Dwell Time, a Critical Factor on Precision in N-in-One Assays Utilizing UPLC-MS/MS

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Introduction

UPLC typically results narrow peaks of less than 0.1 minute in width. As an accurate integration of a chromatography peak requires a minimum of 10 to 15 data points throughout the peak, a short dwell time becomes unavoidable in MS/MS analysis. The situation is more acute when multiple analytes are analyzed simultaneously (n-in-one assays). Here we describe an example of a five-in-one assay we developed. Due to the small peak width of the analytes (i.e., ~ 0.06 minute), the maximum dwell time allowed for each analyte/internal standard (IS) trace was only 30 milliseconds (ms). The impact of short dwell time on assay performance was evaluated.

Method

A UPLC-MS/MS system consisting of a Nexera LC-30AD solvent delivery system (Shimadzu) and an API-5000 mass spectrometer (AB Sciex) was utilized. An extracted LLOQ sample was repeatedly injected by utilizing acquisition methods with different dwell time/number of MRM traces. Repeat injection precision (n=9) was analyzed for each analyte with each method. Then four different acquisition methods were built for the analysis of all five analytes. The precision of the four acquisition methods were analyzed by repeat injection (n=9) of an extracted LQC sample (3×LLOQ) with each of the methods. The precision of the repeat injections was presented in CV%.

Preliminary Data

Large CV% values (8.6-14.8%) were observed when repeatedly injecting the LLOQ sample with a method containing all the ten MRM traces (five for the analytes and five for the ISs), when only 30 ms dwell time was used for each trace. Repeat injection of the same sample with methods containing only one pair of MRM trace (one for the analyte and the other for its IS), with much longer dwell times of 100 ms/trace yielded systematic lower CV% values of 2.4-4.5%. Based on the above results, three different acquisition methods were edited for the analysis of all five analytes. Method 1 had all ten traces together with 30 ms/trace. Repeat injection of the LQC sample with this method resulted high CV% values ranged 5.8-7.7%. In order to extend the dwell time yet still ensure adequate data points, the analysis of the five analytes was split into two injections in method 2. With only two or three analytes analyzed in each injection, the dwell times were extended to 45 or 70 ms, and the CV% values were reduced to 2.7-6.7%. In addition to split injection, method 3 utilized “scheduled” MRM when analyzing three analytes in the same injection. As the dwell times were further extended to 60, 70, or 100 ms. Slight improvement on precision was observed with the CV% values ranged 2.0-4.7%. The split injection with scheduled MRM method was adopted for the five-in-one assay. Three continuous accuracy/precision runs were conducted during the method validation and all runs met the acceptance criteria per FDA guidelines.

Novel Aspect

The data demonstrated dwell time as a critical factor in UPLC-MS/MS assays involving multiple analytes.