

GC & LC Application Note



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**Comparison of the manual
and automated generation
of calibration standards**



Comparison of the manual and automated generation of calibration standards

Thomas Funke, Chemical and Veterinary Analytical Institute
Münsterland-Emscher-Lippe (CVUA-MEL), Münster, Germany



Introduction

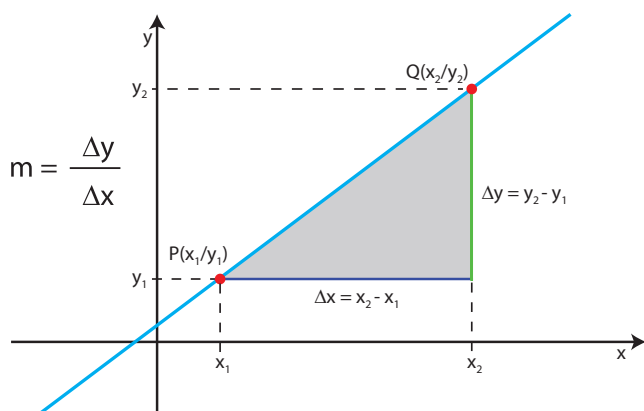
This application note compares the manual and automated generation of calibration standards in terms of their statistical figures of merit.

Calibrations

- are performed regularly in many laboratories and are time consuming
- are used to quantify an analyte or substance
- generate a mathematical model to describe the signal amplitude as a function of the concentration of the analyte using standard solutions of different known concentrations
- are a validation step
- have a limited validity (time, device, measurement parameters, working range, etc.)

Linear correlation

Search for the best fitting type curve ($y=mx+b$) using the least



squares method.

Assumption: Errors of y-values greater than x-values.

The sum of the squares of the vertical deviations between the measuring points is minimized.

Definitions

Accuracy: Difference between intended and actual volume

Precision: Reproducibility of repeated processes (here volume of liquid addition)

Coefficient of correlation r: compares the variation of data points of the regression curve to the total variation of the process.

Coefficient of determination r²: the closer r² is to 1, the higher is the probability of a linear correlation.

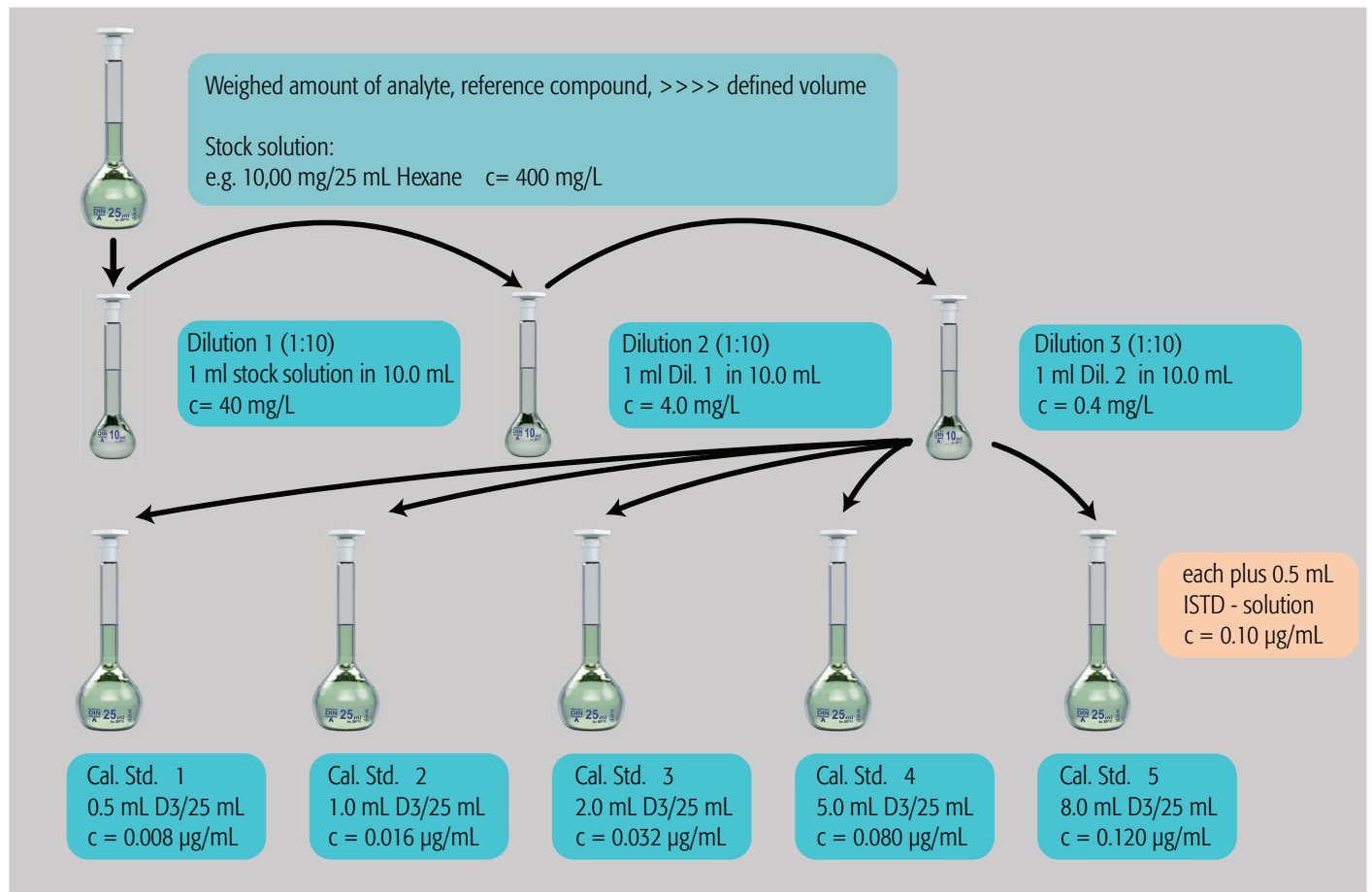
Residual variation Sy: measures the proportion to which a mathematical model accounts for the variation of a given data set.

Standard deviation of the process Sx0 (=Sy/m): a measure for the performance of the analytical process - since the standard deviation of the residues Sy is also a measure of the precision and the slope m represents a measure of sensitivity, Sx0 can be directly compared.

Coefficient of variation for the process Vx0: a relative measure for the precision - since it is formed from the standard deviation of the method Sx0 and the mean value Mw, it is also referred to as a relative standard deviation of the procedure.

$$V_{x0} = \frac{S_{x0}}{M_w} \cdot 100\%$$

Standard manual procedure to generate calibration solutions using volumetric pipettes



Accuracy of some volumetric pipetts

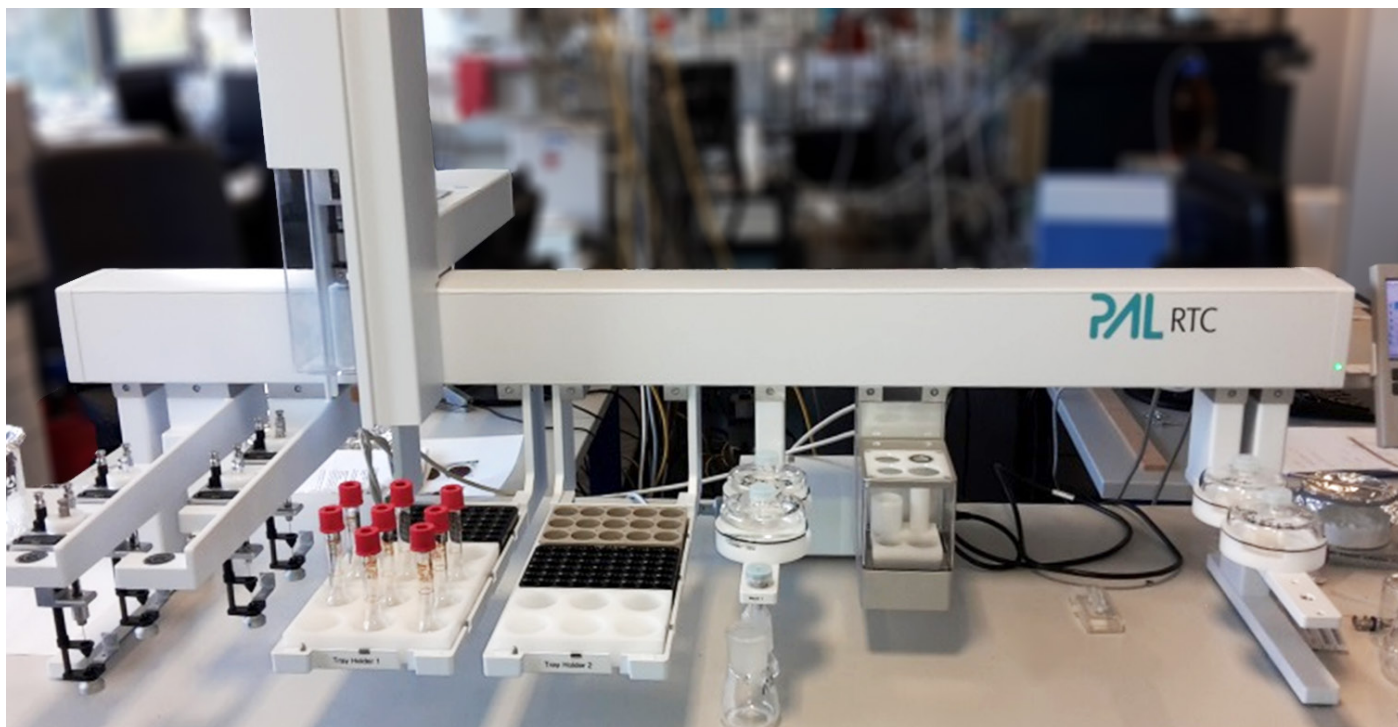
Nominal volume [mL]	Class A		Class AS		Class B	
	max. error [%]	time to drain [s]	max. error [%]	time to drain [s]	max. error [%]	time to drain [s]
0,5	± 1	10–20	± 1	4–8	± 2	4–20
1	$\pm 0,7$	10–20	$\pm 0,7$	5–9	$\pm 1,5$	5–20
10	$\pm 0,2$	15–40	$\pm 0,2$	8–12	$\pm 0,4$	8–40
50	$\pm 0,1$	30–60	$\pm 0,1$	13–18	$\pm 0,2$	13–60
100	$\pm 0,08$	40–60	$\pm 0,08$	25–30	$\pm 0,16$	25–60

Accuracy of positive displacement syringes

Accuracy $\pm 1 \%$ of nominal volume (depending on syringe volume/parameters)

Volume range available for the PAL RTC is 0.5 – 10'000 μL

Automated workflows on the PAL RTC



For the automated workflows on the PAL RTC the following tools (<http://www.palsystem.com/index.php?id=284>) and modules (<http://www.palsystem.com/index.php?id=194>) were used:

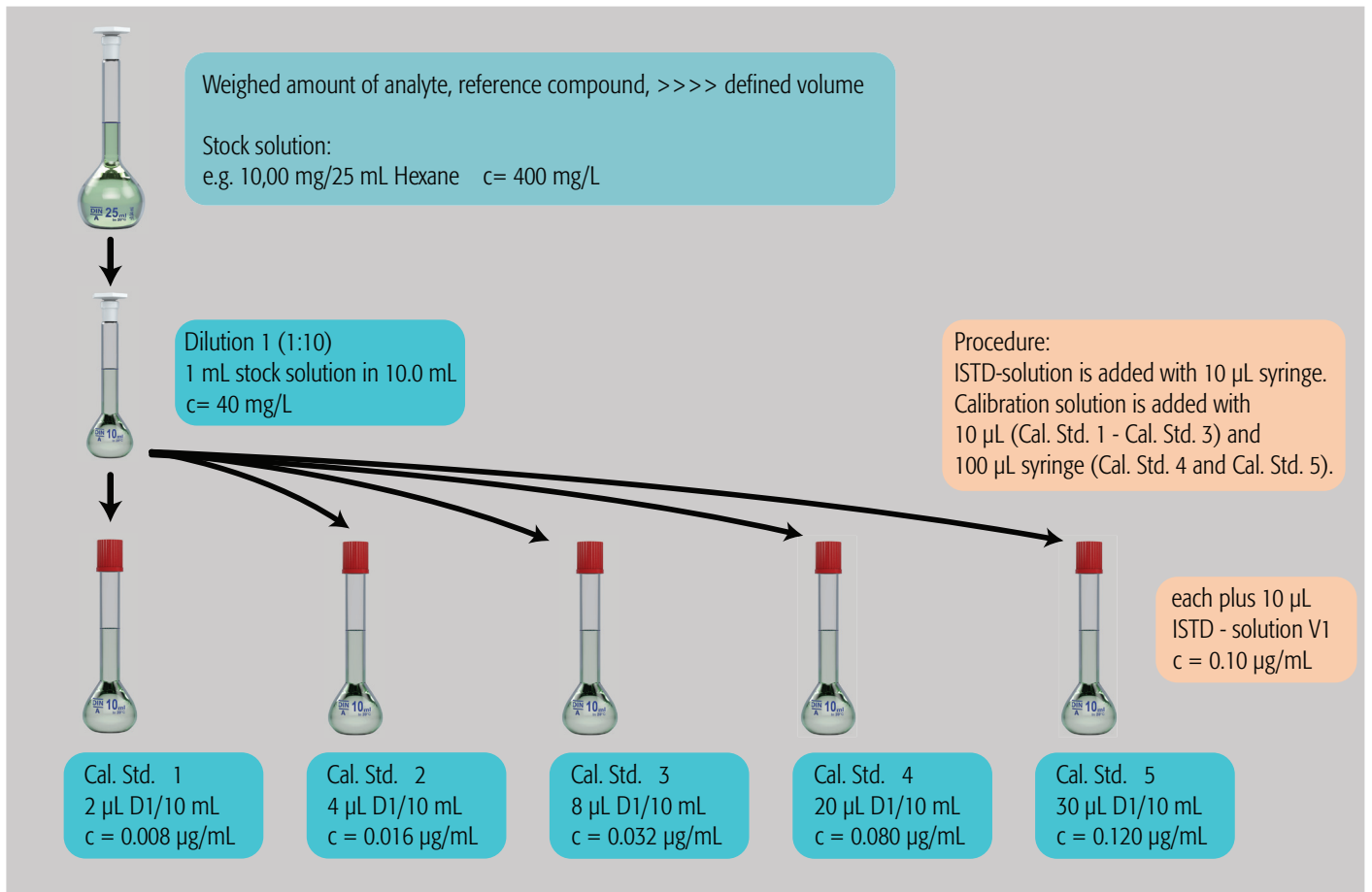
- Park Station
- D7/57 Syringe Tool for 10 μ L syringe
- D7/57 Syringe Tool for 100 μ L syringe
- D8/57 Syringe Tool for 1000 μ L syringe
- 2 Trayholders
- Standard VT15 and VT54 Racks
- Custom Racks for 10 mL volumetric flasks
- 10 mL volumetric flasks from LABC, Hennef, Germany (<http://www.labc.de/>)
- 2 Large Wash Stations
- Vortex Mixer Module

The automated workflows 1 and 2 were realized with the PAL Sample Control software (<http://www.palsystem.com/index.php?id=243>). PAL Sample Control allows to operate both standalone workstations as well as online systems (LC, LC-MS, GC, GC-MS).

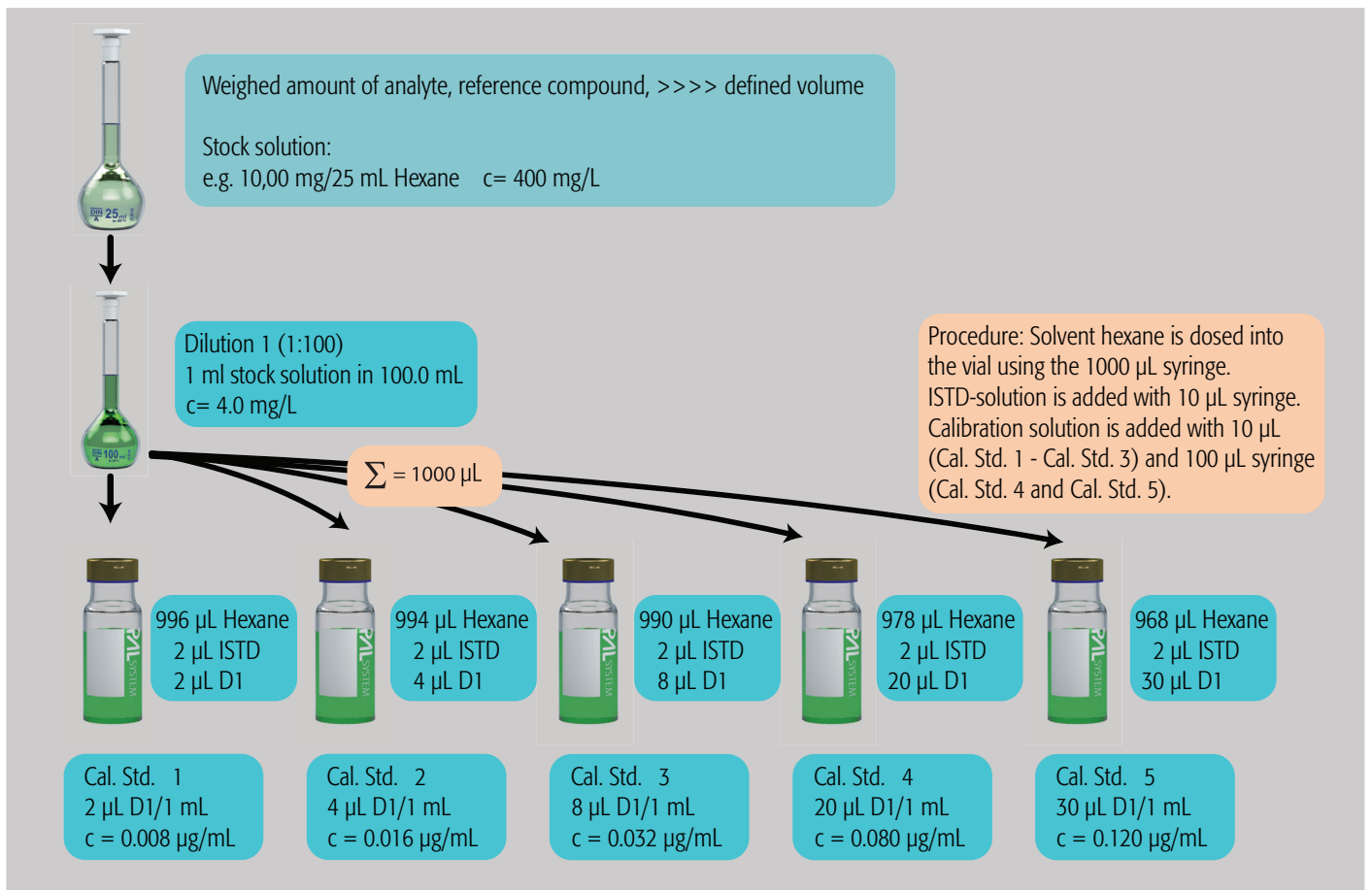
Automatic weighing is possible with the Chronos software and a software plug-in from Axel Semrau GmbH. A separate application note is available (<http://www.palsystem.com/index.php?id=280>).



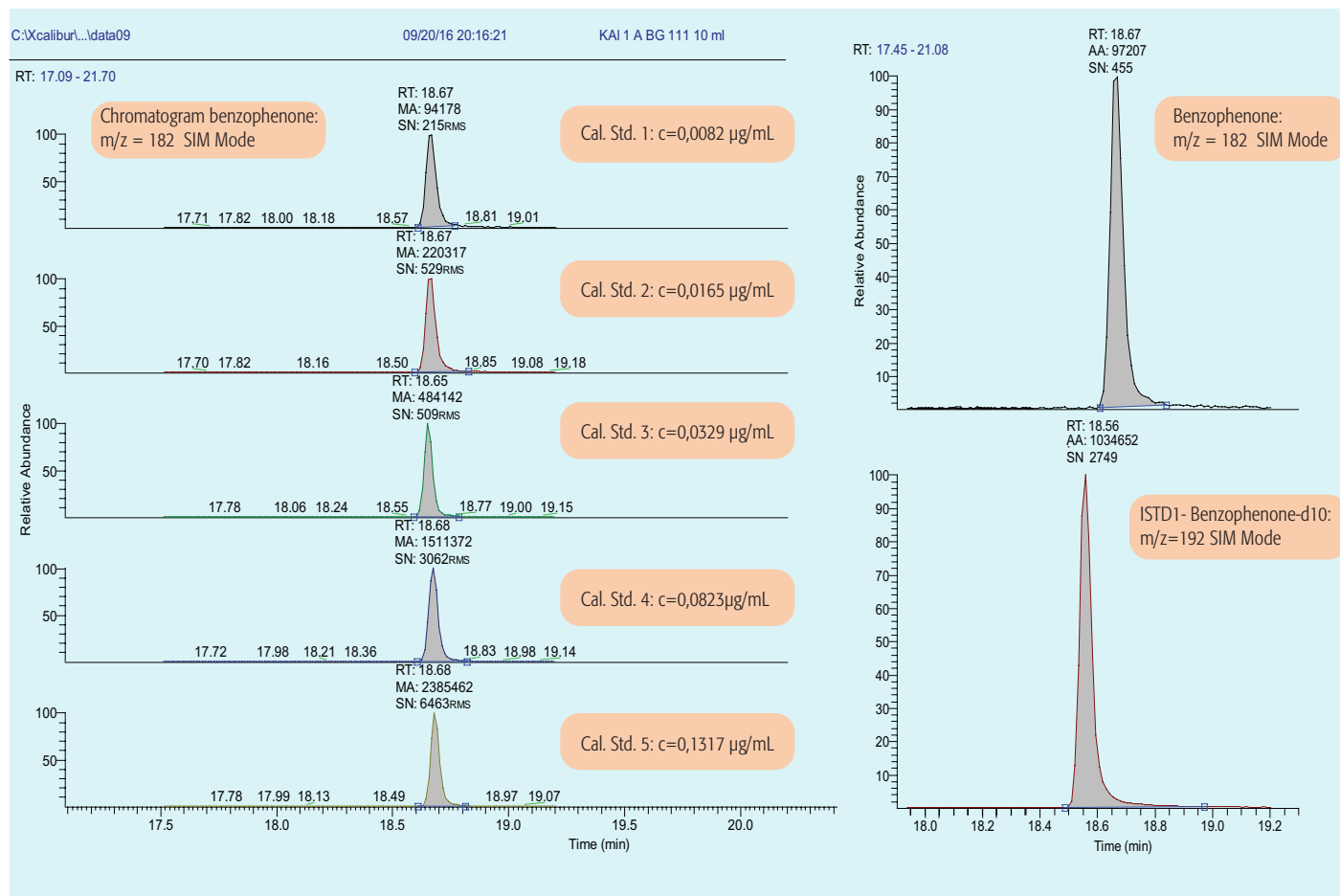
Automated workflow 1 using 10 mL flasks



Automated workflow 2 using 2 mL vials

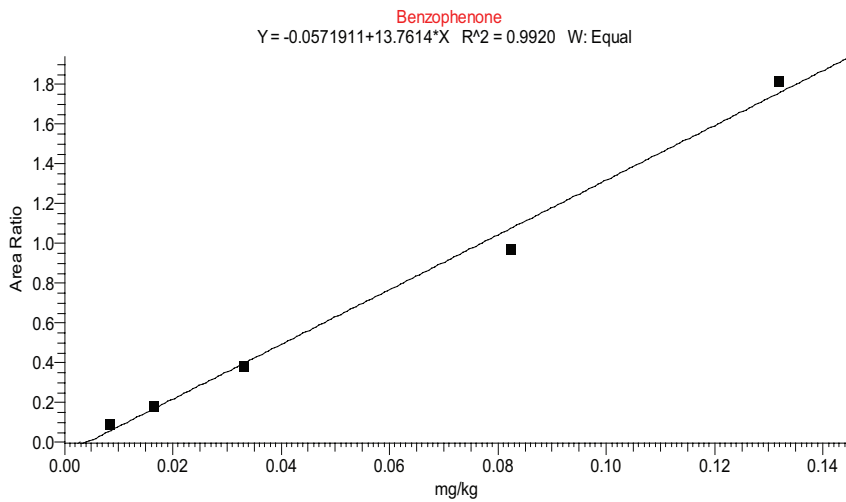


Example of GC/MS data of different concentrations of benzophenone



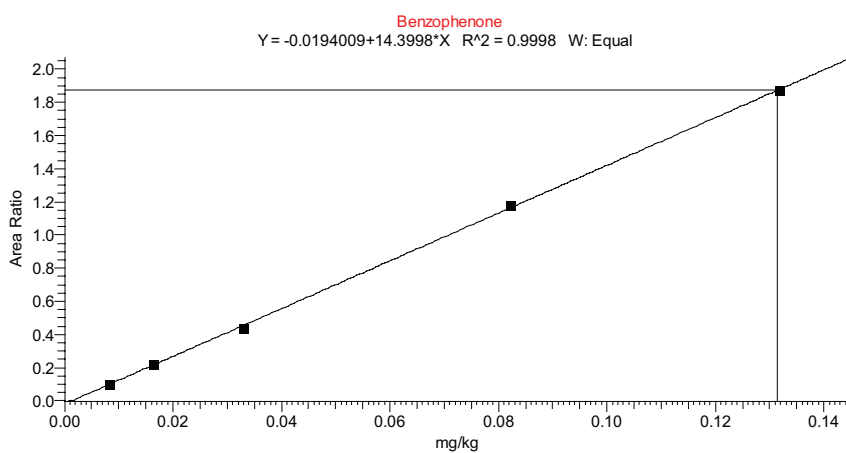
Component Name	Curve Index	Weighting Index	Origin Index	Equation				
Benzophenone	Linear	Equal	Ignore	$Y = -0.0194009 + 14.3998 * X$ $R^2 = 0.9998$				
Filename	Sample Type	Sample Name	Integ. Type	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount
data09	Std Bracket Sample	KAI 1 A BG 111 10 ml	Method Settings	100056	974033	0,103	0,0082	0,0085
data10	Std Bracket Sample	KAI 2 A BG 111 10 ml	Method Settings	228383	1027645	0,222	0,0165	0,0168
data11	Std Bracket Sample	KAI 3 A BG 111 10 ml	Method Settings	489830	1117880	0,438	0,0329	0,0318
data12	Std Bracket Sample	KAI 4 A BG 111 10 ml	Method Settings	1522545	1290800	1,180	0,0823	0,0833
data13	Std Bracket Sample	KAI 5 A BG 111 10 ml	Method Settings	2433599	1299926	1,872	0,1317	0,1314

Comparison of statistical performance figures for Benzophenone calibration curves



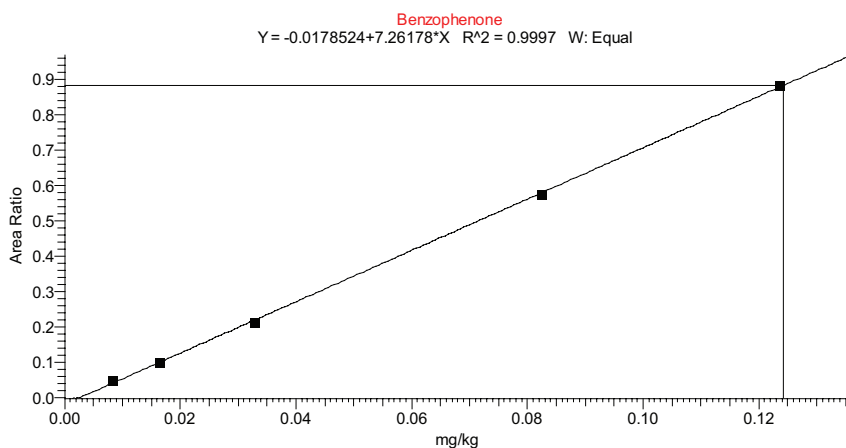
Manual process:

$y = 13,761 * X - 0,00571$
 Residual variation (S_y) = 0,0722
 Area Ratio Standard deviation of the process (S_{x0}) = 0,0053 µg/mL
 Coefficient of variation for the process (V_{x0}) = 9,71 %
 $r = 0,9962$



Automatic workflow with 10 mL vol. flask:

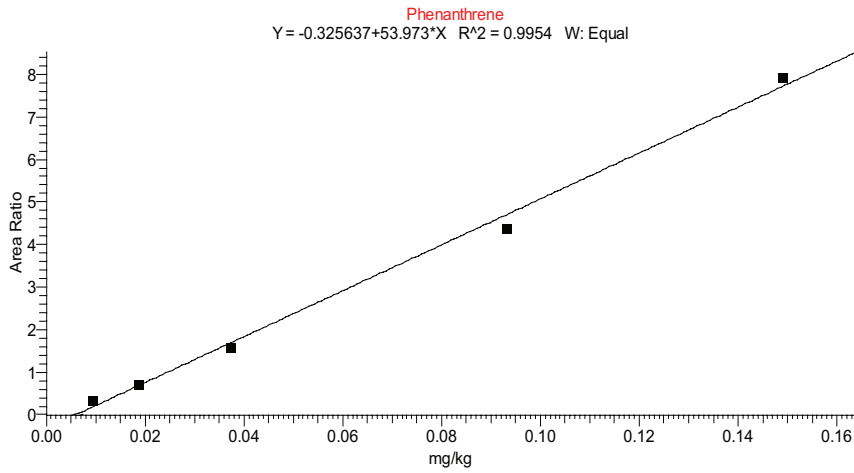
$y = 14,3998 * X - 0,0194$
 Residual variation (S_y) = 0,0133
 Area Ratio Standard deviation of the process (S_{x0}) = 0,0009 µg/mL
 Coefficient of variation for the process (V_{x0}) = 1,70 %
 $r = 0,9999$



Automatic workflow with 2 mL vial :

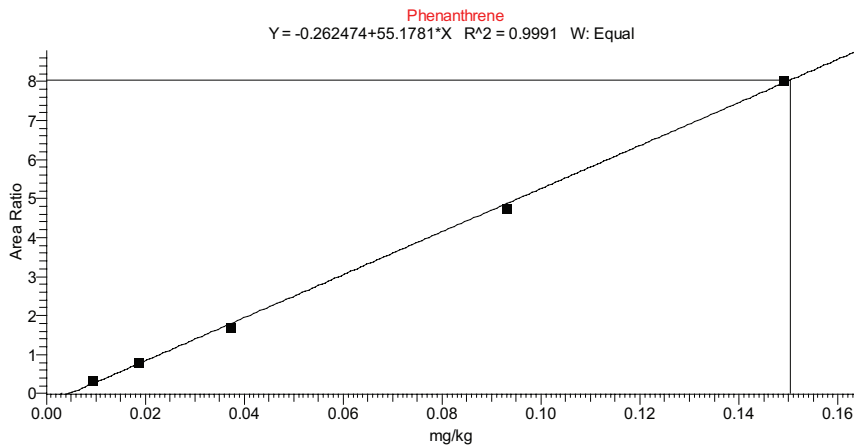
$y = 7,261 * X - 0,0178$ (*2 = 14,54)
 Residual variation (S_y) = 0,0083
 Area Ratio Standard deviation of the process (S_{x0}) = 0,0011 µg/mL
 Coefficient of variation for the process (V_{x0}) = 2,18 %
 $r = 0,9998$

Comparison of statistical performance figures for Phenanthrene calibration curves



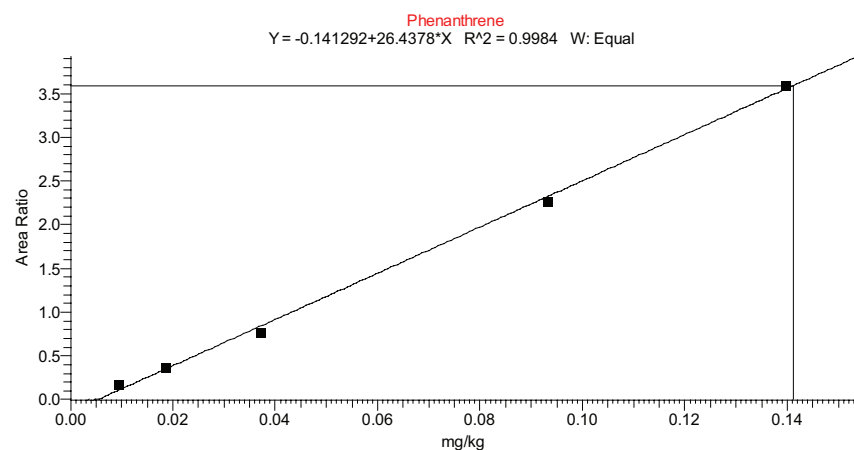
Manual process:

$y = 53,973 * X - 0,3256$
 Residual variation (S_y) = 0,2465
 Area Ratio Standard deviation of the process (S_{x0}) = 0,0046 $\mu\text{g/mL}$
 Coefficient of variation for the process (V_{x0}) = 7,45 %
 $r = 0,9977$



Automatic workflow with 10 mL vol. flask:

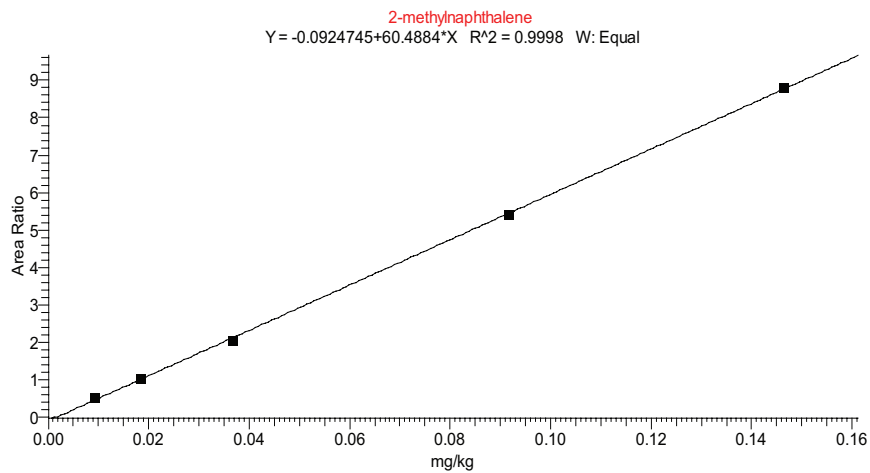
$y = 55,178 * X - 0,2625$
 Residual variation (S_y) = 0,1105
 Area Ratio Standard deviation of the process (S_{x0}) = 0,0020 $\mu\text{g/mL}$
 Coefficient of variation for the process (V_{x0}) = 3,26 %
 $r = 0,9996$



Automatic workflow with 2 mL vial:

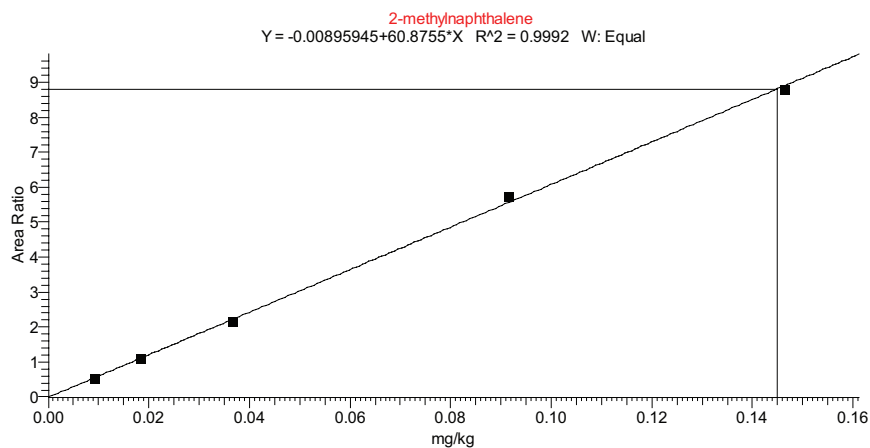
$y = 26,438 * X - 0,1413$ (*2 = 52,72)
 Residual variation (S_y) = 0,0650
 Area Ratio Standard deviation of the process (S_{x0}) = 0,0025 $\mu\text{g/mL}$
 Coefficient of variation for the process (V_{x0}) = 4,13 %
 $r = 0,9993$

Comparison of statistical performance figures for 2-methylnaphthalene calibration curves



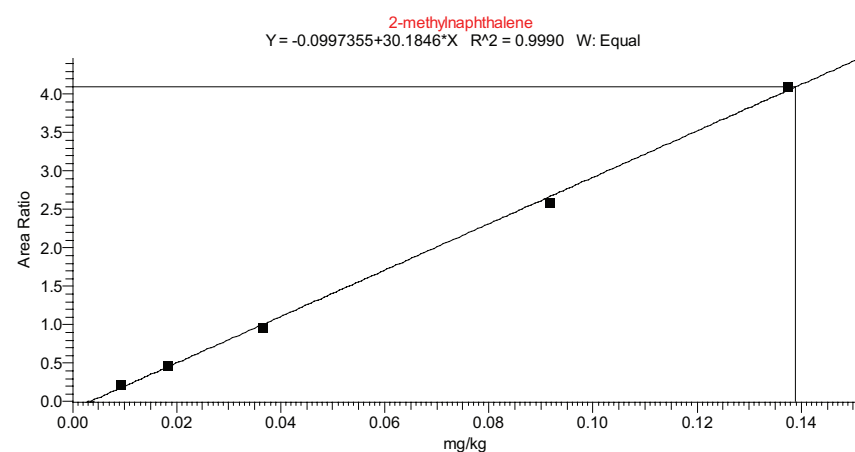
Manual process:

$y = 60,488 \cdot X - 0,0092$
 Residual variation (S_y) = 0,0824
 Area Ratio Process standard deviation (S_{x0}) = 0,0014 $\mu\text{g/mL}$
 Coefficient of variation for the process (V_{x0}) = 2,26 %
 $r = 0,9998$



Automatic workflow with 10 mL vol. flask:

$y = 60,875 \cdot X - 0,00895$
 Residual variation (S_y) = 0,1027
 Area Ratio Process standard deviation (S_{x0}) = 0,0017 $\mu\text{g/mL}$
 Coefficient of variation for the process (V_{x0}) = 2,79 %
 $r = 0,9997$



Automatic workflow with 2 mL vial:

$y = 30,185 \cdot X - 0,00997$ (*2 = 60,328)
 Residual variation (S_y) = 0,0732
 Area Ratio Process standard deviation (S_{x0}) = 0,0024 $\mu\text{g/mL}$
 Coefficient of variation for the process (V_{x0}) = 4,14 %
 $r = 0,9993$

Precision (6-fold determination of the analytes)

Results	Benzophenone			Phenanthrene			2-methylnaphthalene		
	RTC workflow 1	RTC workflow 2	Manual	RTC workflow 1	RTC workflow 2	Manual	RTC workflow 1	RTC workflow 2	Manual
	10 mL	1 mL		10 mL	1 mL		10 mL	1 mL	
1	0,0088	0,0094	0,0088	0,0114	0,0080	0,0118	0,0089	0,0084	0,0090
2	0,0085	0,0094	0,0085	0,0113	0,0079	0,0116	0,0090	0,0084	0,0089
3	0,0085	0,0096	0,0084	0,0115	0,0079	0,0115	0,0086	0,0082	0,0088
4	0,0084	0,0095	0,0087	0,0114	0,0079	0,0115	0,0088	0,0082	0,0089
5	0,0086	0,0095	0,0086	0,0115	0,0079	0,0114	0,0088	0,0083	0,0088
6	0,0089	0,0094	0,0089	0,0114	0,0079	0,0116	0,0088	0,0084	0,0090
significance level	5 %	5 %	5 %	5 %	5 %	5 %	5 %	5 %	5 %
mean value	0,0086	0,0095	0,0087	0,0114	0,0079	0,0116	0,0088	0,0084	0,0089
median	0,0086	0,0095	0,0087	0,0114	0,0079	0,0116	0,0088	0,0084	0,0089
range (R)	0,0005	0,0002	0,0005	0,0002	0,0001	0,0004	0,0004	0,0003	0,0002
standard deviation (s)	0,0002	0,0001	0,0002	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001
coefficient of variation (VK)	2,25%	0,86%	2,16%	0,66%	0,52%	1,18%	1,51%	1,13 %	1,04%
repeatability	0,0002	0,0002	0,0005	0,0002	0,0001	0,0004	0,0004	0,0004	0,0003
confidence range (VBx)	0,0001	0,0001	0,0002	0,0001	0,0001	0,0001	0,0001	0,0002	0,0001

Conclusions

- The statistical specifications for the manual and automatic (PAL RTC) generation of calibrations models are comparable, with the automated process being more reproducible.
- The automatic generation is fully traceable.
- The automatic handling frees up the operators for more valuable tasks.
- Calibration curves can be generated automatically while a sequence is running. Instable calibration samples can always be prepared freshly from stable stock solutions.
- The demonstrated small volume workflow allows for the use of certified standard solutions. At the same time it reduces the consumption of solvents.

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CTC Analytics AG
 Industriestrasse 20
 CH-4222 Zwingen
 Switzerland
 T +41 61 765 81 00
 Contact: info@ctc.ch