Application Note

Ultrafast Workflow
for ICH Linearity Studies

An Ultrafast Workflow for ICH Linearity Studies using Automated Standard Preparation and UHPLC

Introduction

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 5 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 randomizing potential sources of error (e.g. a possible incorrect weighing for 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 5 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.

Methods

Some chromatography methods have more than 1 analyte, and each one 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.

Quantos powder and liquid dosing system

A better way is to automate the preparation of the standards. This can be achieved using the Quantos system from METTLER TOLEDO. This can 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 factor and that it is possible to use the statistically correct approach of preparing each standard concentration individually.

In this experiment we tested the linearity of 5 analytes in a soft drinks analysis. The analytes studied were Acesulfame K, Saccharin, Caffeine, Vanillin, and Benzoate. 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 table 1.

This analysis was performed on the Dionex UltiMate® 3000 Basic Automated System – an entry level system that is fully UHPLC compatible. It 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

METTLER TOLEDO

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.

Laboratories also need to check 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 to try and 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 particular analysis, it is estimated that the use of spreadsheets, and the associated review step, would take 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 enter in their concentrations – a process that takes only 5 minutes. Figure 2 shows the automatically generated report for the analyte "Saccharin".

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

Table 1: Concentrations of soft drinks analytes prepared by the Quantos QB1-L

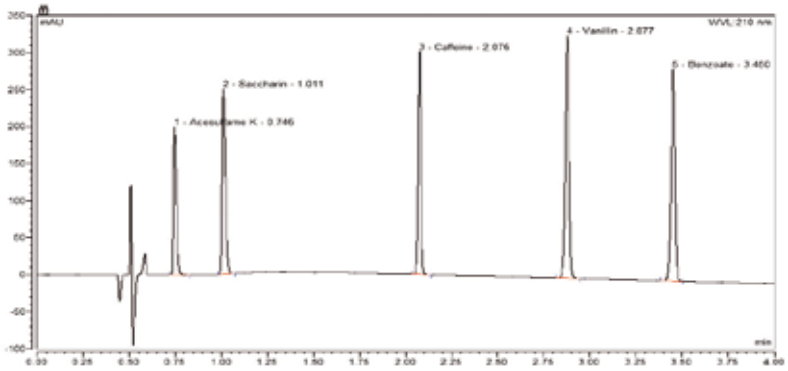

Figure 1: Analysis of 5 soft drinks analytes in less than 5 minutes

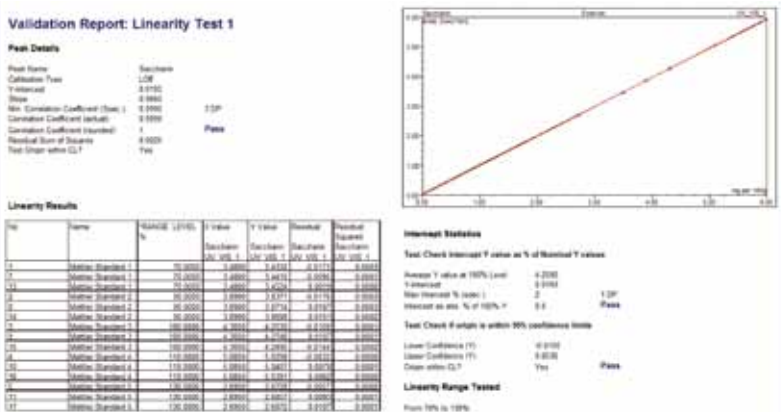

Figure 2: Linearity report for Saccharin

UltiMate 3000-RSLC-System

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

Table 2: Correlation coefficients of all analytes

Results

The combination of the Quantos and the Dionex UltiMate 3000 system produced outstanding results for the linearity experiment, as can be seen in table 2. It shows the correlation coefficient for all analytes – each of which as an R^2 value greater than 0.999.

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**

Table 3: Time taken to perform a linearity experiment using the traditional process and the new automated process

In addition, the automation of the linearity workflow through the combination of the Quantos dosing system from METTLER TOLEDO, and the UltiMate 3000 system and Chromeleon software from Dionex allows laboratories to realize significant productivity benefits – as shown in the table above.

Conclusions

As we can see from table 3, the time taken to perform the experiment using the traditional process is 12.5 hours, whereas this can be performed in only 130 minutes by following the automated process that is made possible through a combination of instruments and software tools from METTLER TOLEDO and Dionex.

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