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Comparison of the manual and automated generation of calibration standards

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Introduction

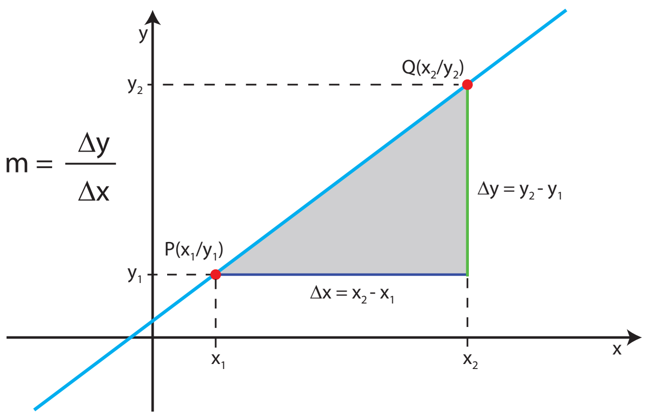
This application note compares the manual and automated generation of calibration standards in terms of their statistical figures of merit.

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

<u>Accuracy</u>: Difference between intended and actual volume

<u>Precision</u>: Reproducibility of repeated processes (here volume of liquid addition)

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

<u>Coeffiecient of determination r^2</u>: the closer r^2 is to 1, the higher is the probability of a linear correlation.

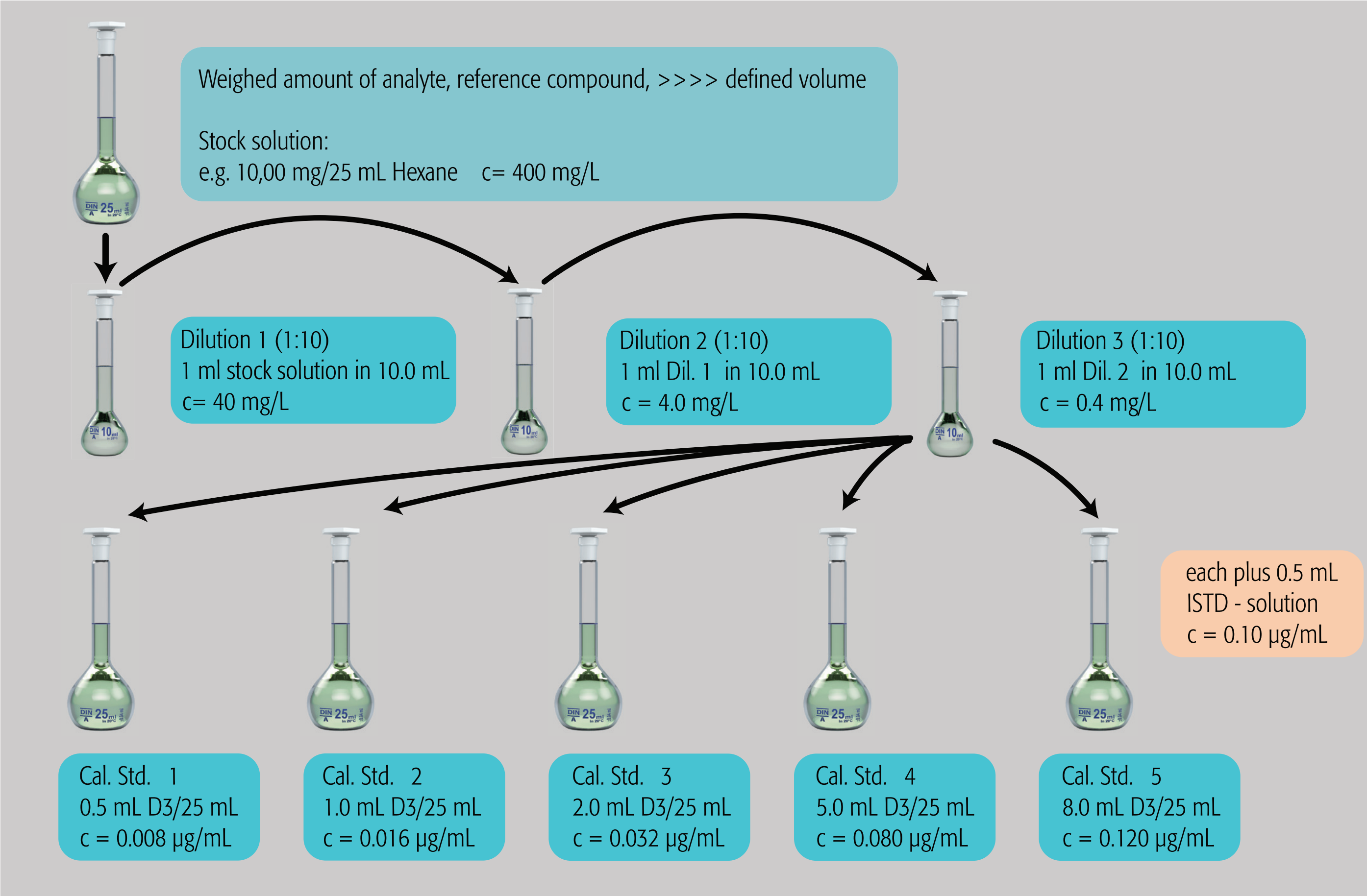
<u>Residual variation Sy</u>: measures the proportion to which a mathematical model accounts for the variation of a given data set.

<u>Standard deviation of the process Sx0 (=Sy/m)</u>: 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.

<u>Coefficient of variation for the process Vx0</u>: 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.

$$Vx0 = \frac{Sx0}{Mw}\ 100\%$$

Standard manual procedure to generate calibration solutions using volumetric pipettes



Accuracy of some volumetric pipetts

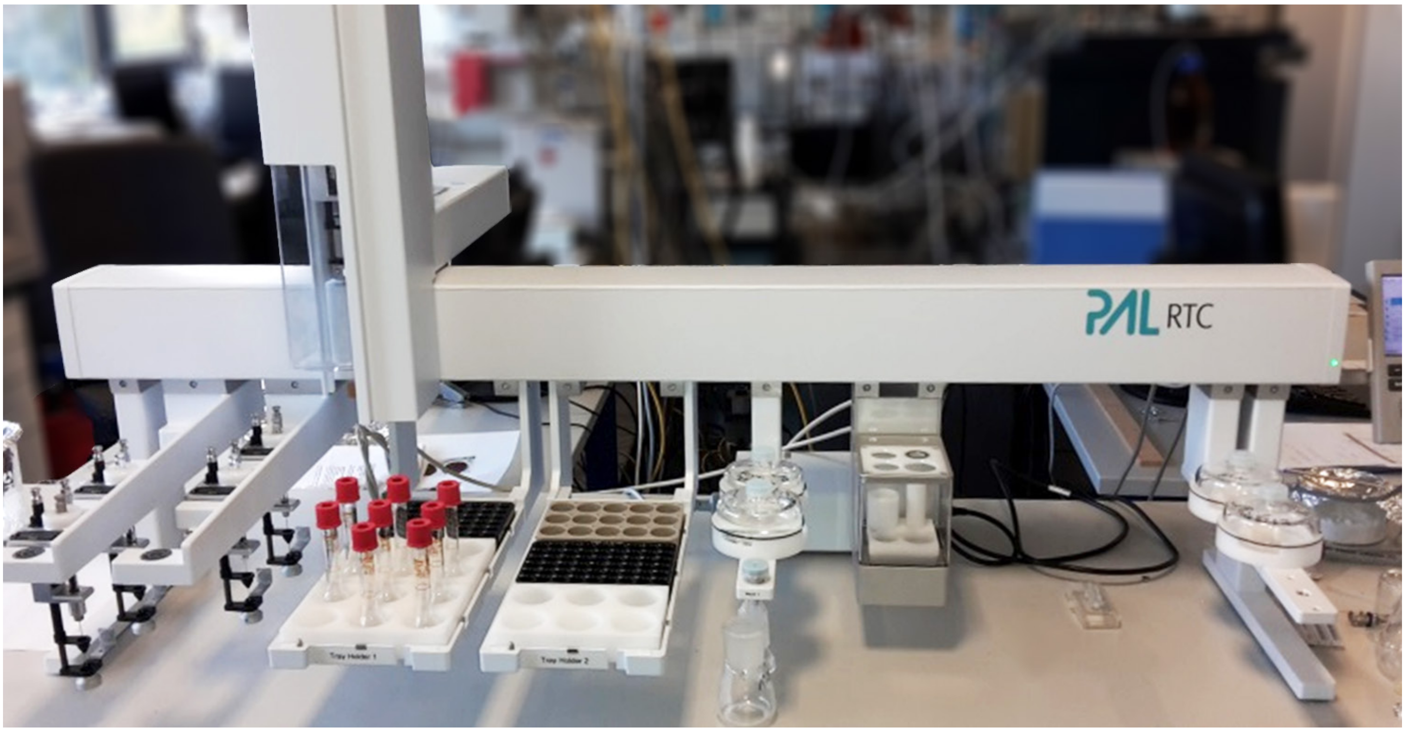
Nominal volume [mL]	Class A		Class AS		Class B	
	max. error [%]	time to drain [s]	max. error [%]	time to drain [s]	max. error [%]	time to drain [s]
0,5	± 1	10–20	± 1	4–8	± 2	4–20
1	± 0,7	10–20	± 0,7	5–9	± 1,5	5–20
10	± 0,2	15–40	± 0,2	8–12	± 0,4	8–40
50	± 0,1	30–60	± 0,1	13–18	± 0,2	13–60
100	± 0,08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

Accuracy of positive displacement syringes

Accuracy ±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



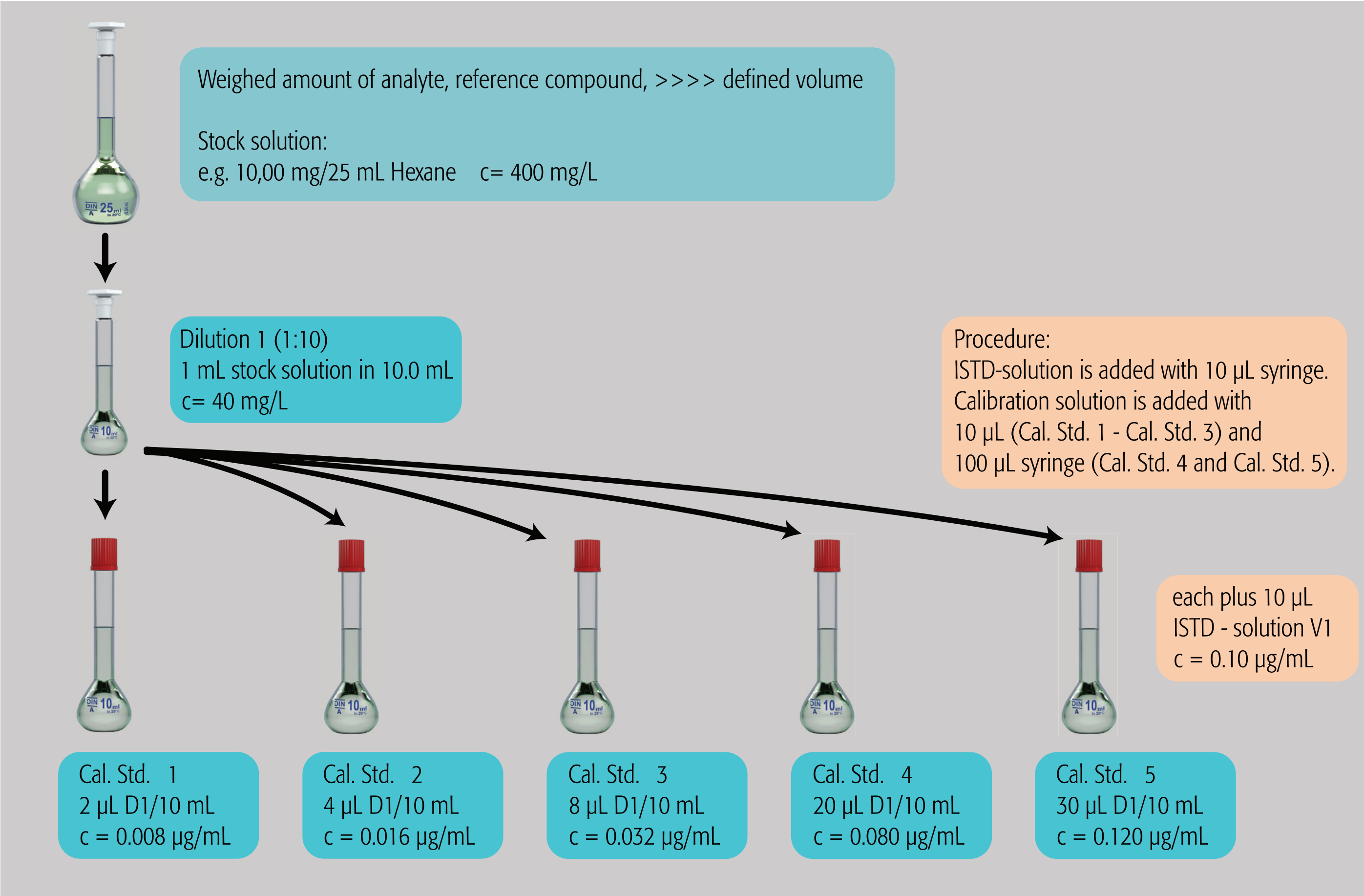
For the automated workflows on the PAL RTC the follwing tools (http://www.palsystem.com/index.php?id=284) and modules (http://www.palsystem.com/index.php?id=194) were used:

- Park Station
- D7/57 Syringe Tool for 10 µL syringe
- D7/57 Syringe Tool for 100 µL syringe
- D8/57 Syringe Tool for 1000 µL 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

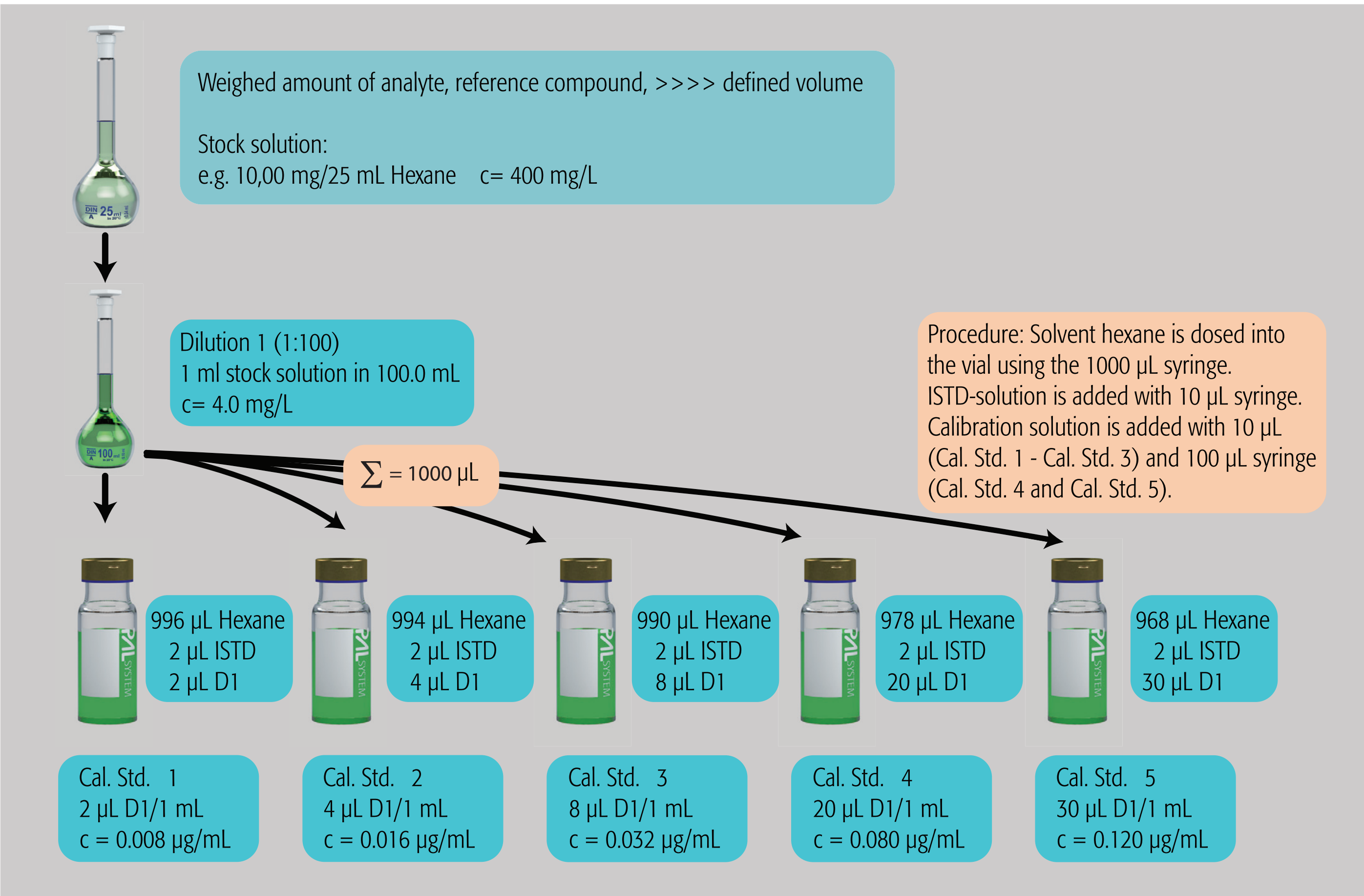
The automated workflows 1 and 2 were realized with the PAL Sample Control software (http://www.palsystem.com/index.php?id=243). PAL Sample Control allows to operate both standalone workstations as well as online systems (LC, LC-MS, GC, GC-MS).

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).

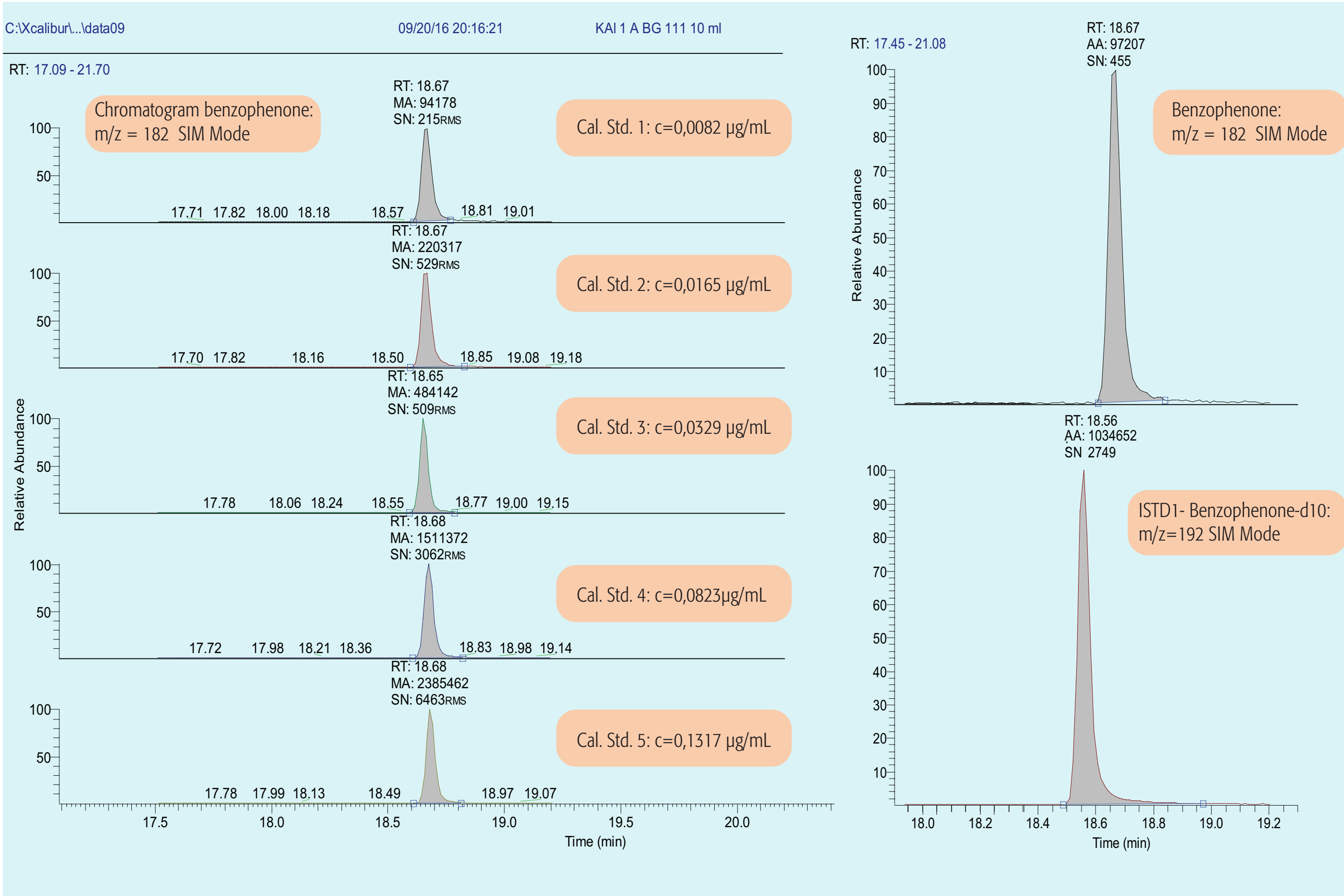
Automated workflow 1 using 10 mL flasks



Automated workflow 2 using 2 mL vials

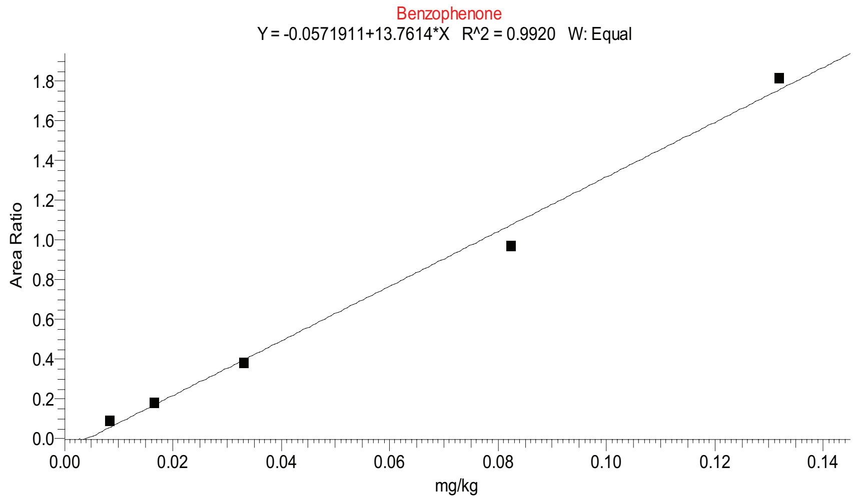


Example of GC/MS data of different concentrations of benzophenone



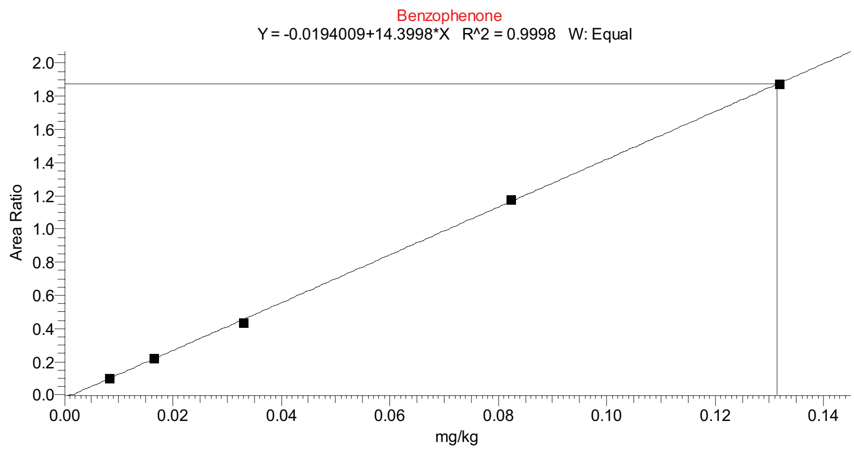
Component Name		Curve Index	Weighting Index	Origin Index	Equation			
Benzophenone		Linear	Equal	Ignore	Y = -0.0194009+14.3998*X R^2 = 0.9998			
Filename	Sample Type	Sample Name	Integ. Type	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount
data09	Std Bracket Sample	KAI 1 A BG 111 10 ml	Method 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



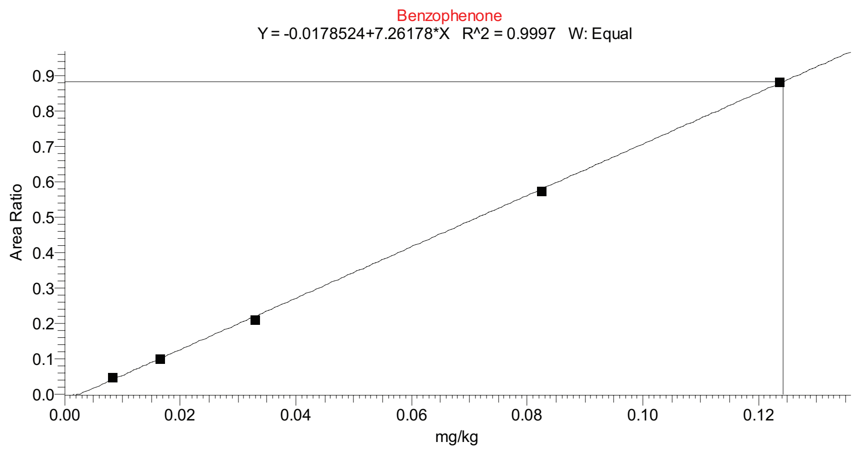
Manual process:

y= 13,761*X-0,00571
Residual variation (Sy) = 0,0722
Area Ratio Standard deviation of the process (Sx0) = 0,0053 µg/mL
Coefficient of variation for the process (Vx0) = 9,71 %
r = 0,9962



Automatic workflow with 10 mL vol. flask:

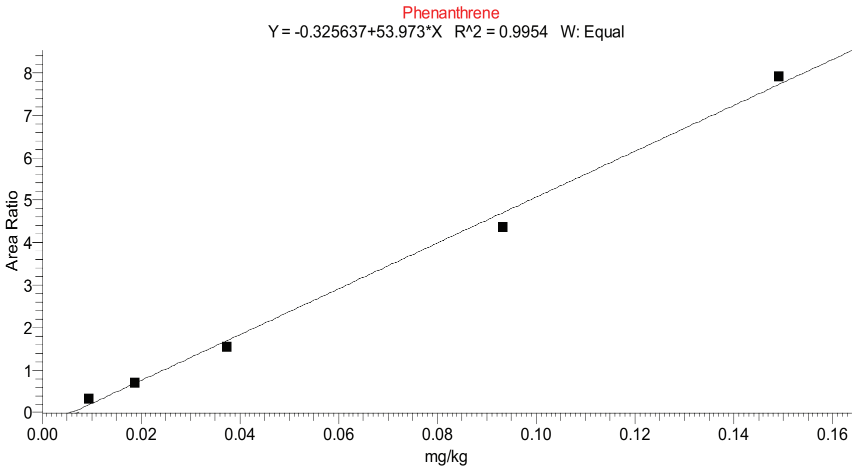
y= 14,3998*X-0,0194
Residual variation (Sy) = 0,0133
Area Ratio Standard deviation of the process (Sx0) = 0,0009 µg/mL
Coefficient of variation for the process (Vx0) = 1,70 %
r = 0,9999



Automatic workflow with 2 mL vial :

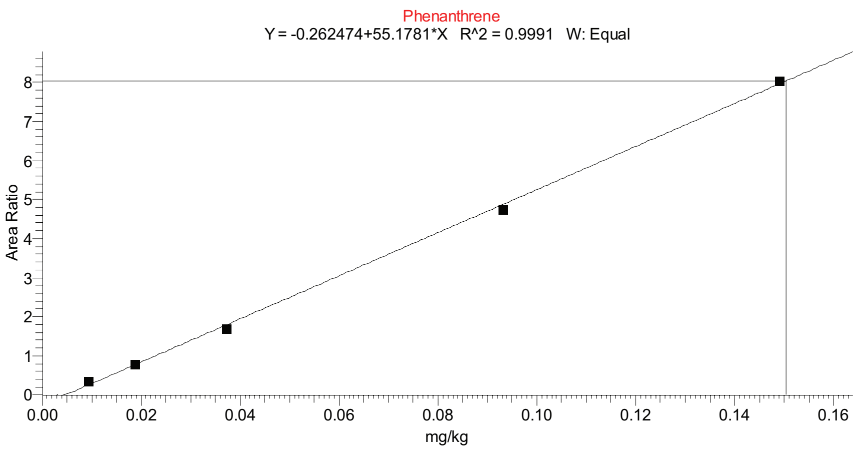
y= 7,261*X-0,0178 (*2= 14,54)
Residual variation (Sy) = 0,0083
Area Ratio Standard deviation of the process (Sx0) = 0,0011 µg/mL
Coefficient of variation for the process (Vx0) = 2,18 %
r = 0,9998

Comparison of statistical performance figures for Phenanthrene calibration curves



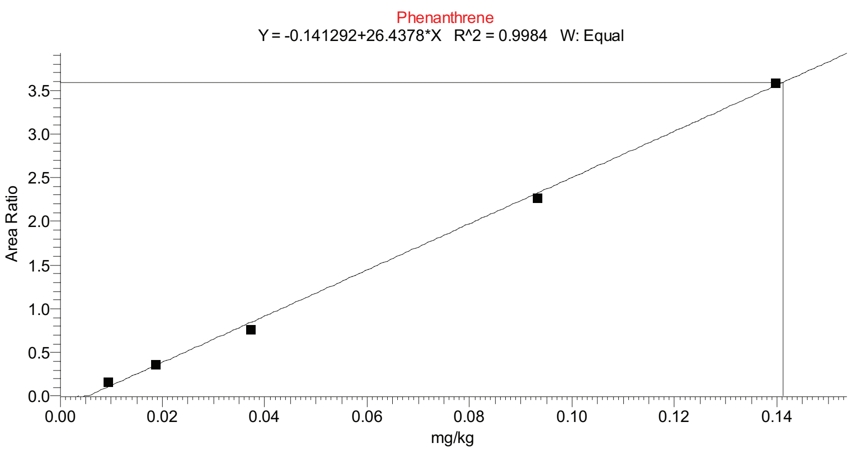
Manual process:

y= 53,973*X-0,3256
Residual variation (Sy) = 0,2465
Area Ratio Standard deviation of the process (Sx0) = 0,0046 µg/mL
Coefficient of variation for the process (Vx0) = 7,45 %
r = 0,9977



Automatic workflow with 10 mL vol. flask:

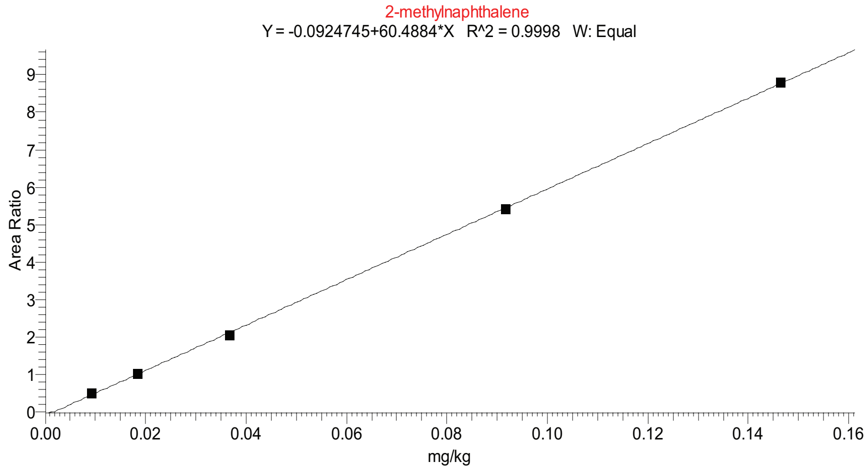
y= 55,178*X-0,2625
Residual variation (Sy) = 0,1105
Area Ratio Standard deviation of the process (Sx0) = 0,0020 µg/mL
Coefficient of variation for the process (Vx0) = 3,26 %
r = 0,9996



Automatic workflow with 2 mL vial:

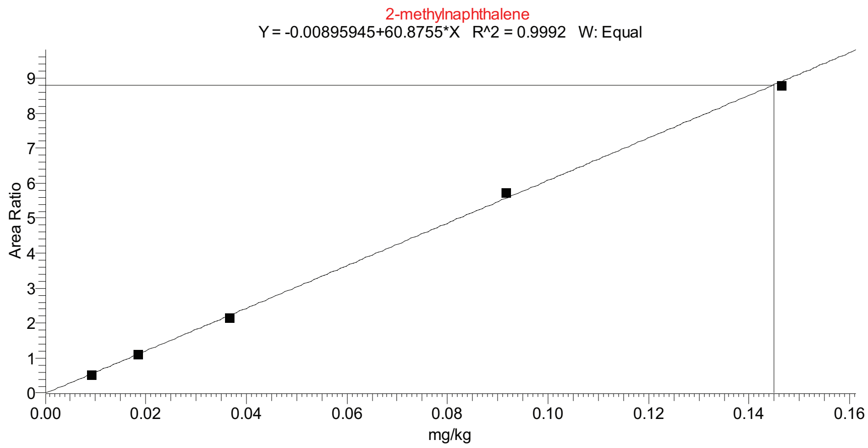
y= 26,438*X-0,1413 (*2= 52,72)
Residual variation (Sy) = 0,0650
Area Ratio Standard deviation of the process (Sx0) = 0,0025 µg/mL
Coefficient of variation for the process (Vx0) = 4,13 %
r = 0,9993

Comparison of statistical performance figures for 2-methylnaphthalene calibration curves



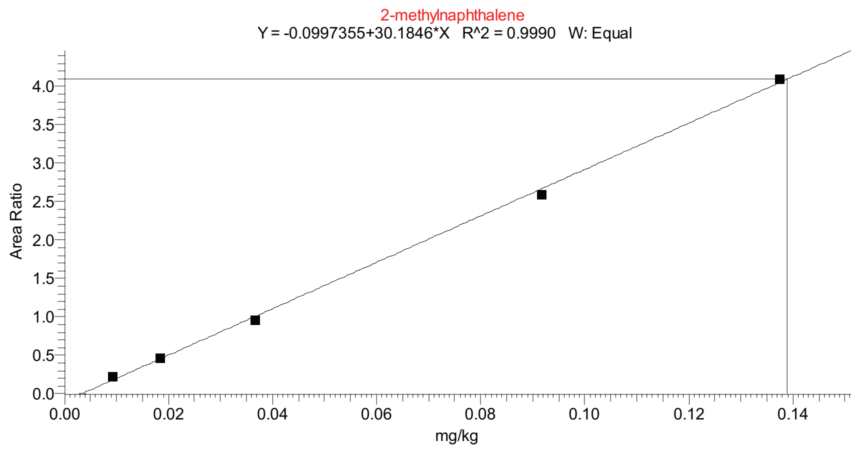
Manual process:

y= 60,488*X - 0,0092
Residual variation (Sy) = 0,0824
Area Ratio Process standard deviation (Sx0) = 0,0014 µg/mL
Coefficient of variation for the process (Vx0) = 2,26 %
r = 0,9998



Automatic workflow with 10 mL vol. flask:

y= 60,875*X-0,00895
Residual variation (Sy) = 0,1027
Area Ratio Process standard deviation (Sx0) = 0,0017 µg/mL
Coefficient of variation for the process (Vx0) = 2,79 %
r = 0,9997



Automatic workflow with 2 mL vial:

y= 30,185*X-0,00997 (*2= 60,328)
Residual variation (Sy) = 0,0732
Area Ratio Process standard deviation (Sx0) = 0,0024 µg/mL
Coefficient of variation for the process (Vx0) = 4,14 %
r = 0,9993

Precision (6-fold determination of the analytes)

	Benzophenone			Phenanthrene			2-methylnaphthalene		
Results	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 varaition (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.

Legal Statements

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