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: 2.34
BAS epsilon™ amperometric detector	Serial #: 101
PE Nelson 500 IVM	Serial #: 2290050040
Computer	Dell L600c Celeron ™ 600, 256 MB RAM
Operating System	Windows XP Professional
Microsoft Excel Software	Version: 2000 (9.0.2720)

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	809575	6101024	809644	6102183
2	809600	6102268	809621	6102787
3	809394	6098335	809619	6101624
4	809479	6099931	809398	6096980
5	809506	6100256	809523	6100204
6	809537	6101129	809540	6100561
7	809471	6099373	809545	6099962
8	809507	6100527	809780	6100326
9	809544	6101151	809626	6099750

10	809598	6102249	809536	6100405

Peak	one3 Heights	one3 Areas	one4 Heights	one4 Areas
1	809422	6097118	809553	6100948
2	809374	6096206	809463	6099141
3	809395	6097000	809537	6101047
4	809480	6099115	809501	6099884
5	809496	6100092	809445	6099125
6	809539	6100957	809524	6100411
7	809538	6100357	809467	6099880
8	809446	6099042	809496	6099961
9	809592	6101132	809497	6100912
10	809532	6100541	809528	6100621

Peak	one5 Heights	one5 Areas	one6 Heights	one6 Areas
1	809522	6100406	809452	6099067
2	809463	6099939	809448	6099766
3	809619	6100354	809482	6100188
4	809619	6099540	809574	6101796
5	809540	6100666	809423	6099572
6	809555	6100525	809464	6099658
7	809483	6100369	809476	6100120
8	809438	6099667	809474	6100249
9	809397	6097344	809553	6101197
10	809548	6100657	809598	6102476

Peak	one7 Heights	one7 Areas	one8 Heights	one8 Areas
1	809566	6100687	809633	6100295
2	809591	6100911	809707	6100125
3	809509	6100231	809639	6100374
4	809511	6100052	809541	6100213
5	809553	6100895	809493	6098026
6	809537	6100294	809563	6100181
7	809459	6099362	809563	6100346
8	809643	6102369	809477	6097522
9	809573	6101232	809532	6100200

10	809509	6098606	809506	6099700

Peak	one9 Heights	one9 Areas	one10 Heights	one10 Areas
1	809610	6101946	809499	6099819
2	809616	6101503	809527	6099760
3	809536	6100157	809401	6098798
4	809434	6098459	809602	6101727
5	809532	6099754	809460	6097796
6	809411	6098313	809522	6099999
7	809475	6099056	809511	6099476
8	809512	6099819	809648	6100298
9	809493	6099483	809624	6100414
10	809543	6101013	809634	6100857

Summary Table for Precision Tests:

	% RSD	
	Heights	**Areas**
one1	0.008	0.020
one2	0.012	0.026
one3	0.009	0.029
one4	0.004	0.012
one5	0.009	0.016
one6	0.007	0.018
one7	0.006	0.017
one8	0.009	0.017
one9	0.008	0.020
one10	0.010	0.018

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	809457	811214	809550
2	405600	404723	405600	404785
3	202807	202448	202807	202442
4	101396	101159	101396	101273
5	50706	50622	50706	50611
6	25345	25372	25345	25306
7	12680	12854	12680	12633

	two3		two4	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	809450	811214	809480
2	405600	404793	405600	404718
3	202807	202404	202807	202462
4	101396	101228	101396	101344
5	50706	50724	50706	50800
6	25345	25417	25345	25346
7	12680	12701	12680	12615

	two5		two6	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	809523	811214	809498

2	405600	404646	405600	404744
3	202807	202415	202807	202343
4	101396	101238	101396	101199
5	50706	50547	50706	50548
6	25345	25437	25345	25344
7	12680	12735	12680	12677

	two7		two8	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	809583	811214	809600
2	405600	405008	405600	404796
3	202807	202660	202807	202445
4	101396	101202	101396	101168
5	50706	50587	50706	50577
6	25345	25301	25345	25347
7	12680	12700	12680	12680

	two9		two10	
Peak	**expected**	**actual**	**expected**	**actual**
1	811214	809506	811214	809604
2	405600	404754	405600	404810
3	202807	202273	202807	202409
4	101396	101075	101396	101240
5	50706	50570	50706	50626
6	25345	25350	25345	25345
7	12680	12678	12680	12684

Summary Table for Accuracy Tests

Run	**Slope**	**% Accuracy Error**
1	0.998	0.229
2	0.998	0.207
3	0.998	0.228
4	0.998	0.227
5	0.998	0.221
6	0.998	0.213
7	0.998	0.197
8	0.998	0.201
9	0.998	0.209
10	0.998	0.203

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	0.00217	0.00205	0.00217	0.00214	0.00208
2	0.00216	0.00201	0.00199	0.00217	0.00235
3	0.00177	0.00180	0.00199	0.00170	0.00193
4	0.00234	0.00121	0.00166	0.00051	0.00156
5	0.00166	0.00187	0.00035	0.00185	0.00314
6	0.00107	0.00154	0.00284	0.00004	0.00363
7	0.01372	0.00371	0.00166	0.00513	0.00434

	Linearity Errors				
Peak	**two6**	**two7**	**two8**	**two9**	**two10**
1	0.00212	0.00201	0.00199	0.00211	0.00198
2	0.00211	0.00146	0.00198	0.00209	0.00195
3	0.00229	0.00072	0.00178	0.00263	0.00196
4	0.00194	0.00191	0.00225	0.00317	0.00154
5	0.00312	0.00235	0.00254	0.00268	0.00158
6	0.00004	0.00174	0.00008	0.00020	0.00000
7	0.00024	0.00158	0.00000	0.00016	0.00032

Summary Table for Linearity Tests

Run	% Linearity Error
two1	0.355
two2	0.203
two3	0.181
two4	0.194
two5	0.272
two6	0.169
two7	0.168
two8	0.152
two9	0.186
two10	0.133

Linearity Errors must all be ≤ 1.0%.

Pass: √

Fail:

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	811614	0.00049	811236	0.00003
2	405600	405766	0.00041	405880	0.00069
3	202807	202891	0.00041	202753	0.00027
4	101396	101571	0.00173	101474	0.00077
5	50706	50629	0.00152	50993	0.00566
6	25345	25286	0.00233	25598	0.00998
7	12680	12366	0.02476	12467	0.01680

		two4		two5	
Peak	expected	actual	error	actual	error
1	811214	811271	0.00007	811551	0.00042
2	405600	405816	0.00053	405571	0.00007
3	202807	202832	0.00012	202853	0.00023
4	101396	101457	0.00060	101520	0.00122
5	50706	50723	0.00034	50484	0.00438
6	25345	25356	0.00043	25654	0.01219
7	12680	12421	0.02043	12625	0.00434

		two6		two7	
Peak	expected	actual	error	actual	error
1	811214	811419	0.00025	811356	0.00018
2	405600	405777	0.00044	405844	0.00060
3	202807	202680	0.00063	202855	0.00024
4	101396	101380	0.00016	101394	0.00002
5	50706	50496	0.00414	50628	0.00154
6	25345	25370	0.00099	25364	0.00075
7	12680	12415	0.02090	12460	0.01735

		two8		two9	
Peak	expected	actual	error	actual	error
1	811214	811509	0.00036	811332	0.00015
2	405600	405863	0.00065	405715	0.00028
3	202807	202852	0.00022	202479	0.00162
4	101396	101346	0.00049	100985	0.00405
5	50706	50607	0.00195	50420	0.00564
6	25345	25439	0.00371	25355	0.00039
7	12680	12449	0.01822	12473	0.01632

two10

Peak	expected	actual	error
1	811214	811328	0.00014
2	405600	405817	0.00054
3	202807	202739	0.00034
4	101396	101459	0.00062
5	50706	50669	0.00073
6	25345	25375	0.00118
7	12680	12422	0.02035

% Calculation Error = 100*average error per run

Run	% Calculation Error
two2	0.452
two3	0.488
two4	0.322
two5	0.326
two6	0.393
two7	0.295
two8	0.366
two9	0.407
two10	0.341

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	811307	0.00011	811207	0.00001
2	405600	405662	0.00015	405670	0.00017
3	202807	202801	0.00003	202763	0.00022
4	101396	101510	0.00112	101465	0.00068
5	50706	50695	0.00022	50808	0.00201
6	25345	25279	0.00260	25390	0.00178
7	12680	12462	0.01719	12529	0.01191

		two4		two5	
Peak	**expected**	**actual**	**error**	**actual**	**error**
1	811214	811237	0.00003	811280	0.00008
2	405600	405595	0.00001	405522	0.00019
3	202807	202821	0.00007	202774	0.00016
4	101396	101582	0.00183	101475	0.00078
5	50706	50884	0.00351	50631	0.00148
6	25345	25319	0.00103	25410	0.00256
7	12680	12444	0.01861	12563	0.00923

		two6		two7	
Peak	**expected**	**actual**	**error**	**actual**	**error**
1	811214	811255	0.00005	811341	0.00016
2	405600	405621	0.00005	405885	0.00070
3	202807	202702	0.00052	203020	0.00105
4	101396	101436	0.00039	101439	0.00042
5	50706	50632	0.00146	50671	0.00069
6	25345	25317	0.00110	25274	0.00280
7	12680	12506	0.01372	12528	0.01199

		two8		two9	
Peak	**expected**	**actual**	**error**	**actual**	**error**
1	811214	811357	0.00018	811263	0.00006
2	405600	405673	0.00018	405631	0.00008
3	202807	202804	0.00001	202632	0.00086
4	101396	101405	0.00009	101312	0.00083
5	50706	50660	0.00091	50654	0.00103
6	25345	25320	0.00099	25323	0.00087
7	12680	12509	0.01349	12507	0.01364

		two10	
Peak	**expected**	**actual**	**error**
1	811214	811362	0.00018
2	405600	405687	0.00021
3	202807	202768	0.00019
4	101396	101477	0.00080
5	50706	50710	0.00008
6	25345	25318	0.00107
7	12680	12512	0.01325

% Calculation Error = 100*average error per run

Run	**% Calculation Error**
two2	0.306
two3	0.240
two4	0.358
two5	0.207
two6	0.247
two7	0.254
two8	0.226
two9	0.248
two10	0.225

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 _____

Date 15-Apr-02