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.

METHODS

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%.

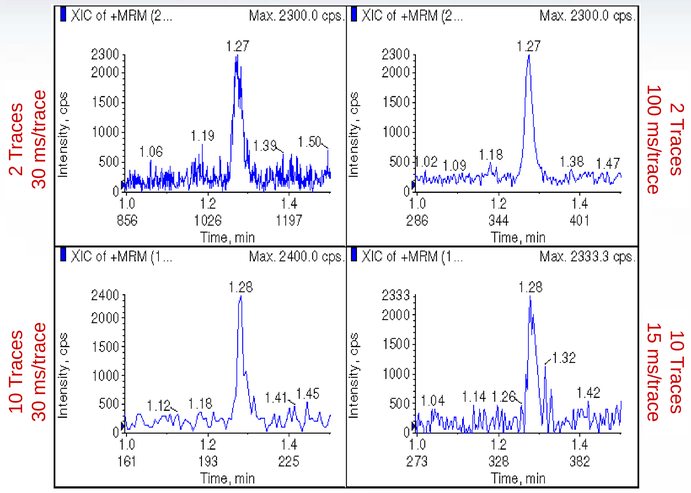
RESULTS

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 (n=9) with four MRM methods: Method 1 and 2 containing all the ten MRM traces (five for the analytes and five for the ISs), with only 15 or 30 ms dwell time was used for each trace; Method 3 and 4 containing only one pair of MRM trace (one for the analyte and the other for its IS), with dwell times of 30 or 100 ms/trace.

Method Precision Comparison of the Four MRM Methods

Scan Conditions	CV%				
	A1	A2	A3	A4	A5
10 Traces, 15 ms/trace Total cycle time ~200 ms	11.1%	5.0%	10.4%	10.0%	10.2%
10 Traces, 30 ms/trace Total cycle time ~350 ms	11.6%	8.8%	8.6%	9.7%	14.8%
2 Traces, 30 ms/trace Total cycle time ~70 ms	4.0%	5.5%	3.8%	6.5%	8.2%
2 Traces, 100 ms/trace Total cycle time ~210 ms	4.5%	4.5%	3.3%	2.4%	3.8%

Chromatogram Comparison of the Four MRM Methods



Method Accuracy Comparison of the Four MRM Methods

Scan Conditions	Average Peak Area Ratio				
	A1	A2	A3	A4	A5
10 Traces, 15 ms/trace Total cycle time ~200 ms	0.0317	0.0264	0.0343	0.0340	0.0394
10 Traces, 30 ms/trace Total cycle time ~350 ms	0.0320	0.0252	0.0334	0.0333	0.0409
2 Traces, 30 ms/trace Total cycle time ~70 ms	0.0341	0.0274	0.0369	0.0361	0.0482
2 Traces, 100 ms/trace Total cycle time ~210 ms	0.0326	0.0262	0.0327	0.0344	0.0415

An extracted LQC Sample was repeatedly injected (n=9) with four MRM methods: Method 1 and 2 containing all the ten MRM traces (five for the analytes and five for the ISs); Method 3 and 4 utilized split injection and thus only analyzing 2 or 3 analytes in each injection. Method 4 further utilized scheduled MRM, so the maximum number of MRM traces in each MRM period is not more than 4.

Method Precision Comparison of the Four MRM Methods

Scan Conditions	CV%				
	A1	A2	A3	A4	A5
10 Traces, 20 ms/trace, 15 ms pause time	6.0	5.4	6.3	8.0	9.2
10 Traces, 30 ms/trace, 5 ms pause time	7.7	5.8	6.1	6.4	7.4
Split Injection, 4 or 6 traces/period, 45 or 70 ms/trace	3.2	3.4	2.7	6.7	3.9
Split Injection w/ Scheduled MRM, 2 or 4 traces/period, 60, 70 or 100 ms/trace	3.8	2.0	4.7	2.1	3.1

CONCLUSIONS

Adequate dwell time is essential to achieve better precision as demonstrated in this work by adopting the split injection with scheduled MRM method for the five-in-one assay using UPLC and API-5000. Three continuous accuracy/precision runs were conducted during the method validation and all runs met the acceptance criteria .
Having higher scanning speed instrument may prevent the need for the split injection.

Accuracy & Precision	LLOQ QC Level				
	A1	A2	A3	A4	A5
Intraday %RE	-18.7 to -0.9	-8.9 to -2.0	-16.1 to 1.0	-14.6 to 9.0	-9.6 to 4.0
Intraday %CV	0.7 to 4.6	5.8 to 10.8	2.7 to 5.9	3.9 to 5.8	2.1 to 10.2
Interday %RE	-8.6	-5.4	-10.2	-6.0	-2.7
Interday %CV	10.1	9.1	10.3	12.6	8.6
Accuracy & Precision	Other QC Levels				
	A1	A2	A3	A4	A5
Intraday %RE	-11.6 to 8.0	-6.7 to 7.3	-10.3 to 4.7	-10.7 to 11.3	-8.5 to 9.0
Intraday %CV	0.7 to 4.6	0.8 to 4.3	1.0 to 5.4	0.7 to 4.9	0.9 to 4.6
Interday %RE	-4.9 to 1.7	0.0 to 1.0	-4.0 to -0.7	-5.6 to 4.0	-1.9 to 4.0
Interday %CV	5.5 to 7.1	5.1 to 6.6	4.1 to 7.8	4.2 to 7.4	5.0 to 6.8

NOVEL ASPECT

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