Technical Overview

Instrument Detection Limit at Ultrashort Dwell Times Demonstrated on the Agilent 6495C Triple Quadrupole LC/MS

Authors

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Abstract

This Technical Overview presents the measurement of the instrument detection limit (IDL) at two different conditions of MRM dwell times: 1) with sufficient ion sampling time, and 2) with minimum allowed ion sampling time. The workflow used an Agilent 1290 Infinity LC and an Agilent 6495C triple quadrupole LC/MS. Results show that even with short MRM dwell times, low IDLs were still confidently achievable with this experimental setup.

Introduction

To demonstrate suitability for robustness and reliability in routine applications, instead of "one-shot" metrics such as signal-to-noise (S/N), instrument sensitivity is primarily characterized using the IDL.[1] This specification is more rigorous and is determined using the ion statistics of replicate injections.[2] IDL is based on the method detection limit (MDL), thoroughly defined by the US EPA and other governing bodies.[3] "The MDL is the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results." A key difference between the IDL and MDL is that the IDL is separate from the "analysis method" of an application but still determines the absolute lowest level of analyte the instrument can detect without the interference of sample matrix.

The IDL is the minimum level of analyte that results in a statistically differentiable signal from the instrument's overall noise baseline. Instead of using a single injection at high concentration (such as is the case with a S/N measurement), the IDL is determined using replicate measurements at low levels, typically with a %RSD of 10 to 20%. The RSD of response output (chromatographic peak area) is used in the IDL calculation.

A confidence limit is predefined, typically at 99% confidence, for which a t-statistic is applied as a multiplication factor to determine the IDL on-column amount. This confidence arises from the statistical variation of a series of injections around the instrument's ability to reliably produce replicable data. The result of the IDL measurement is a statistically meaningful instrument performance specification, rather than the extrapolation of a single measurement at a high injection amount.

The IDL is calculated using the equation:

IDL = (t-statistic) × (amount on column) × (%RSD/100)

The calculated IDL states the minimum amount injected on column that can be unambiguously distinguished from the baseline noise of the chromatograph with 99% confidence, not attributable to random spikes in noise, or the minimum point at which the instrument can reliably replicate data.

Effects of ion sampling rate on signal variability: Why would the IDL change with various dwell times?

An important parameter in the discussion of instrument sensitivity is the "MRM dwell time", which influences the stochastic sampling of the ion beam. Generally, the IDL is characterized by a considerable amount of time dedicated to sampling the ion beam, allowing a stable and consistent flux of ions to hit the detector. At lowered instrument dwell times (typically <5 ms), ion beam sampling becomes less precise due to insufficient number of of ions involved in the measurement.

Number of ions ∝ ion flux × dwell time

Deficiencies in inadequate sampling time (that is, reduced MRM dwell times) manifest themselves as decreased signal stability (increased %RSD), even if the analyte is introduced at a constant rate and high concentration. Figure 1 presents this observation, where the %RSD of the ion signal increases at decreasing dwell times.

This Application Note characterizes IDL performance when the instrument is operated at extremely short dwell times (1 and 0.5 ms).

The experiments carried out for this paper were acquired on and apply to the 6495C LC/TQ. Although the performance metrics may vary between instrument type and model, the overall concept may be applied to any other LC/MS or GC/MS mass spectrometer.

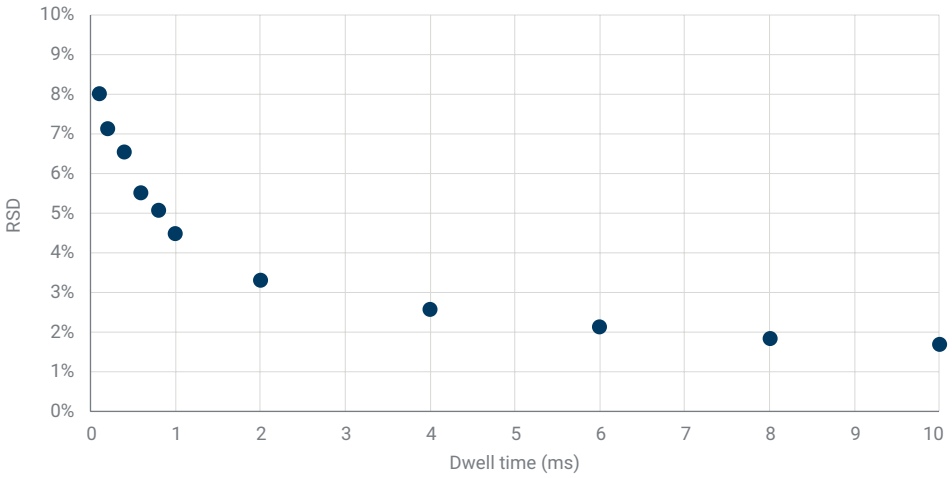


Figure 1. ESI-L tune mix infusion acquired at various MRM dwell times. Signal was acquired in MRM mode using the transition 622.0 → 622.0 with CE = 0 V.

Experimental

Instrumentation and reagents

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity autosampler (G4226A)
- Agilent 1200 Series autosampler thermostat (G1330B)
- Agilent ZORBAX Eclipse Plus RRHD C18, 2.5 × 50 mm, 1.8 µm (p/n 959757-902)
- Agilent 6495C triple quadrupole LC/MS (G6495C)
- ESI/APCI positive ion performance standard (G1946-85004)

Method parameters

Table 1. LC and MS parameters.

LC Parameters	
Flow Rate	0.4 mL/min
Solvent A	H_2O w/ 0.1% FA
Solvent B	ACN w/ 0.1% FA
Gradient	Time (minutes) %B 0.00 10 0.20 10 1.50 100 2.50 100 2.51 10
Stop Time	3 minutes
Post Time	0.30 minutes
MS Parameters	
AJS parameters	
Sheath Gas Temperature	400 ˚C
Sheath Gas Flow	11 L/min
Drying Gas Temperature	325 ˚C
Drying Gas Flow	11 L/min
Capillary Voltage	4,000 V
Nozzle Voltage	0 V
iFunnel parameters	
High-Pressure RF	200 Vpp
Low-Pressure RF	110 Vpp
MRM Transition	609.3 → 195.1
Dummy Transition	610.3 → 196.1
Fragmentor	166 V
Collision Energy	42 V
Duty Cycle	201 ms
Dwell Time	200 , 1, or 0.5 ms

MRM dwell times for IDL measurements

Measurements using the primary MRM transition of reserpine (*m/z* 609.3 → 195.1) were acquired at 200, 1, and 0.5 ms dwell time. However, to ensure that the same number of chromatographic peak data points were collected, a dummy transition of *m/z* 610.3 → 196.1 was included so that the overall duty cycle of the instrument was 201 ms (shown in Table 2).

Table 2. Primary and dummy MRM dwell times used equating to the same instrument duty cycle for all experiments.

MRM Dwell 609.3 → 195.1	Dummy MRM Dwell 610.3 → 196.1	Total Cycle Time
200.0 ms	1.0 ms	201.0 ms
1.0 ms	200.0 ms	201.0 ms
0.5 ms	200.5 ms	201.0 ms

Results and discussion

Demonstration of IDL using sufficient ion sampling

To demonstrate IDL characterizations using the ideal ion sampling scenario, an acquisition using an MRM dwell time of 200 ms was used.

In this case, 1 fg reserpine was injected on-column and repeated 10 times in MRM mode of acquisition. The %RSD of the chromatographic peak area was determined to be 17.62%. For n = 10 injections, the single-tailed t-statistic at 99% confidence was found to be 2.821. Putting these parameters into the equation, the IDL at 200 ms dwell time = 0.49 fg.

Given that the ion beam has been sufficiently sampled for 200 ms, uncertainty in the measurement arises due to the physical absence of ions, producing a chromatographic peak in close proximity to baseline noise. Inspecting the overlaid chromatograms in Figure 2, it may appear that injecting anything less than 0.49 fg on-column would not provide enough ion current.

With injections less than the IDL, signal produced will become statistically confounded with the chromatographic baseline, and it cannot be determined with ≥99% confidence that the signal is distinct from the noise.

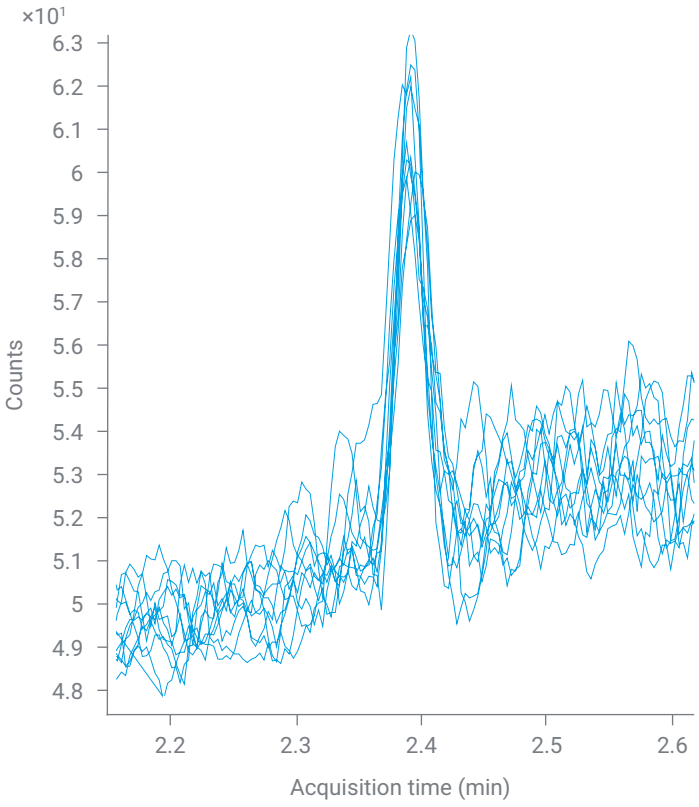
Demonstration of IDL at extremely short dwell times

Referring to Figures 3A and 4A, sufficient ion current is produced, demonstrating that the instrument can detect these on-column amounts with adequate "visual" S/N. However, due to insufficient ion sampling time, uncertainty in the measurement arises from the stochastic variation of number of ions hitting the detector, thus affecting chromatographic peak area variability.

In line with guidance for characterizing IDL, the injected on-column amount of reserpine was targeted to produce a %RSD between 10 and 20%. For 1 ms dwell time, 100 fg reserpine was used, resulting in $IDL_{1\ ms}$ = 52.4 fg, while for 0.5 ms dwell time, 250 fg reserpine was used, resulting in $IDL_{0.5\ ms}$ = 119.5 fg.

Both cases produced a chromatographic peak far above a reasonable S/N threshold, however, the variation in chromatographic peak area fell between ~40 to 50%, which is deemed unacceptable for confident quantitation (Figures 3B and 4B).

When examining additional measurements at short dwell times far below the characterized IDL (10 fg at 1 ms; 50 fg at 0.5 ms), some injection replicates produced sufficient chromatographic S/N (Figures 3C and 4C). However, when replicated over a series of injections, the chromatographic peak variability was obviously unacceptable at %RSD≈146.07% and %RSD≈99.35%, rendering the data unsuitable for reliable quantitative analysis.



Injection No.	Area
1	22
2	20
3	28
4	21
5	25
6	24
7	20
8	14
9	23
10	19
Average	21.6
St. dev.	3.8
%RSD	17.62%

IDL = (2.821) × (1 fg) × (0.1762)

IDL = 0.49 fg on-column

Figure 2. Replicate (n = 10) injections of 1 fg reserpine on-column using a 200 ms MRM dwell time.

1 ms MRM dwell time

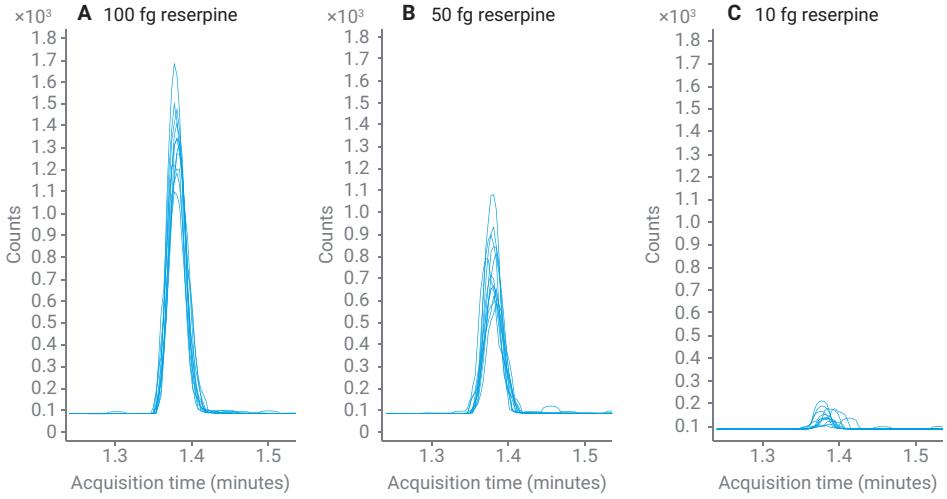


Figure 3. Replicate (n = 10) injections of various amounts of reserpine on-column using 1 ms MRM dwell times.

Table 3. Replicate (n = 10) injections of 100, 50, and 10 fg reserpine on-column using 1 ms MRM dwell times.

Injection No.	Area (100 fg)	Area (50 fg)	Area (10 fg)
1	1,791	935	13
2	1,650	448	169
3	1,211	516	202
4	1,902	609	18
5	1,387	189	1
6	1,727	626	11
7	1,159	917	111
8	1,657	836	6
9	2,130	531	0
10	1,747	430	0
Average	1,636.1	603.7	53.1
St. dev.	303.7	236.1	77.6
%RSD	18.56%	39.11%	146.07%

IDL = (2.821) × (100 fg) × (18.56%/100) = 52.4 fg on-column

0.5 ms dwell time

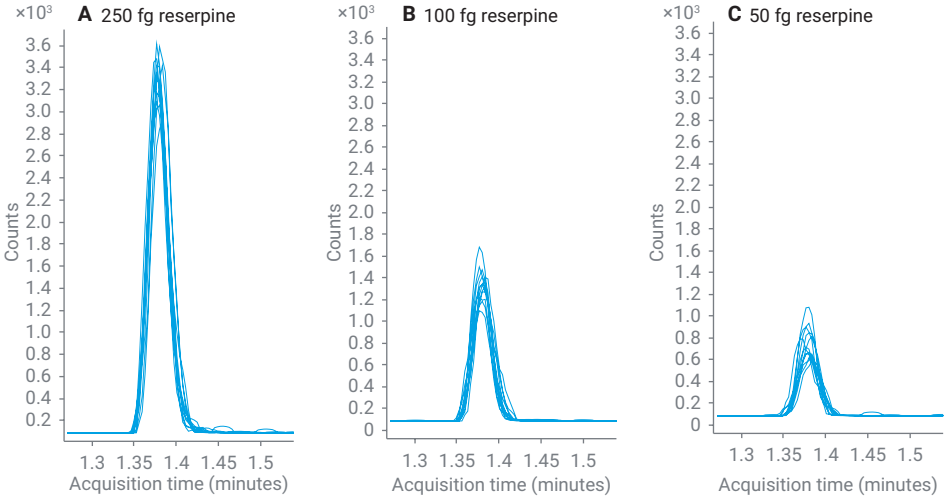


Figure 4. Replicate (n = 10) injections of various amounts of reserpine on-column using 0.5 ms MRM dwell times.

Table 4. Replicate (n = 10) injections of 250, 100, and 50 fg reserpine on-column using 0.5 ms MRM dwell times.

Injection No.	Area (250 fg)	Area (100 fg)	Area (50 fg)
1	4,868	1,722	511
2	3,118	1,548	8
3	2,825	665	227
4	4,476	477	139
5	4,041	1,292	13
6	4,002	2,072	126
7	3,380	709	82
8	3,769	966	135
9	4,234	2,105	239
10	4,643	2,251	31
Average	3,935.6	1,380.7	151.1
St. dev.	667.1	655.3	150.1
%RSD	16.95%	47.46%	99.35%

IDL = (2.821) × (250 fg) × (16.95%/100) = 119.5 fg on-column

Conclusion

This Technical Overview summarizes the characterization of the IDL at two different MRM dwell time regimes: (1) at 200 ms providing sufficient ion sampling time; or (2) at 1 and 0.5 ms with inadequate sampling time of the ion beam. Table 5 shows the results.

When operated at extremely short dwell times such as 1 or 0.5 ms, IDL characterization relies on the instrument's ability to reproducibly sample and stabilize the ion beam within the restricted time frame. Although the MRM dwell times are reduced, components along the ion optics rail were designed to transmit as many ions as possible. This results in reasonable performance (low IDLs), even at the shortest MRM dwell times.

Table 5. Replicate (n = 10) injections of 100, 50, and 10 fg reserpine on-column.

Dwell Time	Injection Amount	%RSD	IDL
200 ms	1 fg	17.62%	0.49 fg
1 ms	100 fg	18.56%	52.4 fg
0.5 ms	250 fg	16.95%	119.5 fg

IDL characterization was carried out to demonstrate the changes in sensitivity with extremely short dwell times (demonstrated on the 6495C LC/TQ). This was done to provide the customer with an expectation of sensitivity changes when running extremely challenging methods for high-throughput and routine applications.

Although using extremely short dwell times is not a generally recommended practice, Agilent recognizes that customers are facing various challenges, demanding scientific accuracy and confident results while maximizing sample throughput to keep up with the cost of running the lab.

References

1. Sheehan, T.; Yost, R. What's the Most Meaningful Standard for Mass Spectrometry: Instrument Detection Limit or Signal-to-Noise Ratio? *Spectroscopy* **2018**, *13*, 16–22.
2. Wells, G.; *et al.* Signal, Noise, and Detection Limits in Mass Spectrometry. Agilent Technologies Application Note, publication number 5990-7651EN, **2011**.
3. Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. Environmental Protection Agency. (**2016**). doi:EPA 821-R-16-006.

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