Workflow

ICH Linearity Studies using Automated Standard Preparation and UHPLC

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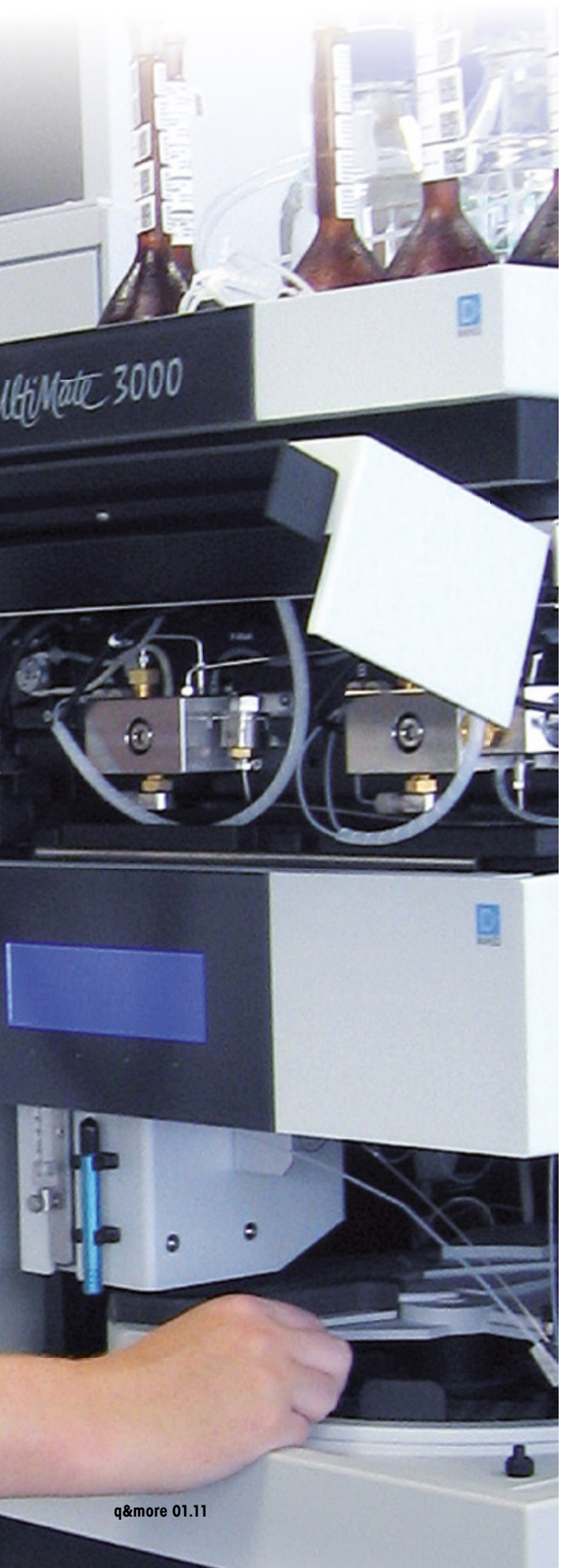
Fraser McLeod studied chemistry at the University of Strathclyde, Glasgow, Scotland, where he graduated with an MSc in Analytical Chemistry. He subsequently worked in the pharmaceutical industry for 10 years, primarily in analytical development, before he became a software solutions consultant for the industry. Fraser McLeod is a highly sought after expert in the area of software applications to improve productivity in laboratories as well as the productivity of system policies, for instance validation according to 21 CFR Part 11 of the FDA (US Food and Drug Administration). After his career move to Dionex he was initially in charge of installing the Chromeleon software in large companies, but was soon promoted to Product Manager for Chromeleon. For the last two years Fraser McLeod has held the position of Head of Product Management of the Life Sciences Business Unit; in this position he is responsible for the introduction of the UltiMate 3000 RSLC and RSLCnano-systems, for Chromeleon software and the new UHPLC+ systems.



According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), it is necessary to test analytical methods for their linearity as part of a method validation study. The typical process for this is to prepare standards for five different calibration levels, and to make triplicate injections of each of these standards. The concentration range used is typically 70–130 % of the nominal concentration.

Statistically it is recommended that each concentration level is prepared individually. This has the effect of randomising potential sources of error (e.g. a possible incorrect weighing of one of the standards). However, this is typically not done as it is a time consuming process. Instead, many laboratories prepare a stock solution, and then dilute this down to the five different concentration levels. This has the benefit of being the fastest way of preparing standards, but has a major disadvantage: any error in the stock solution will be carried through to the diluted standards.

Many chromatography methods determine more than one analyte, and each analyte must be tested for linearity. This substantially increases the time (and chance of error) for manual preparation and also increases the chance of error for the stock standard/dilution approach. This means that all approaches have drawbacks.

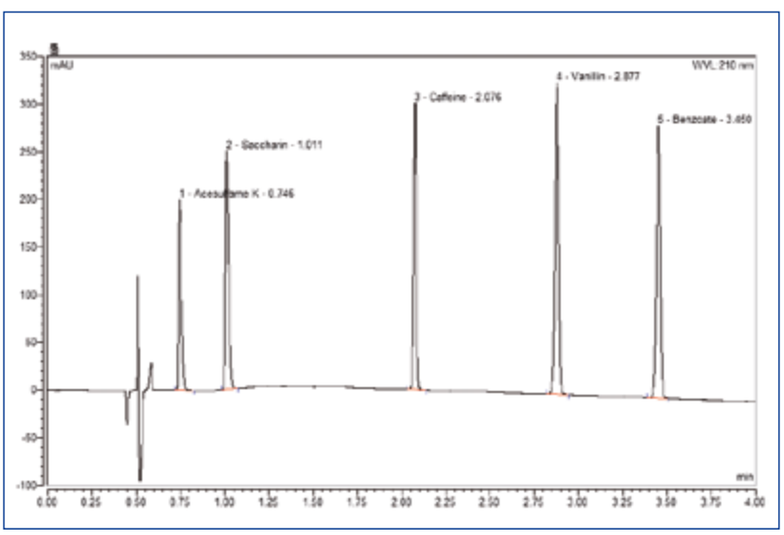


Fig. 1 Analysis of 5 soft drinks analytes in less than 5 minutes

Tab. 1 Concentrations of soft drinks analytes prepared by the Quantos QB1-L-System

Analyte	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Acesulfame-K	7.390	9.730	9.930	11.450	13.650
Saccharin	2.695	3.480	3.890	4.305	5.085
Caffeine	2.800	3.700	3.995	4.355	5.220
Vanillin	6.285	8.110	8.995	9.860	11.655
Benzoate	14.050	18.100	19.965	21.910	25.855

Tab. 2 Correlation coefficients of all analytes

Analyte	R^2
Acesulfame-K	0.99973
Saccharin	0.99989
Caffeine	0.99939
Vanillin	0.99971
Benzoate	0.99964

To automate the preparation of the standards, using the new Quantos QB1-L system from Mettler-Toledo is a more suitable method. The system makes it possible to automatically dispense and weigh analytes into HPLC vials or volumetric flasks. It can also weigh in the appropriate amount of diluents in order to provide a gravimetric solution. For linearity studies this has many advantages, but the major one is that the time issue is no longer a critical factor and that it is possible to use a statistically correct approach of preparing each standard concentration individually.

In this experiment we tested the linearity of five analytes in a soft drinks analysis. The analytes were acesulfame K, saccharin, caffeine, vanillin and benzoic acid. The Quantos system automatically weighed the correct amount of each analyte, and then the correct amount of diluent (90:10 water: methanol) to provide the concentrations shown in Tab. 1. The time taken to prepare these standards was only 50 minutes. This is a significant reduction in time, compared directly to the manual preparation (approx. 3 hour for the stock solution approach, and 4 hours for the manual approach in single steps).

Once the solutions are prepared, the actual analysis follows. In classical HPLC this could take about 30 minutes per analysis. This means that the total run time for the linearity experiment would be 7.5 hours (5 calibration levels x 3 injections per level x 30 minutes). However, with UHPLC it is possible to decrease this run time substantially. Figure 1 shows the chromatographic analysis of all 5 analytes. All peaks are separated in less than 4 minutes, and the total run time is only 5 minutes. This means that all injections can be performed in only 1.25 hours.

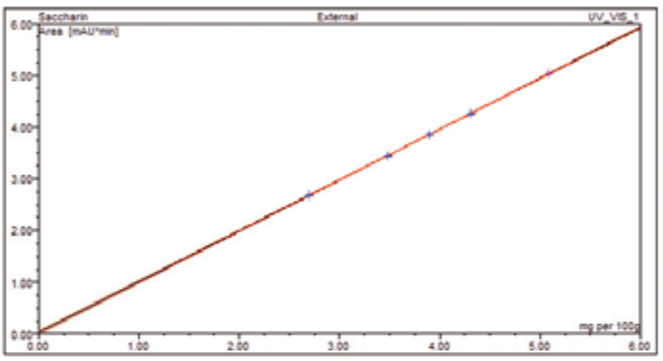
This analysis was performed on the Dionex UltiMate® 3000 Basic Automated System – an entry level system that is fully UHPLC compatible. The system supports pressures up to 620 bar and flow rates as high as 10 ml/min, and is ideal for running fast routine analyses. Further speed-up of the analysis would be possible with the UltiMate 3000 Rapid Separation LC System, as this supports pressures as high as 1000 bar.

Once all data has been acquired it is necessary to calculate the results. ICH requires that the following values are reported; correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares. Further, it needs to be checked that the correlation coefficient is within the limits expected of the method (typically >= 0.999). Performing these calculations can be a time consuming task. Some laboratories use Excel spreadsheets in an attempt to speed-up the process, but even this can be time consuming as users typically need to manually transcribe values into the spreadsheet and another person has to review this transcription.
For this sample analysis, the use of spreadsheets, and the associated review step, would take about 2 hours.
The Chromeleon® Chromatography Data System from Dionex can fully automate this task, and immediately generate all results for all analytes. All that is required is for the user to name the peaks and input their concen-

Validation Report: Linearity Test 1

Peak Details

Peak Name	Saccharin	
Calibration Type	LOff	
Y-Intercept	0.0193	
Slope	0.9860	
Min. Correlation Coefficient (Spec.)	0.9990	3 DP
Correlation Coefficient (actual)	0.9999	
Correlation Coefficient (rounded)	1	Pass
Residual Sum of Squares	0.0020	
Test Origin within CL?	Yes	



Linearity Results

No.	Name	%RANGE LEVEL %	X Value Saccharin UV_VIS_1	Y Value Saccharin UV_VIS_1	Residual Saccharin UV_VIS_1	Residual Squared Saccharin UV_VIS_1
1	Mettler Standard 1	70.0000	3.4800	3.4332	-0.0173	0.0003
7	Mettler Standard 1	70.0000	3.4800	3.4410	-0.0095	0.0001
13	Mettler Standard 1	70.0000	3.4800	3.4524	0.0019	0.0000
2	Mettler Standard 2	90.0000	3.8900	3.8371	-0.0176	0.0003
8	Mettler Standard 2	90.0000	3.8900	3.8714	0.0167	0.0003
14	Mettler Standard 2	90.0000	3.8900	3.8698	0.0151	0.0002
3	Mettler Standard 3	100.0000	4.3050	4.2530	-0.0109	0.0001
9	Mettler Standard 3	100.0000	4.3050	4.2746	0.0107	0.0001
15	Mettler Standard 3	100.0000	4.3050	4.2495	-0.0144	0.0002
4	Mettler Standard 4	110.0000	5.0850	5.0298	-0.0032	0.0000
10	Mettler Standard 4	110.0000	5.0850	5.0407	0.0078	0.0001
16	Mettler Standard 4	110.0000	5.0850	5.0391	0.0062	0.0000
5	Mettler Standard 5	130.0000	2.6950	2.6708	-0.0057	0.0000
11	Mettler Standard 5	130.0000	2.6950	2.6857	0.0093	0.0001
17	Mettler Standard 5	130.0000	2.6950	2.6872	0.0107	0.0001

Intercept Statistics

Test: Check intercept Y value as % of Nominal Y values

Average Y value at 100% Level	4.2590	
Y-Intercept	0.0193	
Max Intercept % (spec.)	2	1 DP
Intercept as abs. % of 100% Y	0.5	Pass

Test: Check if origin is within 95% confidence limits

Lower Confidence (Y)	-0.0150	
Upper Confidence (Y)	0.0536	
Origin within CL?	Yes	Pass

Linearity Range Tested

From 70% to 130%

Fig. 2 Linearity report for Saccharin

tration data – a process that takes only 5 minutes. Figure 2 shows the automatically generated report for the analyte "Saccharin".

By combining both systems an outstanding result for the linearity experiment could be achieved (see Tab. 2). The R2 value for all analytes is greater than 0.999.

In addition, the automation of the linearity workflow through the combined use of Quantos QB1-L from Mettler-Toledo together with the UltiMate 3000 system and Chromeleon software provides vast potential in realising significant productivity advantages (see Tab. 3).

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Tab. 3 Time required to perform a linearity experiment using the traditional process and the new automated process

Step	Time Taken (Old Process)	Time Taken (New Process)
Sample Prep	180 minutes	50 minutes
Analysis	450 minutes	75 minutes
Results	120 minutes	5 minutes
Total	**750 minutes**	**130 minutes**