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GC Application Note

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Dilution/standard addition: Efficient and traceable.

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Dilution / standard addition: Efficient and traceable.

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Introduction

Dilution is a tedious, repetitive task that is performed daily in many labs. It is typically done either for the preparation of calibration curves or for sample preparation prior to injection. In many cases the serial dilutions steps are done manually. Depending on the number of samples to be prepared this can be a full time job. Automation helps to increase productivity, but also to reduce errors related to manual handling. The productivity is further increased if the system used can also perform the injection. The PAL RTC can change tools like syringes automatically and therefore perform all dilution steps. After sample preparation it can also inject the sample into the GC or LC. This feature opens the door to new fields of applications.

The article presents a dilution method developed to prepare calibration curves. The method is explained and results obtained with the method are shown.

Objective

The objective was to prepare serial dilutions with high efficiency, good accuracy and precision, low error rate and the possibility to log acitivities during operation of the instrument.
The following parameters were defined:

- Up to 14 calibration vials to be prepared over 5 decades of concentration
- Addition of an internal standard (ISTD) should be possible
- No syringe should dispense less than 10 % of its total volume
- All calibration solutions must end up with the same volume
- All of the dilution levels should be user selectable
- The method must be easy to use

PAL RTC configuration and method specifications

A PAL RTC with extended x-axis (figures 1a and 1b) with the following tools and modules was used:

- 2 park stations
- 6 syringes: 2 x 1 mL; 2 x100 µL; 2 x 10 µL
- Vortex mixer
- 2 tray holders with 3 x VT54 trays and 3 x VT15 trays
- Fast wash station
- Solvent module
- PAL Sample Control software

Up to 14 samples were prepared for a calibration curve from 100 to 0.01 µg/mL

Figure 1a: Tool station with the 6 syringes

Figure 1b: The PAL RTC with (from left to right) incubator, fast wash station, VT54 trays, VT15 trays, vortex mixer, standard wash, solvent station, bar code reader.

(5 decades). To remain within the 10 % of syringe volume three solutions were prepared by serial dilutions.
Dedicated syringes can be used for concentration ranges as well as for internal standard and injection.

Method

The method consists of preparing sub solutions by serial dilution of a stock solution. These sub solutions are then used to obtain the calibration solutions. The total volume of the sub solution is fixed at 5 mL to ensure enough solution for the dilutions.
Each sub solution can then be diluted into a maximum of 6 vials (calibration solution) at a settable volume. The total volume of the calibration solutions is settable in the method. A software tool was developed to calculate the different volumes to be added.

Sub solution preparation (figure 3)

- From the stock solution at 4 mg/mL solution A is prepared at 400 µg/mL
- The solution A is vortexed
- Then solution B is prepared at 40 µg/mL from solution A
- The solution B is vortexed
- Then solution C is prepared at 4 µg/mL from solution B
- The solution C is vortexed

Calibration solution preparation (figure 4)

- From sub solution A, 3 vials are prepared at 400, 200 and 100 µg/mL.
- From sub solution B, 3 vials are prepared at 40, 20 and 10 µg/mL.
- From sub solution C, 3 vials are prepared at 4, 2 and 1 µg/mL.

Results

A mixture of 14 components was analyzed by GC (14 compound GC-O mix, Brechbuehler AG, list of compunds see on the right side).
A Trace 1310 GC (Thermo Scientific) configured with a split splitless injector module and FID was used.

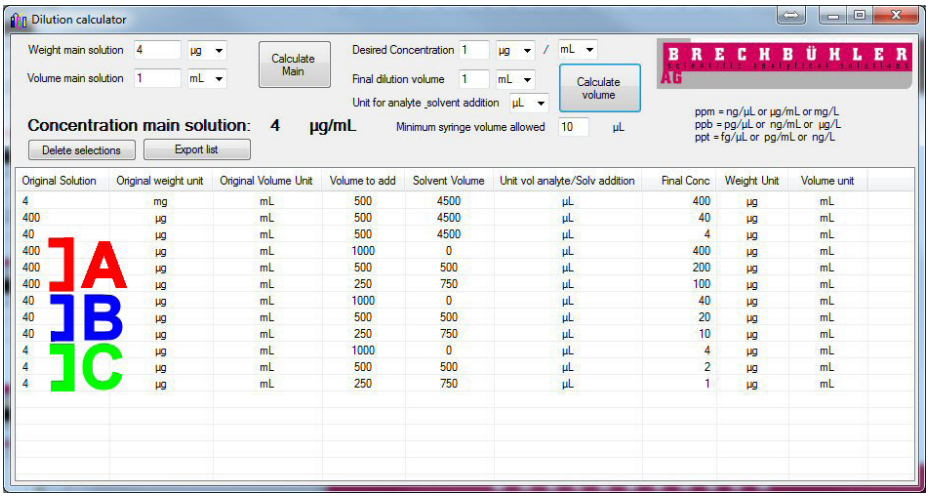

Figure 2: A screenshot of the Dilution Calculator showing the list of the dilutions performed.

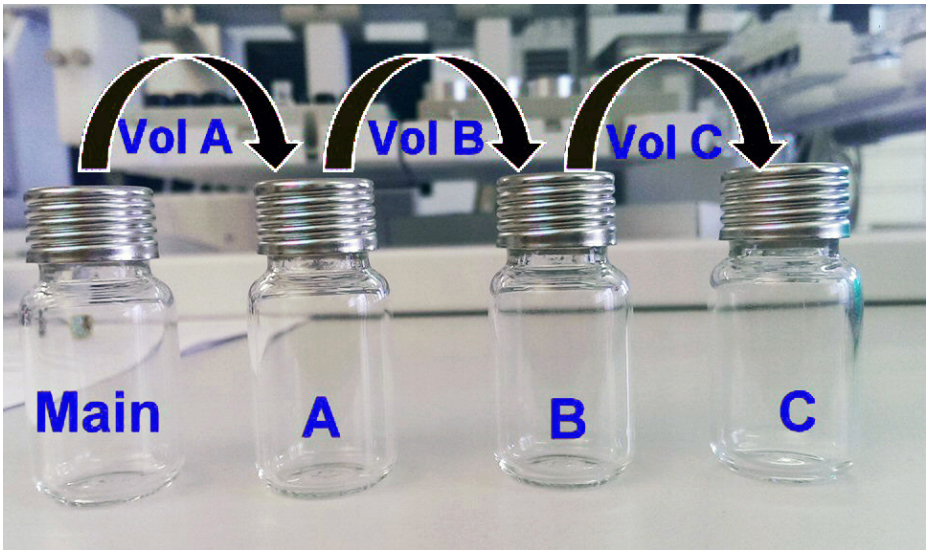

Figure 3: Sub solution preparation

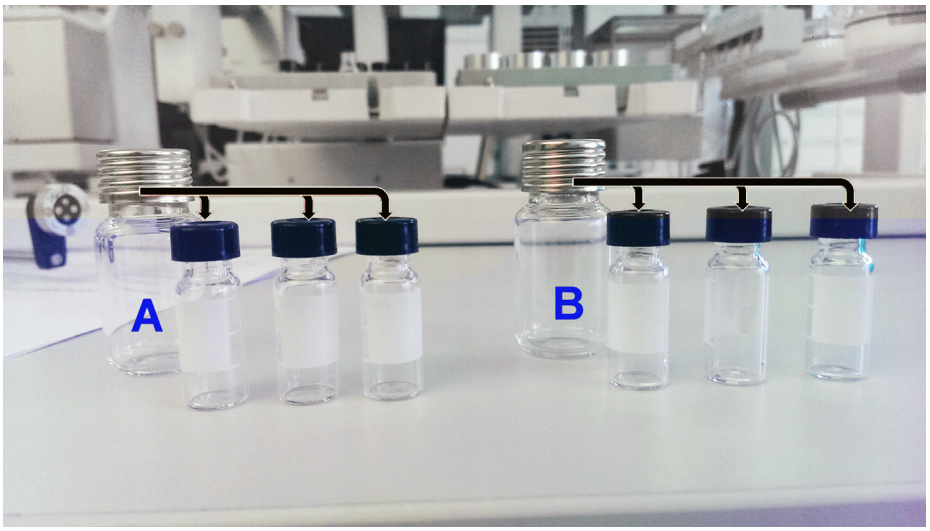

Figure 4: Calibration solution preparation

- The column was a ZB1 (Phenomenex) 15 m x 0.25 mm ID; 0.25 µm film thickness.
- The injector was set to 260 °C for split injection 20:1.
- The FID was set at 270 °C.
- The oven was programmed as follows: 4 min @ 40 °C, then to 260 °C @15 °C/min, hold 1.5 min.
- The data system was Chromeleon (Thermo Scientific).

All samples were injected with the PAL RTC using a standard liquid injection method. The results were processed with Chromeleon.

Figure 5 shows the chromatogram obtained with a 40 µg/mL solution, table 1 summarizes the calibration results.

Compound Name	R square
Butyl acetate	0.9999
Cyclohexanone	0.9998
Ethyl valerate	0.9996
Benzaldehyde	0.9996
Beta-pinene	0.9995
C10	0.9995
Limonene	0.9995
Linalool	0.9995
Benzyl acetate	0.9995
Menthol	0.9995
Citronellol	0.9995
Geraniol	0.9995
Coumarin	0.9997
Alpha Ionone	0.9995

Table 1: R^2 obtained with the prepared calibration

Full traceability

The log file of the steps performed allows to trace every step during the operation (figure 8). This is essential, especially in a regulated lab environment.

Discussion

In this method no syringe was used below 10 % of its full capacity ensuring a very good accuracy. To prevent cross contamination a 1 mL syringe was used for the dilutions and a second 1 mL syringe was used for the addition of solvent. For volumes below 100 µL, a 100 µL syringe was used. A 10 µL syringe was reserved for the injection into a GC and a second 10 µL syringe for the addition of internal standards, if required.

Conclusion

The dilution method for the PAL RTC presented here produced excellent results. Once the sample list is generated and launched no human intervention is required.

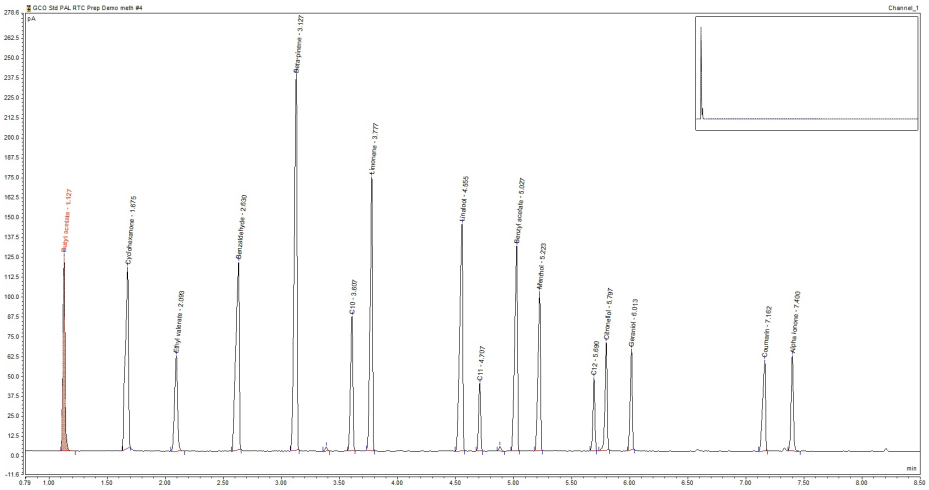

Figure 5: Chromatogram of the 14 component mix

Calibration Details	Butyl acetate		
Calibration Type	Lin, WithOffset	Offset (C0)	0.0020
Evaluation Type	Area	Slope (C1)	0.0078
Number of Calibration Points	9	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9999

Calibration Plot	Butyl acetate

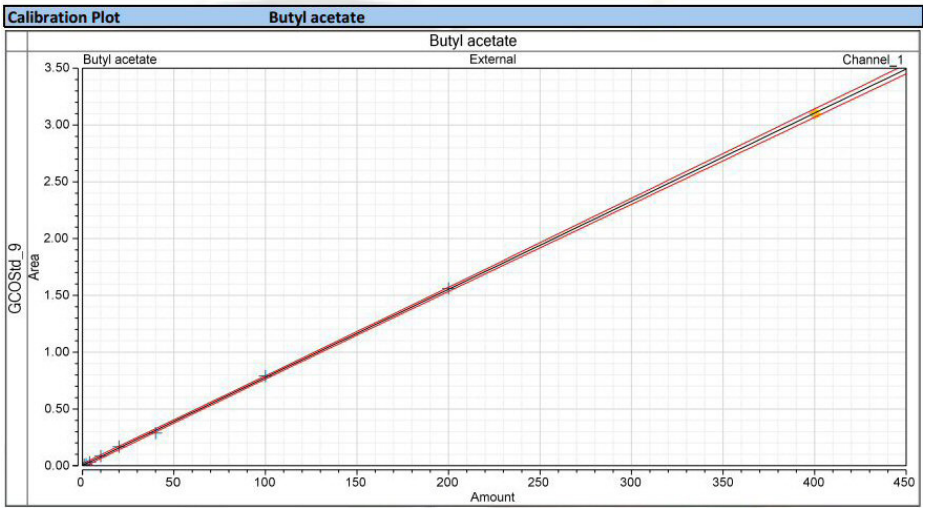

Figure 6: Butyl acetate calibration curve R^2= 0.9999 (best)

Calibration Details	Limonene		
Calibration Type	Lin, WithOffset	Offset (C0)	0.0064
Evaluation Type	Area	Slope (C1)	0.0144
Number of Calibration Points	9	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9995

Calibration Plot	Limonene

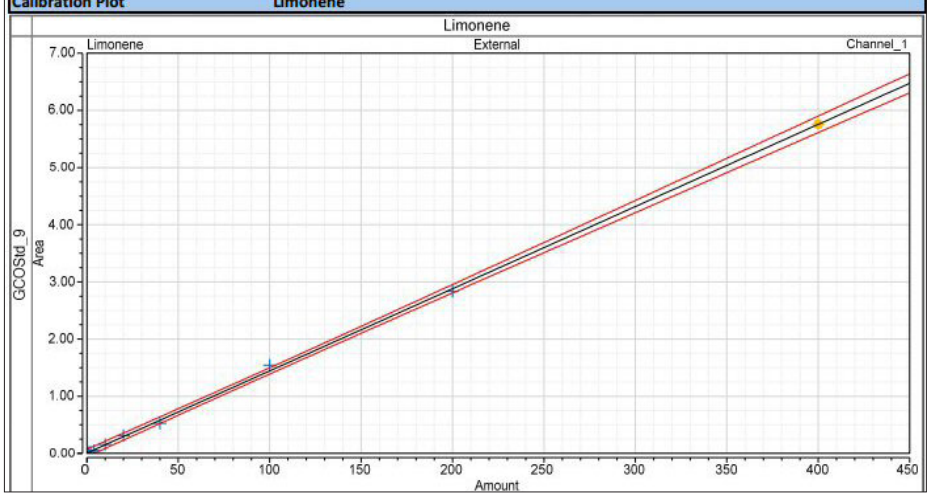

Figure 7: Calibration curve for alpha ionone (r^2=0.9995)

The programming of the method is based on simple tasks. Some advanced programming was used to enhance the functionality (Dilution Calculator). The PAL Sample Control software gives great flexibility for method development. Very important is also the fact, that all activities of the PAL RTC are logged and therefore traceable.

Start	Duration	Task	Action	Done
00:00:00	00:02:25	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:02:25	00:00:40	Transfer	Tray Holder 2:Slot1:1 -> Tray Holder 2:Slot1:2 - Volume: 250 µL	
00:03:05	00:02:20	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:05:25	00:02:50	Transfer	Solvent Module1:1 -> Tray Holder 2:Slot1:2 - Volume: 4750 µL	
00:08:15	00:00:20	VortexVial	Vortex vial from Tray Holder 2:Slot1:2 (1500 rpm, 20 s)	
00:08:35	00:02:40	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:11:15	00:00:40	Transfer	Tray Holder 2:Slot1:2 -> Tray Holder 2:Slot1:3 - Volume: 500 µL	
00:11:55	00:02:20	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:14:15	00:02:40	Transfer	Solvent Module1:1 -> Tray Holder 2:Slot1:3 - Volume: 4500 µL	
00:16:55	00:00:20	VortexVial	Vortex vial from Tray Holder 2:Slot1:3 (2000 rpm, 20 s)	
00:17:15	00:02:20	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:19:35	00:00:43	Transfer	Tray Holder 2:Slot1:3 -> Tray Holder 2:Slot1:4 - Volume: 500 µL	
00:20:18	00:02:25	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:22:43	00:02:40	Transfer	Solvent Module1:1 -> Tray Holder 2:Slot1:4 - Volume: 4500 µL	
00:25:23	00:00:20	VortexVial	Vortex vial from Tray Holder 2:Slot1:4 (2000 rpm, 20 s)	
00:25:43	00:02:26	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:28:09	00:00:40	Transfer	Tray Holder 2:Slot1:2 -> Tray Holder 1:Slot1:1 - Volume: 1000 µL	
00:28:49	00:00:40	Transfer	Tray Holder 2:Slot1:2 -> Tray Holder 1:Slot1:2 - Volume: 600 µL	
00:29:29	00:00:40	Transfer	Tray Holder 2:Slot1:2 -> Tray Holder 1:Slot1:3 - Volume: 200 µL	
00:30:09	00:02:20	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:32:29	00:00:40	Transfer	Solvent Module1:1 -> Tray Holder 1:Slot1:1 - Volume: 0 µL	
00:33:09	00:00:40	Transfer	Solvent Module1:1 -> Tray Holder 1:Slot1:2 - Volume: 400 µL	
00:33:49	00:00:40	Transfer	Solvent Module1:1 -> Tray Holder 1:Slot1:3 - Volume: 800 µL	
00:34:29	00:02:20	CleanSyringe	Station: Fast Wash 1:1, Cycles: 3, Fill level: 100%	
00:36:49	00:00:40	Transfer	Tray Holder 2:Slot1:3 -> Tray Holder 1:Slot1:4 - Volume: 1000 µL	
00:37:29	00:00:40	Transfer	Tray Holder 2:Slot1:3 -> Tray Holder 1:Slot1:5 - Volume: 600 µL	
00:38:09	00:00:40	Transfer	Tray Holder 2:Slot1:3 -> Tray Holder 1:Slot1:6 - Volume: 200 µL	

Report Export

Figure 8: Snapshot of the log (partial) for the dilution method

Acknowledgments

CTC Analytics AG would like to acknowledge and extend thanks to the author of this paper:

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Legal Statements

Imprint

Date of print: 10.2014

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