Poster Reprint

ASMS 2026
WP 221

Rapid LC-MS/MS compound method development and sample analysis for DMPK workflows

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Introduction

Bioanalysis – DMPK – studies

Drug metabolism and pharmacokinetics (DMPK) studies are essential to understanding the absorption, distribution, metabolism, and excretion (ADME) of drug candidates and play a critical role in drug discovery and development. The characterization of DMPK properties for modern therapeutics increasingly requires advanced analytical techniques due to long half-lives and low systemic concentrations. DMPK labs need a workflow that includes robust and easy to maintain instrumentation, easy to use and efficient software to enable the analysis of hundreds of compound studies a week. Rapid review of the results is necessary to deliver high quality results in a timely fashion. Training requirements of new lab personnel needs to be minimized on the operation of this workflow to keep lab operations running well.

Bioanalysis - Workflow

Bioanalysis are based on synthetic molecule testing *in vivo* and *in vitro* assays. Depending on the assay, more than 1000 of injections including ~ 700 compounds screening are performed on weekly basis. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) has become a cornerstone of DMPK research, offering high sensitivity, selectivity, and throughput for the quantitative analysis of diverse compound classes. A simplified workflow is showed below.

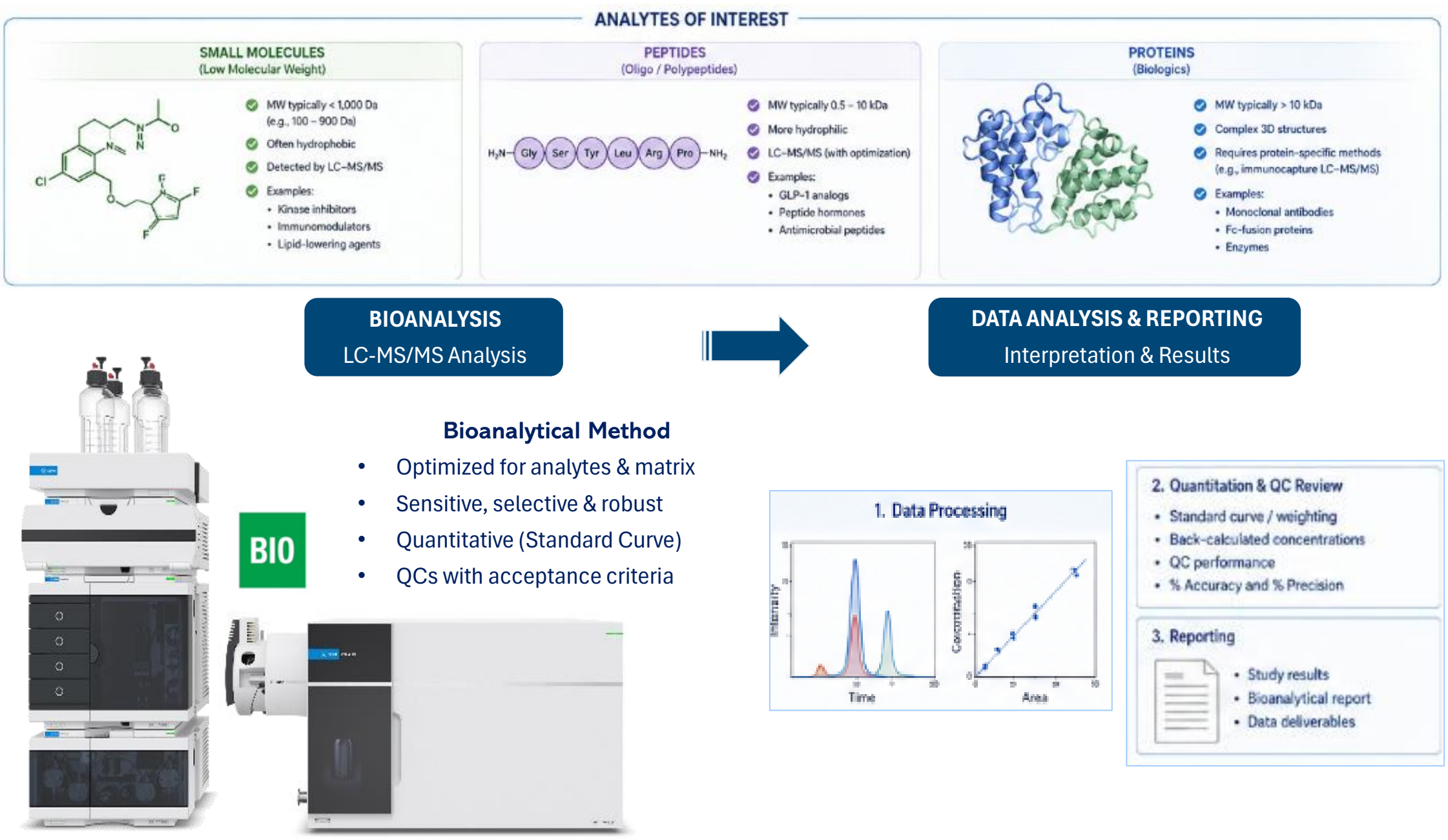


Figure 1. General workflow for Molecules in Bioanalysis studies.

The Agilent solution for DMPK/Bioanalysis analysis

Agilent 6495D triple quadrupole LC/MS systems, combined with the *Sound Analytics LeadScape* solution, are designed to streamline method development and support robust, high-quality bioanalytical assays across multiple modality types. These capabilities enable efficient evaluation of DMPK properties and facilitate the optimization of drug candidates during early discovery. *LeadScape for Agilent* software delivers the speed of developing compound specified methods, rapid sample analysis and review, and enterprise method management in an easy – user friendly interface. Additionally, the 6495D LC/TQ does not require any special settings to switch between peptide and small molecules methods.

Experimental

Methods

An Agilent Infinity II Bio UHPLC coupled to an Agilent 6495D triple quadrupole LC/MS (LC/TQ) running MassHunter and LeadScape software was used for the analysis of five compounds and one internal standard. Compound specific methods were rapidly developed using LeadScape. Samples were prepared using eight calibration levels in extracted commercial biological matrix samples along with several spiked with a blinded concentration.

Sound Analytics

LeadScape®

Advantages:

- ✓ **Rapid MRM optimization**
- ✓ **Rapid methods and worklists creation**
- ✓ **Easy data review**
- ✓ **Enterprise-wide compound database**

MRM Optimization

- Fast LC method template
- Compound name and formula/MW
- 2 min per compound
- Storage in local or global Database
- Only performed once

Text file with compound info

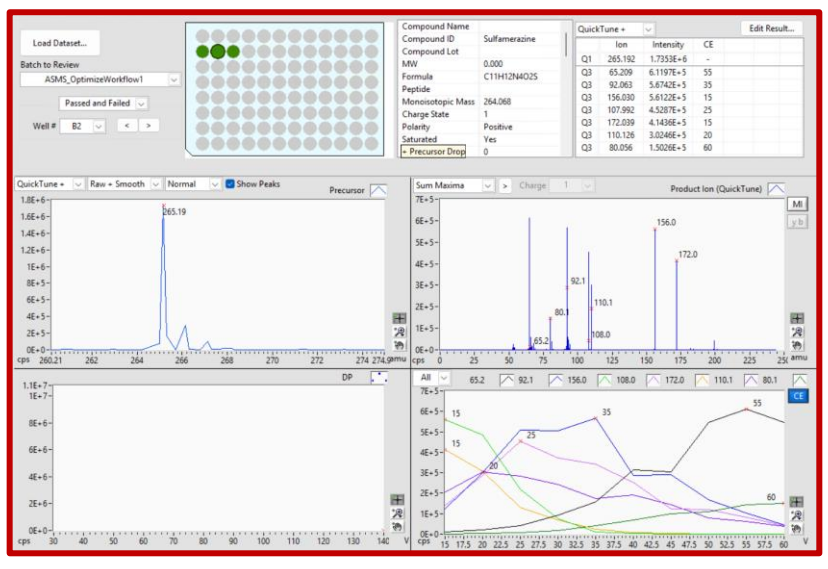
	A	B	C	D	E
1	Compound ID	Vial Position	Plate Code	Plate Location	Formula
2	Sulfadiazine	B1	*96Agilent*	P1	C10H10N4O2S
3	Sulfamerazine	B2	*96Agilent*	P1	C11H12N4O2S
4	Sulfamethazole	B3	*96Agilent*	P1	C10H11N3O3S

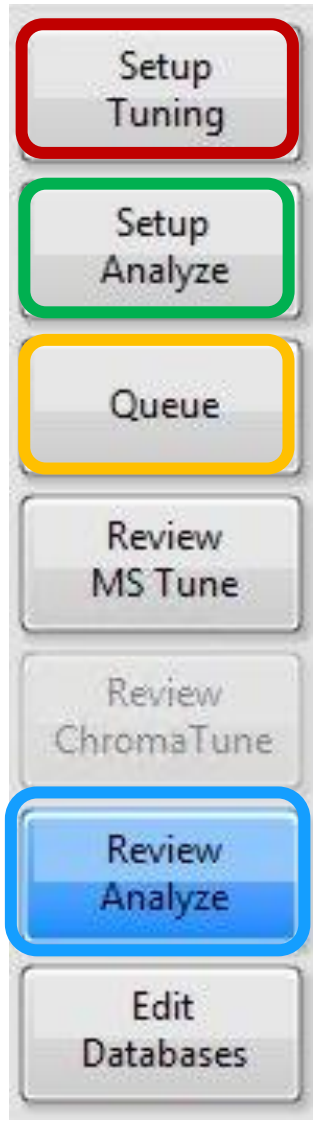
+

Fast LC method

Time [min]	A [%]	B [%]	Flow [mL/min]
0.00	30.00	70.00	0.800
0.03	30.00	70.00	0.800
0.04	30.00	70.00	0.100
0.32	30.00	70.00	0.100
0.33	30.00	70.00	0.800

Review MRM transitions





Fast Chromatography

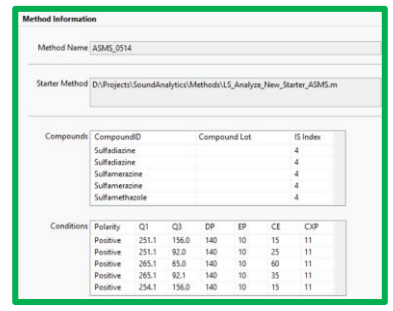
Time [min]	A [%]	B [%]	Flow [mL/min]
0.00	90.00	10.00	0.400
0.20	90.00	10.00	---
1.20	0.00	100.00	---
1.30	0.00	100.00	---
1.35	90.00	10.00	---

+

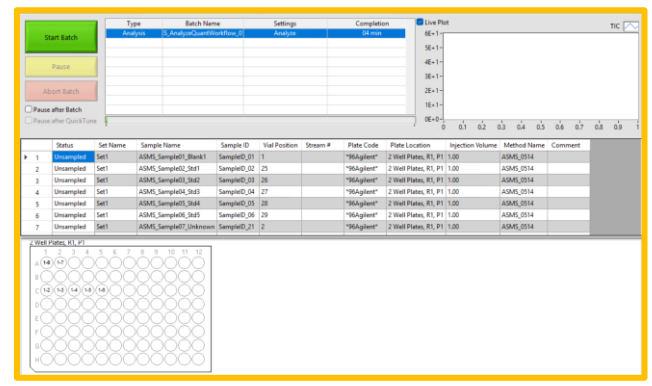
Text file with analysis info

	A	B	C	D	E	F	G	H	I	J	K
1	Sample Name	SampleID	Acquisition Method	Plate Code	Plate Location	Vial Position	Set Name	Data File Name	Quant Type	Compound ID	Concentra
2	ASMS_Sample01_Blank1	SampleID_01	ASMS_0514	*96Agilent*	P1	A1	Set1	Data_0514	Blank	Sulfadiazine	0
3	ASMS_Sample02_Std1	SampleID_02	ASMS_0514	*96Agilent*	P1	C1	Set1	Data_0514	Standard	Sulfadiazine	6.75
4	ASMS_Sample03_Std2	SampleID_03	ASMS_0514	*96Agilent*	P1	C2	Set1	Data_0514	Standard	Sulfadiazine	12.5
5	ASMS_Sample04_Std3	SampleID_04	ASMS_0514	*96Agilent*	P1	C3	Set1	Data_0514	Standard	Sulfadiazine	25
6	ASMS_Sample05_Std4	SampleID_05	ASMS_0514	*96Agilent*	P1	C4	Set1	Data_0514	Standard	Sulfadiazine	50
7	ASMS_Sample06_Std5	SampleID_06	ASMS_0514	*96Agilent*	P1	C5	Set1	Data_0514	Standard	Sulfadiazine	100
8	ASMS_Sample07_Unknown	SampleID_21	ASMS_0514	*96Agilent*	P1	A2	Set1	Data_0514	Unknown	Sulfadiazine	0
9	ASMS_Sample08_Blank2	SampleID_22	ASMS_0514	*96Agilent*	P1	A1	Set1	Data_0514	Blank	Sulfadiazine	0

Methods created



Automated worklists created



Automated Method Creation

- Uses MRMs from database to merge with LC-MS/MS method template
- Only need compound ID and positions
- Creates 100s of methods in < 1min from database.
- Creates automated worklists.

Data Review

- Thousands of runs reviewed for less than 2 hours
- Screening review or quantitation analysis available.

Review results

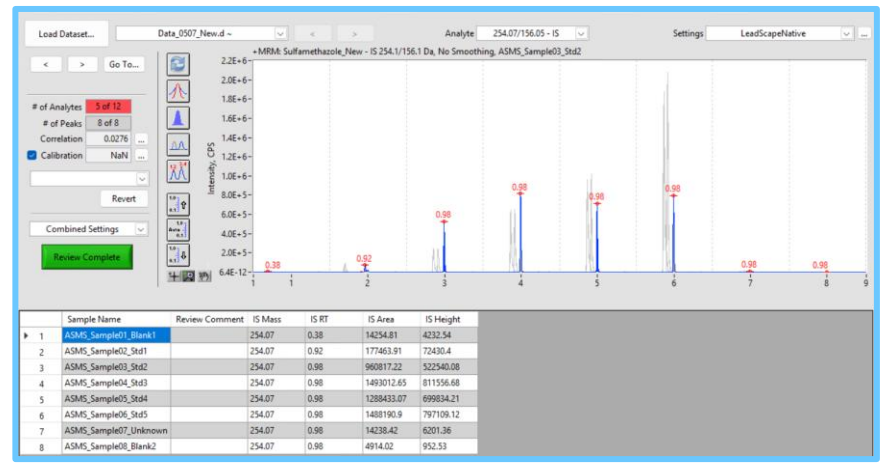


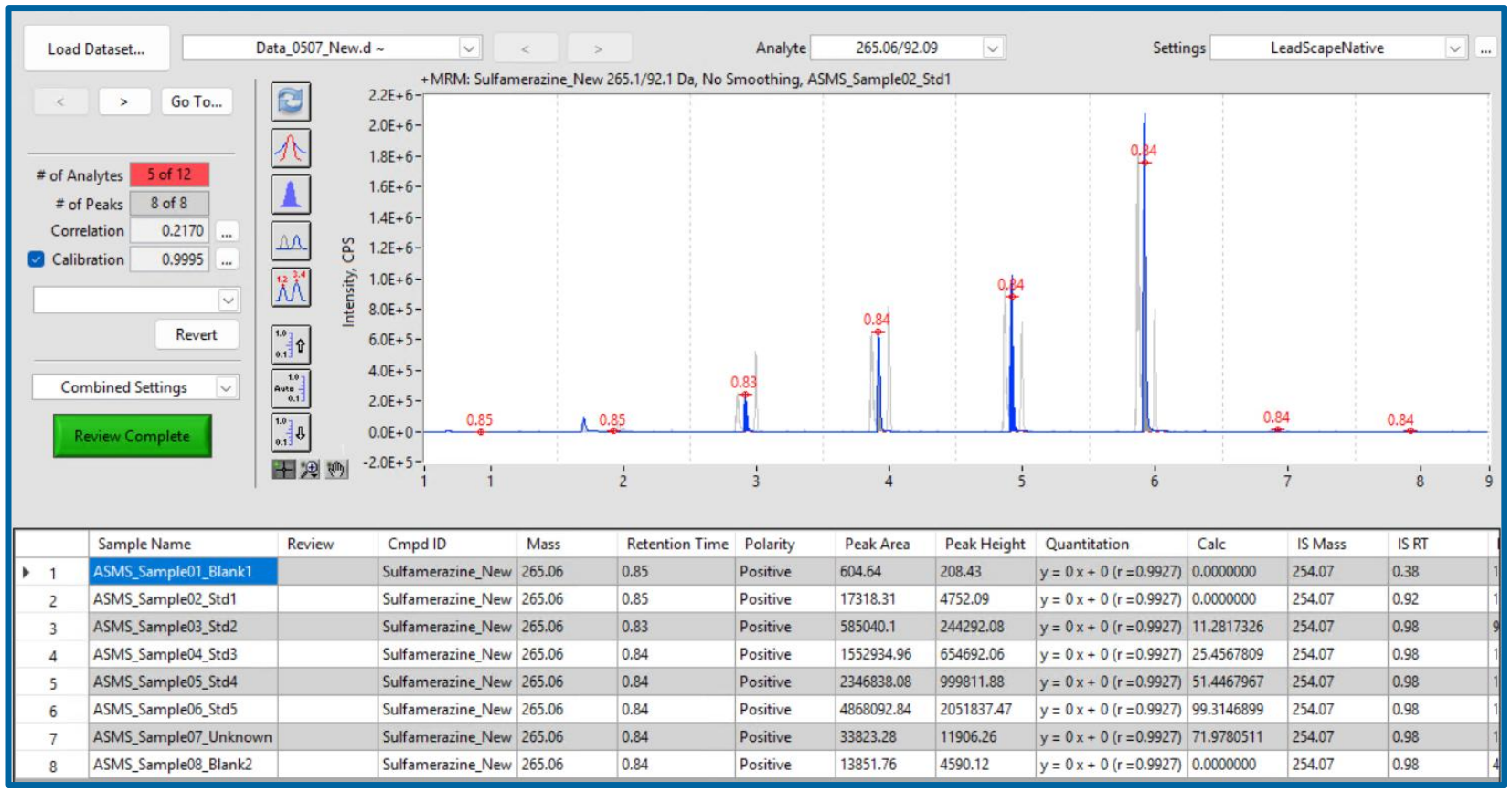
Figure 2. LeadScape for Agilent workflow.

Results and Discussion

From Methods creation to Review data – Few steps: multiple runs

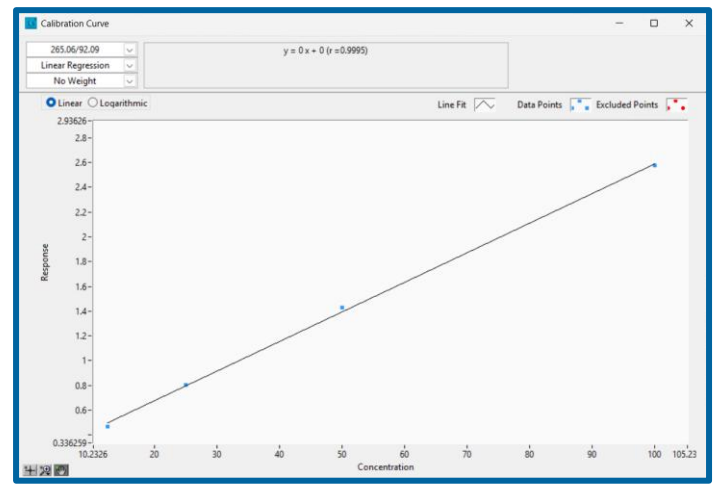
The combination of automated method generation and the high sensitivity of the 6495D LC/TQ instrument resulted in accurate and precise quantitative data at low concentration levels commonly encountered in DMPK studies. Sample acquisition and processing were completed quickly, demonstrating the efficiency of the software-driven workflow. Overall, these results highlight the advantages of combining advanced mass spectrometry instrumentation with intelligent software solutions to support fast, reliable, and high-quality data generation for DMPK applications.

Entire batch display.



	Sample Name	Review	Cmpd ID	Mass	Retention Time	Polarity	Peak Area	Peak Height	Quantitation	Calc	IS Mass	IS RT
1	ASMS_Sample01_Blank1		Sulfamerazine_New	265.06	0.85	Positive	604.64	208.43	y = 0 x + 0 (r =0.9927)	0.0000000	254.07	0.38
2	ASMS_Sample02_Std1		Sulfamerazine_New	265.06	0.85	Positive	17318.31	4752.09	y = 0 x + 0 (r =0.9927)	0.0000000	254.07	0.92
3	ASMS_Sample03_Std2		Sulfamerazine_New	265.06	0.83	Positive	585040.1	244292.08	y = 0 x + 0 (r =0.9927)	11.2817326	254.07	0.98
4	ASMS_Sample04_Std3		Sulfamerazine_New	265.06	0.84	Positive	1552934.96	654692.06	y = 0 x + 0 (r =0.9927)	25.4567809	254.07	0.98
5	ASMS_Sample05_Std4		Sulfamerazine_New	265.06	0.84	Positive	2346838.08	999811.88	y = 0 x + 0 (r =0.9927)	51.4467967	254.07	0.98
6	ASMS_Sample06_Std5		Sulfamerazine_New	265.06	0.84	Positive	4868092.84	2051837.47	y = 0 x + 0 (r =0.9927)	99.3146899	254.07	0.98
7	ASMS_Sample07_Unknown		Sulfamerazine_New	265.06	0.84	Positive	33823.28	11906.26	y = 0 x + 0 (r =0.9927)	71.9780511	254.07	0.98
8	ASMS_Sample08_Blank2		Sulfamerazine_New	265.06	0.84	Positive	13851.76	4590.12	y = 0 x + 0 (r =0.9927)	0.0000000	254.07	0.98

Calibration Curves.



Individual data file review.

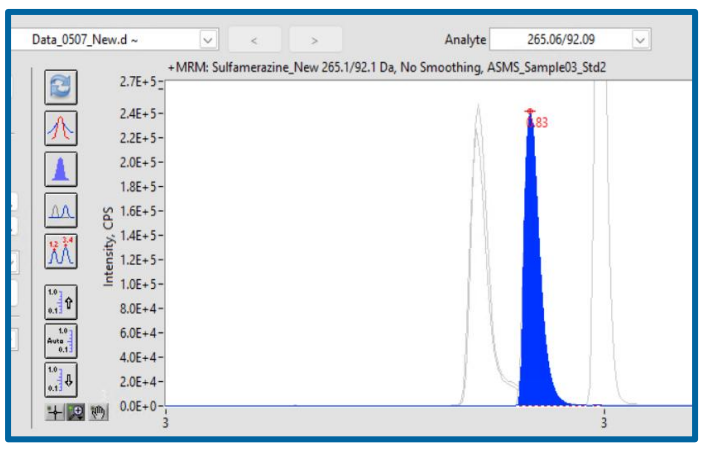


Figure 3. Easy to review individual chromatograms vs entire batch and calibration curves.

Conclusions

- **User-friendly workflow:** A fast, reliable, and efficient bioanalysis workflow generated high-quality data using LeadScape for Agilent.
- **Excellent performance:** The 6495D LC/TQ demonstrated the sensitivity and the speed necessary for DMPK laboratories.
- **High Throughput:** The integration of LeadScape software with the Agilent LC/MS 6495D LC/TQ system enabled rapid optimization and acquisition of MRM transitions, significantly reducing method development time. Compound specific acquisition methods were generated quickly and applied to the study set efficiently, allowing multiple compounds to be analyzed within a single system setup. This approach eliminated time-consuming manual steps typically associated with method setup and sample organization.

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DE-014949

Published in USA, June 30, ,2026

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