0.00014
2	405600	405526	0.00018	405587	0.00003
3	202807	202857	0.00025	202732	0.00037
4	101396	101465	0.00068	101491	0.00094
5	50706	50648	0.00114	50661	0.00089
6	25345	25458	0.00446	25389	0.00174
7	12680	12885	0.01617	12744	0.00505

		two6		two7	
Peak	expected	actual	error	actual	error
1	811214	812328	0.00137	811162	0.00006
2	405600	405541	0.00015	405510	0.00022
3	202807	202789	0.00009	202705	0.00050
4	101396	101461	0.00064	101510	0.00112
5	50706	50615	0.00179	50676	0.00059
6	25345	25352	0.00028	25374	0.00114
7	12680	12688	0.00063	12649	0.00244

		two8		two9	
Peak	expected	actual	error	actual	error
1	811214	811208	0.00001	811219	0.00001
2	405600	405581	0.00005	406149	0.00135
3	202807	202750	0.00028	202739	0.00034
4	101396	101454	0.00057	101637	0.00238
5	50706	50664	0.00083	50616	0.00177
6	25345	25403	0.00229	25622	0.01093
7	12680	12829	0.01175	12705	0.00197

		two10	
Peak	expected	actual	error
1	811214	811206	0.00001
2	405600	406006	0.00100
3	202807	202785	0.00011
4	101396	101488	0.00091
5	50706	50699	0.00014
6	25345	25404	0.00233
7	12680	12696	0.00126

Summary Table for Calculated Amount by Area Tests

% Calculation Error = 100*average error per run

Run	% Calculation Error
two2	0.130
two3	0.054
two4	0.327
two5	0.131
two6	0.071
two7	0.087
two8	0.225
two9	0.268
two10	0.082

Calculation Errors must all be ≤ 1.0%. **Pass:** √
Fail:

XII. Calculated Amount (by Height) Test

Using Trial2 run two1, create a one-level standards file in ChromGraph Report, using height. Use the expected values for Trial2 in part IX for the 'amount' entries for each peak. Process the other Trial2 runs against this standards file and enter the calculated amounts below. Calculation Error is determined by the following formula:

Calculation Error = abs(Y-Yo)/Yo

Where Y = amount calculated by ChromGraph, Yo = expected amount (IVM spec's)

		two2		two3	
Peak	**expected**	**actual**	**error**	**actual**	**error**
1	811214	811146	0.00008	811228	0.00002
2	405600	405432	0.00041	405404	0.00048
3	202807	202777	0.00015	202805	0.00001
4	101396	101464	0.00067	101394	0.00002
5	50706	50695	0.00022	50697	0.00018
6	25345	25312	0.00130	25311	0.00134
7	12680	12687	0.00055	12677	0.00024

Peak	expected	two4 actual	two4 error	two5 actual	two5 error
1	811214	811221	0.00001	811188	0.00003
2	405600	405411	0.00047	405368	0.00057
3	202807	202833	0.00013	202775	0.00016
4	101396	101318	0.00077	101386	0.00010
5	50706	50692	0.00028	50691	0.00030
6	25345	25356	0.00043	25329	0.00063
7	12680	12737	0.00450	12683	0.00024

Peak	expected	two6 actual	two6 error	two7 actual	two7 error
1	811214	811506	0.00036	811199	0.00002
2	405600	405427	0.00043	405407	0.00048
3	202807	202816	0.00004	202790	0.00008
4	101396	101394	0.00002	101401	0.00005
5	50706	50691	0.00030	50699	0.00014
6	25345	25321	0.00095	25324	0.00083
7	12680	12680	0.00000	12672	0.00063

Peak	expected	two8 actual	two8 error	two9 actual	two9 error
1	811214	811220	0.00001	811166	0.00006
2	405600	405430	0.00042	405529	0.00018
3	202807	202813	0.00003	202752	0.00027
4	101396	101375	0.00021	101438	0.00041
5	50706	50681	0.00049	50691	0.00030
6	25345	25331	0.00055	25407	0.00245
7	12680	12733	0.00418	12692	0.00095

Peak	expected	two10 actual	two10 error
1	811214	811214	0.00000
2	405600	405600	0.00000
3	202807	202807	0.00000
4	101396	101396	0.00000
5	50706	50706	0.00000
6	25345	25345	0.00000
7	12680	12680	0.00000

Summary Table for Calculated Amount by Height Tests

% Calculation Error = 100*average error per run

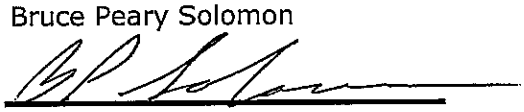
Run	% Calculation Error
two2	0.048
two3	0.033
two4	0.094
two5	0.029
two6	0.030
two7	0.032
two8	0.084
two9	0.066
two10	0.000

Calculation Errors must all be ≤ 1.0%. **Pass:** √
Fail:

XIII. Certification

I certify that the tests above were performed as described, and that the indicated results were truly and fairly obtained.

Name Bruce Peary Solomon

Signature 

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