Chromatography Technical Note No AS58

Time Efficient Automated On-line Construction of Calibration Curves using the GERSTEL MPS Prepstation

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Introduction

This note follows on from Anatune application notes AS30, AS45, AS53, AS55 and AS56 and extends that work by automating the addition of solutions of standards, internal standards and surrogates to calibration and analytical samples in a more timely fashion. At the beginning of the prep-sequence the MPS Prepstation added an appropriate volume of standard solution to each of the calibration vials. The internal standards and surrogate solutions were subsequently added to the required vials in a "just-in-time" manner. This enables accurate and reproducible volumes to be added to each vial prior to injecting the sample.

By proving this application for the analysis of VOCs in water, the spiking of less volatile standards, internal standards and surrogates should be at least as reproducible as the results achieved here.

The use of the Anatune 160 position tray allowed the manual spiking of 160 samples at the 100µg/L level for each of the analytes listed below. The process of spiking 160 samples with internal standards and surrogates and their subsequent on-line headspace analysis took approximately 27 hours.

The process of spiking the standards, internal standards and surrogates into the 8 calibration levels (0, 3, 5, 10, 20, 50, 100 and 200µg/L) and their subsequent headspace analysis took approximately $1^1/_2$ hours. The prep-ahead capability of the Maestro software was un-affected since all of the spiking of standards into their respective vials was performed at the beginning of the prep-sequence.

GERSTEL Maestro software was used to control both MPS rails and was used to write the sample preparation sequence. This software can be integrated into ChemStation, so a single prep-sequence can be used to both add standards, internal standards and surrogates and to perform the sample injection in a "just-in-time" manner. This prevents any possible transcription errors that can occur if several sequences need to be written and synchronised to perform the analysis.

Instrumentation and Methods

- GERSTEL MPS Prepstation, one rail configured for headspace analysis, the second rail for liquid addition
- Agilent 6890N GC with 5975B inert MSD
- GERSTEL Maestro software
- Agilent ChemStation
- Anatune 160 position tray
- Anatune CoolR+
- Anatune OptimisedVOC method as detailed in Anatune application note AS30
- The automated spiking of standards to construct calibration curves as detailed in Anatune application note AS45
- The automated spiking of internal standards and surrogates into water samples and calibration samples as detailed in Anatune application notes AS53 and AS55.
- The automated construction of on-line calibration curves as detailed in Anatune application notes AS56

Compound List

Internal Standards	**Surrogate Compounds**
Pentafluorobenzene	1,2-Dichloroethane-d6
Difluorobenzene	Toluene-d8
Chlorobenzene-d5	4-Bromofluorobenzene
1,4-Difluorobenzene	
Analytes	
Chloroethane	Bromomethane
Trichlorofluoromethane	1,1-Dichloroethene
trans-1,2-Dichloroethene	1,1-Dichloroethane
cis-1,2-Dichloroethene	Chloroform
Bromochloromethane	1,1,1-Trichloroethane
1,1-Dichloropropene	1,2-Dichloroethane
Benzene	1,2-Dichloropropane
Trichloroethene	Bromodichloromethane
Dibromomethane	cis-1,3-Dichloropropene
Toluene	1,1,2-Trichloroethane
Carbon tetrachloride	1,3-Dichloropropane
Tetrachloroethene	Dibromochloromethane
1,2-Dibromomethane	Chlorobenzene
1,1,1,2-Tetrachloroethane	Ethyl benzene
m,p-Xylene	o-Xylene
Styrene	Bromoform
Isopropylbenzene	1,1,2,2-Tetrachloroethane
1,2,3-Trichloropropane	n-Propylbenzene
Bromobenzene	2-Chlorotoluene
1,3,5-Trimethylbenzene	4-Chlorotoluene
tert-Butylbenzene	1,2,4-Trimethylbenzene
sec-Butylbenzene	p-Isopropyltoluene
1,3-Dichlorobenzene	1,4-Dichlorobenzene
n-Butylbenzene	1,2-Dichlorobenzene
1,2-Dibromo-3-chloropropane	1,2,4-Trichlorobenzene
Hexachlorobutadiene	Naphthalene
1,2,3-Trichlorobenzene	

Sample Preparation

Anhydrous sodium sulphate was weighed into 176 vials before adding the required volume of water. One calibration curve was prepared from these vials by manually spiking the required volume of mixed standard, internal standards and surrogates into the vials. The other set of calibration standards were capped after the water was added to the vials and placed on the MPS Prepstation for automated standard, internal standard and surrogate addition. 160 replicate samples were manually spiked to a concentration of 100µg/L before being capped and placed on the MPS Prepstation for automated internal standard and surrogate addition.

Headspace GC-MS Method

Each calibration sample was spiked with the appropriate volume of mixed standard at the start of the prep-sequence. Because the prep-ahead function within the Maestro software is limited to those analyses contained within a single "job", sequential spiking of the standard solution into the calibration vials at the beginning of the prep-sequence is a single job. This then allowed the MPS Prepstation to add the solutions of internal standards and surrogates in a "just-in-time" manner prior to their on-line headspace analysis. Preparing the calibration samples in this way, the process of spiking the standards, internal standards and surrogates into the 8 calibration levels and their subsequent headspace analysis took $1^1/_2$ hours.

Figure 1 shows the MPS Prepstation used for this work

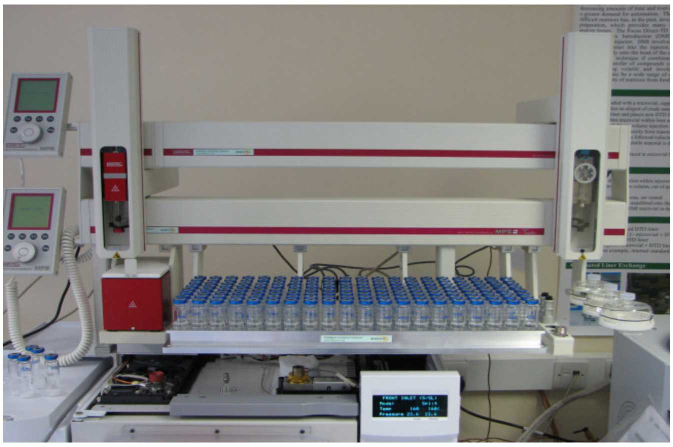

Figure 1

Results

Spiking the standards into the relevant vials at the beginning of the prep-sequence resulted in a saving of approximately $2^1/_2$ hours compared with the work detailed in Anatune application note AS56 where the standard, internal standards and surrogates were all added in a "just-in-time" manner.

Because this application was performed using a headspace method where the vial incubation time is approximately three times longer than the GC method, spiking the standards into the relevant vials at the beginning of the prep-sequence meant that the internal standards and surrogates and the subsequent headspace analysis was a single "job". This meant that the prep-ahead function within Maestro would, after the initial incubation periods, have subsequent vials spiked and incubated for headspace sampling shortly after the GC came ready for injection.

The MCERTS criteria for precision and accuracy state that the %RSD of the surrogate and analyte concentration for a batch of samples must be below 15% and the concentration bias, the difference from the spiked concentration, must be less than 30%. It was decided to work to criteria equivalent to this. The internal standards, surrogates and analytes were spiked at 100µg/L, so a concentration between 70 and 130µg/L meets the regulatory criteria.

Table 1 shows the correlation coefficients obtained for each analytes' calibration curve, the mean concentration, standard deviation and relative standard deviation for each analyte using both standard curves as detailed above.

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Standard curve constructed manually

Internal Standards	r^2	Acceptable regulatory range	Mean concentration (n=160)	SD	% RSD	Bias (%)
Pentafluorobenzene	N/A	N/A	690638.97	45779.47	6.63	N/A
Difluorobenzene	N/A	N/A	1089287.14	72696.26	6.67	N/A
Chlorobenzene-d5	N/A	N/A	462925.03	31711.90	6.85	N/A
1,4-Dichlorobenzene-d4	N/A	N/A	500771.92	32584.90	6.51	N/A
Surrogates						
1,2-Dichloroethane-d6	N/A	70 – 130	97.41	1.92	1.97	-2.59
Toluene-d8	N/A	70 – 130	100.50	0.96	0.96	0.50
4-Bromofluorobenzene	N/A	70 – 130	98.59	1.47	1.50	-1.41
Analytes						
Chloroethane	1.000	70 – 130	90.14	3.88	4.31	-9.86
Bromomethane	1.000	70 – 130	80.09	5.20	6.49	-19.91
Trichlorofluoromethane	1.000	70 – 130	90.44	3.91	4.33	-9.56
1,1-Dichloroethene	1.000	70 – 130	92.28	3.81	4.13	-7.72
trans-1,2-Dichloroethene	1.000	70 – 130	93.20	4.20	4.50	-6.80
1,1-Dichloroethane	1.000	70 – 130	96.09	3.80	3.96	-3.91
cis-1,2-Dichloroethene	1.000	70 – 130	95.14	4.10	4.31	-4.86
Chloroform	1.000	70 – 130	95.68	3.98	4.16	-4.32
Bromochloromethane	1.000	70 – 130	97.72	4.29	4.39	-2.28
1,1,1-Trichloroethane	1.000	70 – 130	95.29	4.03	4.23	-4.71
1,1-Dichloropropene	0.999	70 – 130	94.18	4.10	4.35	-5.82
1,2-Dichloroethane	0.999	70 – 130	95.35	4.04	4.23	-4.65
Benzene	1.000	70 – 130	94.92	3.87	4.08	-5.08
1,2-Dichloropropane	0.999	70 – 130	96.57	4.03	4.17	-3.43
Trichloroethene	0.998	70 – 130	97.17	4.31	4.44	-2.83
Bromodichloromethane	1.000	70 – 130	95.33	4.05	4.25	-4.67
Dibromomethane	0.999	70 – 130	96.39	4.27	4.43	-3.61
cis-1,3-Dichloropropene	0.999	70 – 130	74.78	9.79	13.09	-25.22
Toluene	0.999	70 – 130	91.36	4.13	4.52	-8.64
1,1,2-Trichloroethane	1.000	70 – 130	96.54	4.20	4.35	-3.46
Carbon tetrachloride	1.000	70 – 130	94.93	4.28	4.51	-5.07
1,3-Dichloropropane	1.000	70 – 130	97.51	4.47	4.59	-2.49
Tetrachloroethene	1.000	70 – 130	93.46	5.17	5.53	-6.54
Dibromochloromethane	1.000	70 – 130	97.49	4.51	4.63	-2.51
1,2-Dibromomethane	1.000	70 – 130	97.44	4.48	4.60	-2.56
Chlorobenzene	1.000	70 – 130	94.84	4.84	5.10	-5.16

Standard curve constructed entirely by the MPS 2

Internal Standards	r^2	Acceptable regulatory range	Mean concentration (n=160)	SD	% RSD	Bias (%)
Pentafluorobenzene	N/A	N/A	690638.73	45779.63	6.63	N/A
Difluorobenzene	N/A	N/A	1089288.09	72696.22	6.67	N/A
Chlorobenzene-d5	N/A	N/A	462925.03	31711.90	6.85	N/A
1,4-Dichlorobenzene-d4	N/A	N/A	500771.92	32584.90	6.51	N/A
Surrogates						
1,2-Dichloroethane-d6	N/A	70 – 130	99.25	1.95	1.97	-0.75
Toluene-d8	N/A	70 – 130	100.21	0.96	0.96	0.21
4-Bromofluorobenzene	N/A	70 – 130	100.42	1.50	1.50	0.42
Analytes						
Chloroethane	1.000	70 – 130	95.78	4.13	4.31	-4.22
Bromomethane	1.000	70 – 130	85.79	5.62	6.55	-14.21
Trichlorofluoromethane	1.000	70 – 130	95.82	4.16	4.34	-4.18
1,1-Dichloroethene	1.000	70 – 130	98.70	4.06	4.12	-1.30
trans-1,2-Dichloroethene	1.000	70 – 130	99.72	4.49	4.50	-0.28
1,1-Dichloroethane	1.000	70 – 130	103.32	4.11	3.98	3.32
cis-1,2-Dichloroethene	1.000	70 – 130	101.05	4.37	4.32	1.05
Chloroform	1.000	70 – 130	102.63	4.30	4.19	2.63
Bromochloromethane	1.000	70 – 130	104.21	4.59	4.40	4.21
1,1,1-Trichloroethane	1.000	70 – 130	100.78	4.25	4.22	0.78
1,1-Dichloropropene	1.000	70 – 130	99.96	4.36	4.36	-0.04
1,2-Dichloroethane	1.000	70 – 130	103.00	4.39	4.26	3.00
Benzene	1.000	70 – 130	100.77	4.10	4.07	0.77
1,2-Dichloropropane	1.000	70 – 130	104.14	4.35	4.17	4.14
Trichloroethene	1.000	70 – 130	103.24	4.60	4.46	3.24
Bromodichloromethane	1.000	70 – 130	101.64	4.32	4.25	1.64
Dibromomethane	1.000	70 – 130	104.77	4.65	4.44	4.77
cis-1,3-Dichloropropene	0.999	70 – 130	80.32	10.56	13.15	-19.68
Toluene	1.000	70 – 130	99.86	4.46	4.46	-0.14
1,1,2-Trichloroethane	1.000	70 – 130	104.80	4.56	4.35	4.80
Carbon tetrachloride	1.000	70 – 130	99.60	4.46	4.48	-0.40
1,3-Dichloropropane	1.000	70 – 130	103.06	4.71	4.57	3.06
Tetrachloroethene	1.000	70 – 130	98.11	5.42	5.52	-1.89
Dibromochloromethane	1.000	70 – 130	103.13	4.77	4.62	3.13
1,2-Dibromomethane	1.000	70 – 130	101.05	4.61	4.57	1.05
Chlorobenzene	0.999	70 – 130	99.37	5.04	5.07	-0.63

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Standard curve constructed manually							Standard curve constructed entirely by the MPS 2						
Analytes	**r^2**	**Acceptable regulatory range**	**Mean concentration (n=160)**	**SD**	**% RSD**	**Bias (%)**	**Analytes**	**r^2**	**Acceptable regulatory range**	**Mean concentration (n=160)**	**SD**	**% RSD**	**Bias (%)**
1,1,1,2-Tetrachloroethane	1.000	70 – 130	96.05	4.47	4.65	-3.95	1,1,1,2-Tetrachloroethane	0.999	70 – 130	101.62	4.70	4.63	1.62
Ethyl benzene	1.000	70 – 130	92.39	4.66	5.05	-7.61	Ethyl benzene	0.999	70 – 130	97.54	4.89	5.01	-2.46
m,p-Xylene	0.999	70 – 130	92.07	4.96	5.39	-7.93	m,p-Xylene	0.999	70 – 130	96.73	5.19	5.36	-3.27
o-Xylene	1.000	70 – 130	93.62	4.61	4.92	-6.38	o-Xylene	0.999	70 – 130	98.19	4.83	4.92	-1.81
Styrene	0.999	70 – 130	90.26	5.25	5.82	-9.74	Styrene	0.999	70 – 130	95.39	5.52	5.78	-4.61
Bromoform	1.000	70 – 130	96.97	4.44	4.58	-3.03	Bromoform	1.000	70 – 130	102.89	4.70	4.57	2.89
Isopropylbenzene	1.000	70 – 130	93.34	4.55	4.88	-6.66	Isopropylbenzene	0.999	70 – 130	98.56	4.79	4.86	-1.44
1,1,2,2-Tetrachloroethane	1.000	70 – 130	92.36	5.24	5.68	-7.64	1,1,2,2-Tetrachloroethane	1.000	70 – 130	99.00	5.62	5.68	-1.00
1,2,3-Trichloropropane	1.000	70 – 130	94.90	4.45	4.69	-5.10	1,2,3-Trichloropropane	1.000	70 – 130	101.74	4.80	4.72	1.74
n-Propylbenzene	1.000	70 – 130	91.35	5.31	5.82	-8.65	n-Propylbenzene	0.999	70 – 130	95.55	5.52	5.78	-4.45
Bromobenzene	1.000	70 – 130	92.41	5.05	5.47	-7.59	Bromobenzene	0.999	70 – 130	97.11	5.26	5.42	-2.89
2-Chlorotoluene	1.000	70 – 130	91.75	5.20	5.66	-8.25	2-Chlorotoluene	1.000	70 – 130	98.46	5.57	5.66	-1.54
1,3,5-Trimethylbenzene	1.000	70 – 130	89.94	5.23	5.82	-10.06	1,3,5-Trimethylbenzene	0.999	70 – 130	95.19	5.51	5.78	-4.81
4-Chlorotoluene	1.000	70 – 130	89.38	6.39	7.15	-10.62	4-Chlorotoluene	1.000	70 – 130	94.66	6.76	7.14	-5.34
tert-Butylbenzene	1.000	70 – 130	95.25	4.47	4.69	-4.75	tert-Butylbenzene	0.999	70 – 130	100.58	4.68	4.66	0.58
1,2,4-Trimethylbenzene	0.999	70 – 130	87.50	5.87	6.70	-12.50	1,2,4-Trimethylbenzene	0.999	70 – 130	93.54	6.23	6.66	-6.46
sec-Butylbenzene	0.999	70 – 130	91.57	4.39	4.79	-8.43	sec-Butylbenzene	1.000	70 – 130	96.89	4.65	4.80	-3.11
p-Isopropyltoluene	0.999	70 – 130	87.59	4.97	5.67	-12.41	p-Isopropyltoluene	0.999	70 – 130	92.79	5.26	5.67	-7.21
1,3-Dichlorobenzene	1.000	70 – 130	86.22	6.87	7.97	-13.78	1,3-Dichlorobenzene	0.999	70 – 130	90.70	7.19	7.93	-9.30
1,4-Dichlorobenzene	0.999	70 – 130	87.03	7.34	8.43	-12.97	1,4-Dichlorobenzene	1.000	70 – 130	91.79	7.83	8.53	-8.21
n-Butylbenzene	0.999	70 – 130	85.00	5.51	6.48	-15.00	n-Butylbenzene	1.000	70 – 130	89.95	5.85	6.51	-10.05
1,2-Dichlorobenzene	1.000	70 – 130	89.61	5.71	6.37	-10.39	1,2-Dichlorobenzene	1.000	70 – 130	94.95	6.05	6.37	-5.05
1,2-Dibromo-3-chlorpropane	0.999	70 – 130	97.14	4.45	4.58	-2.86	1,2-Dibromo-3-chlorpropane	0.999	70 – 130	103.38	4.74	4.59	3.38
1,2,4-Trichlorobenzene	1.000	70 – 130	78.70	10.32	13.12	-21.30	1,2,4-Trichlorobenzene	0.999	70 – 130	83.27	10.88	13.07	-16.73
Hexachlorobutadiene	1.000	70 – 130	79.99	8.27	10.33	-20.01	Hexachlorobutadiene	0.998	70 – 130	87.46	9.12	10.43	-12.54
Naphthalene	1.000	70 – 130	87.08	6.56	7.54	-12.92	Naphthalene	0.999	70 – 130	93.57	7.07	7.55	-6.43
1,2,3-Trichlorobenzene	1.000	70 – 130	81.83	8.85	10.82	-18.17	1,2,3-Trichlorobenzene	0.999	70 – 130	88.62	9.59	10.82	-11.38

Table 1 shows comparative data between a manually constructed calibration curve and a calibration curve constructed entirely by the MPS Prepstation prior to spiking the internal standards and surrogates and their headspace analysis in a “just-in-time” manner.

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Conclusions

The spiking of volatile standards, internal standards and surrogates represent the most technically challenging group of compounds, due to the ease with which evaporative losses can occur. This work demonstrates that even in this context, the automation of the addition of standards, internal standards and surrogates into both calibration and analytical samples is a practical proposition, and can be used to save both skilled analyst's valuable time and further reduce the opportunity for human error in the analytical process.

Although the standards were spiked into the calibration vials prior to the "just-in-time" addition of internal standards and surrogates and their subsequent headspace analysis, we have demonstrated that there is negligible difference between the calibration data obtained in this fashion and a calibration curve prepared entirely manually. This approach to the automated preparation of standards confers a worthwhile saving in total time to prepare the calibration curve prior to analysing customer samples compared with adding the standard to the vials in a "just-in-time" manner.

This work demonstrates that the automated addition of standards by the MPS Prepstation and Maestro software enables the construction of calibration curves that have correlation coefficients for individual analytes that are at least as good as and in many cases better than those obtained from standards that have been prepared manually.