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Automated Data-Driven Optimization of MRM Transitions and Collision Energies in LC–MS/MS

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Introduction

Targeted LC–MS/MS methods rely on carefully optimized multiple reaction monitoring (MRM) parameters to achieve high sensitivity, selectivity, and quantitative robustness. Conventional MRM development requires prior knowledge of precursor ions, multiple injections, and iterative manual tuning of collision energies and other parameters, making method development time-consuming, operator-dependent, and poorly scalable. These limitations are particularly restrictive for high-throughput and complex workflows.

To overcome above-mentioned limitations, we present an automated, data-driven MRM optimization strategy that eliminates the need for prior precursor knowledge and minimizes experimental injections. By combining ultra-fast survey scans, retention-time scheduling, and adaptive optimization algorithms, the approach rapidly generates high-confidence MRM tables suitable for targeted quantitative analysis.

Experimental

Sample Preparation and Instrumentation

Agilent Sulfa drug standard mixture (P/N: 5190-0580) was used for benchmarking of the data-driven MRM optimization.

Data was collected using an Agilent 1290 Infinity LC system coupled to a 6495D triple quadrupole LC/MS (Figure 1).

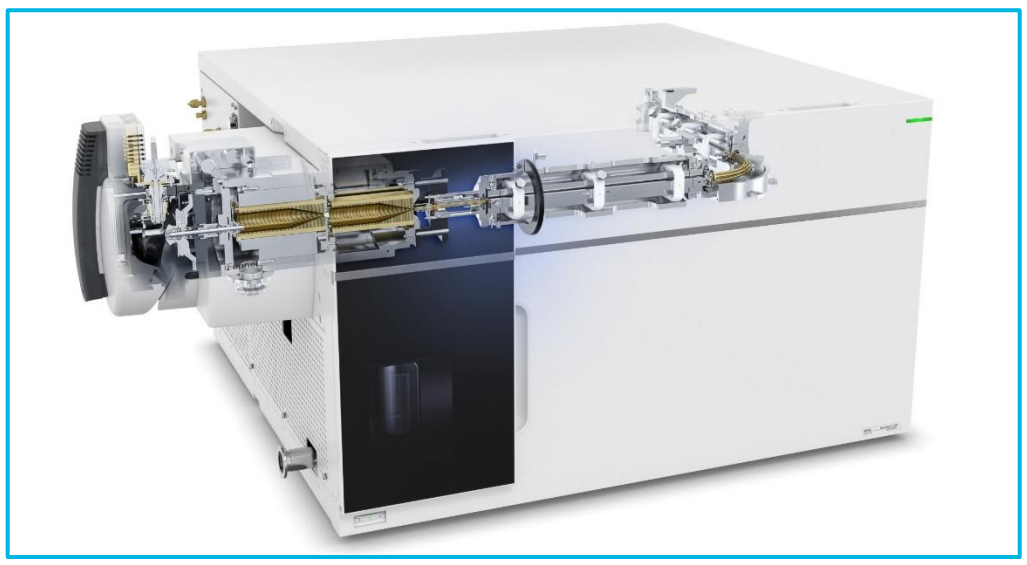


Figure 1. Agilent 6495D triple quadrupole LC/MS equipped with intelligent on-board computer.

Method

All the procedures were implemented in Python 3.1 and in Tune-Calibration-Diagnosis (TCD) environment.

Experimental

Conventional MRM Optimizer

Traditionally, generation and optimization of MRM transitions during analytical method development are performed by selecting a limited number of known precursor ions, acquiring product ion scans, and then iteratively evaluating various MRM-related parameters such as collision energy (Figure 2).

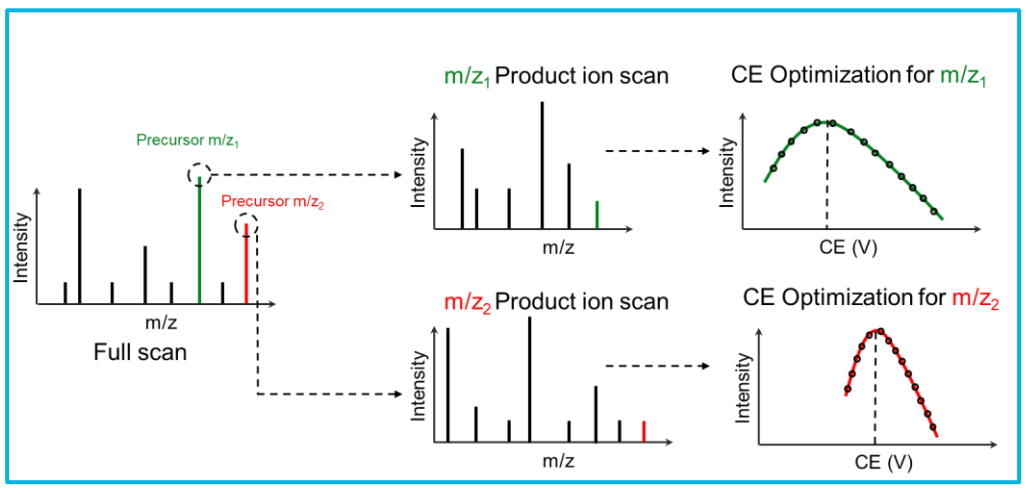


Figure 2. Traditional MRM optimization workflow

Data-Driven MRM Optimizer

Data-driven MRM optimization workflow combines ultra-fast survey scans, retention-time scheduling, and adaptive algorithms to rapidly generate high-confidence MRM transitions with minimal injections and no prior precursor knowledge (Figure 3, and Flow Chart 1).

This workflow represents a fully automated, data-driven strategy for rapid MRM method development in LC-MS/MS, designed to reduce method development time, minimize sample consumption, and eliminate dependence on prior compound knowledge. Algorithms iteratively and adaptively update acquisition parameters to maximize analytical performance while minimizing unnecessary scans or injections.

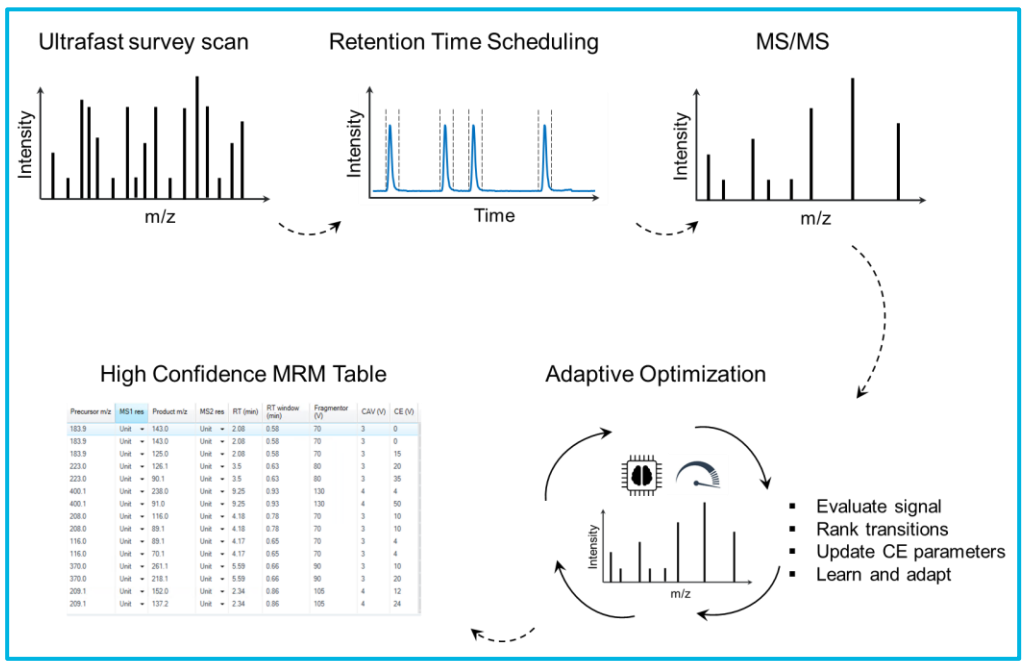


Figure 3. Data-driven MRM optimization workflow.

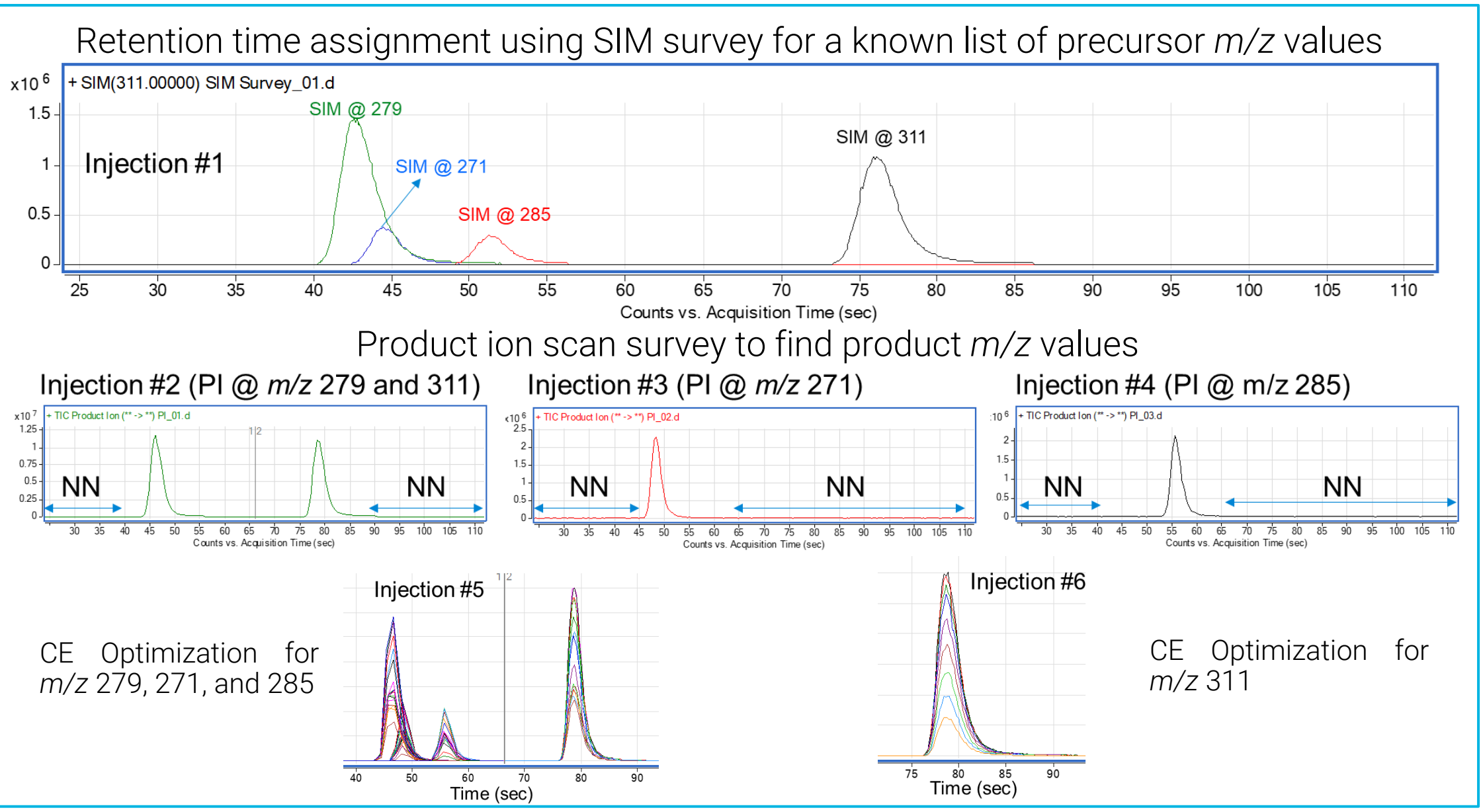


Figure 4. LC/MS/MS chromatograms for Agilent Sulfa drug mixture. Injection numbers correspond to actual sample injections required for optimization using traditional optimization approach. NN: Not Needed.

MRM Transition Optimization Using Traditional Approach

Figure 4 shows the LC/MS/MS chromatograms for a sulfa drug mixture. As shown in Figure 4, a total of four sample injections and LC/MS runs are required to identify the analyte retention time and desired fragment ions for MRM selection and subsequent CE optimization. This is followed by additional 2 injections for CE optimization.

Parameter Optimization Using Reference Normalization

The concept of reference normalization can be better understood by pictorial representation in Figure 4. Figure 4 illustrates the principle of reference-based deconvolution utilized during MRM optimization to isolate fragmentation behavior from chromatographic and ionization effects.

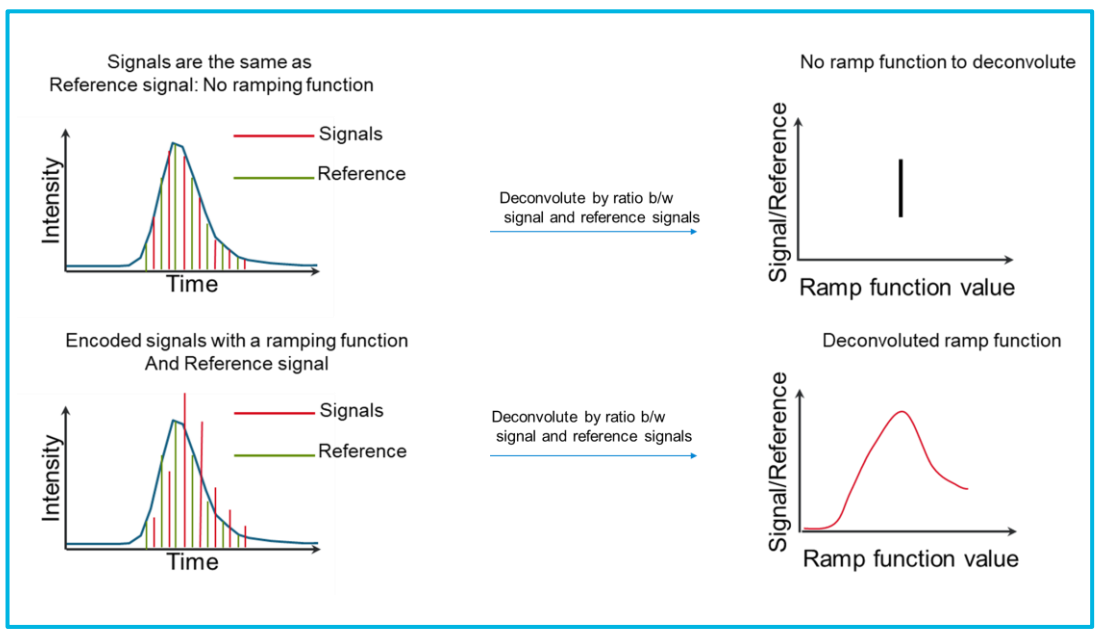


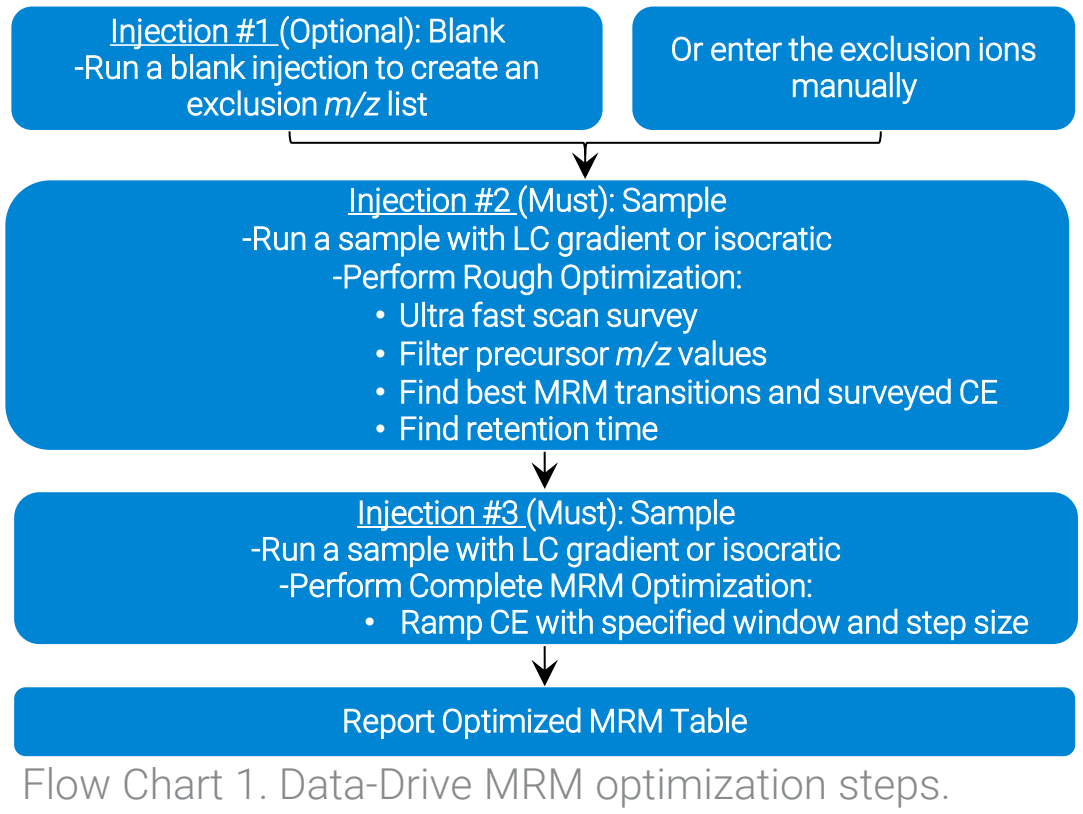
Figure 5. Concept of parameter optimization using reference normalization.

When no ramp function is used, e.g., no CE ramping, the result of the reference normalization is a flat line, and the chromatogram can be reconstructed from the intensity of reference ion as a function of time. However, once a ramp parameter is used during chromatographic separation, the outcome is a CE optimization curve. The reference ion normalization is performed by dividing, at each acquisition time point, a measured product-ion abundance by a corresponding precursor-ion abundance acquired at, approximately, the same retention time. By evaluating relative product-ion intensities instead of absolute intensities, one is able to objectively compare fragment-ion responses from different precursor ions and from different acquisition times during which the product ions are detected. Plotting reference ion intensity versus time constructs the chromatogram.

Results and Discussion

Table 1. MRM optimization results using MH 12.2 optimizer.

Prec Ion (m/z)	MS1 Res	Frag (V)	Prod Ion (m/z)	MS2 Res	CE (V)
271.0	Unit	135	156.0	Unit	12
271.0	Unit	135	108.0	Unit	26
279.0	Unit	135	186.0	Unit	16
279.0	Unit	135	124.1	Unit	24
285.0	Unit	135	156.0	Unit	14
285.0	Unit	135	108.0	Unit	28
311.0	Unit	135	156.0	Unit	22
311.0	Unit	135	108.0	Unit	32



Flow Chart 1. Data-Drive MRM optimization steps.

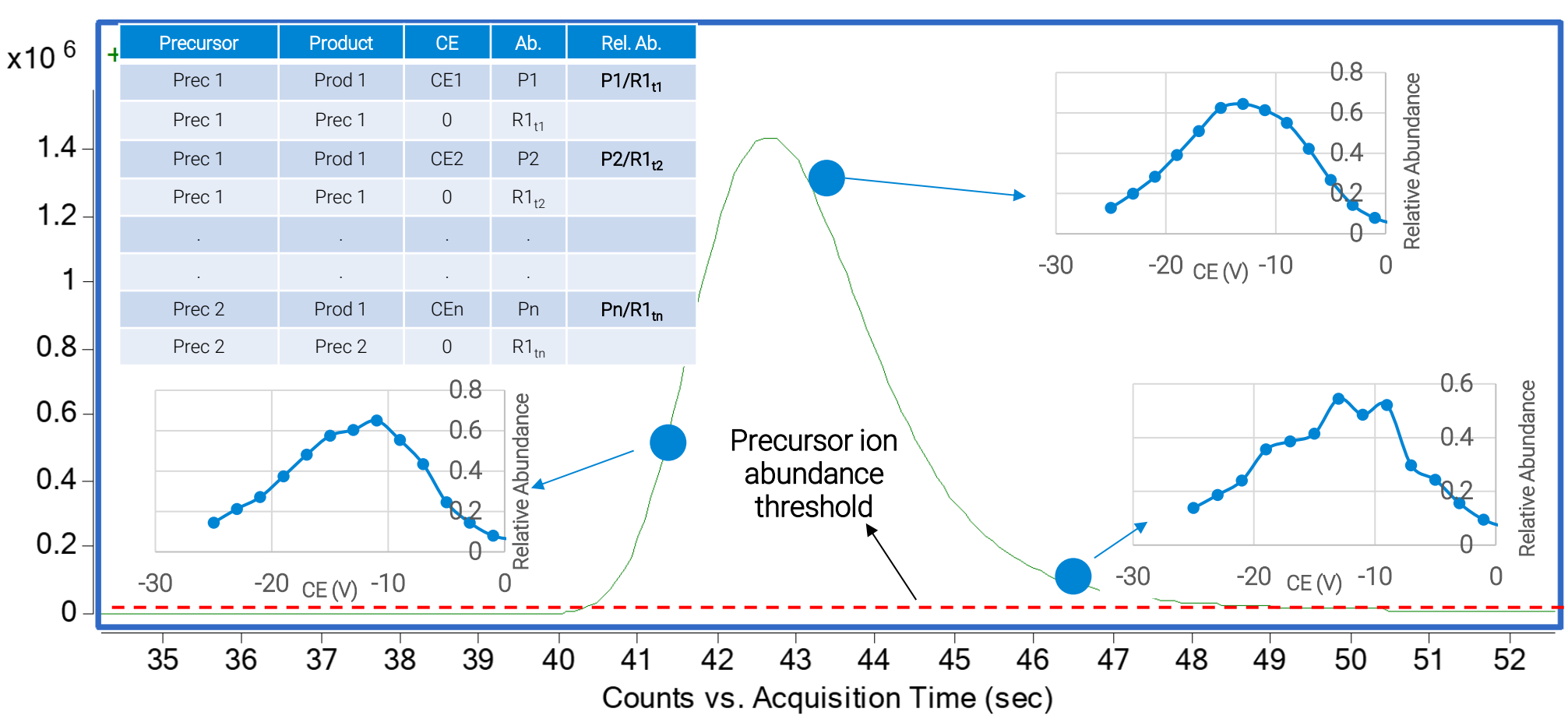


Figure 6. Example of reference normalization for CE optimization using an LC run.

Table 2. MRM optimization results.

Precursor m/z	Product m/z	Optimum CE (V)	Relative Height	Reference Height
247	172.9	-15	0.68	11844.46
247	202.8	-7	0.3	8536.02
279.11	108.2	-31	0.19	136378.81
279.11	186.1	-17	0.34	288478.84
271.11	156.1	-13	0.62	15708.74
271.11	108.2	-25	0.32	36071.77
285.11	156.1	-15	0.69	33717.41
285.11	108.1	-29	0.36	35337.62
311.11	156.1	-21	0.45	66924.29
311.11	108.1	-31	0.19	188852.47

Conclusions

- Combined use of multiplexing and reference normalization allows efficient and shorter optimization time for analytes in a sample mixture. This significantly improves the throughput of optimization workflows.
- Such method optimization has advantage over traditional approaches and reduces time and labor-intensive workflow and does not require prior knowledge of the sample content.

References

US Patent Application No: 19/441,718, 01/06/2026.

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