Evaluation of Mass Accuracy Robustness for the Agilent InfinityLab Pro iQ Plus

Author

Dallas Freitas
Agilent Technologies, Inc.

Abstract

This application note demonstrates the robustness and mass accuracy of the Agilent InfinityLab Pro iQ Plus single quadrupole mass detector, coupled to an Agilent 1260 Infinity II LC system, for the analysis of a multicomponent toxicological mixture. Measurements were obtained over 1,150 repeated injections without cleaning or recalibration. In this study, the analyzed components were found to have peak areas with relative standard deviations (RSDs) of less than 10%, while maintaining a mass accuracy tolerance within ±0.1 Da. This accuracy was further enhanced using Cerno Bioscience MassWorks software, achieving sub-0.01 Da mass accuracy for the analytes.

Introduction

Detecting small-molecule compounds is essential to the operation of cutting-edge instrumentation labs. Mass spectrometry (MS), specifically liquid chromatography/mass spectrometry (LC/MS), is often used to analyze these compound classes and is considered a gold standard for their detection and rapid confirmation.[1] Often, these types of analyses–including those for drugs of abuse–require rapid processing of large sample sets. Therefore, it is vital that instruments can accurately and reliably separate and identify these compounds of interest.

Unit-resolution instruments, such as quadrupole-based mass analyzers, are used in many analytical laboratories due to their low cost and robustness. However, unlike high-resolution mass spectrometers, which can resolve ions with a high mass accuracy of approximately ±0.001 Da, quadrupole mass analyzers typically can only resolve ions within a lower accuracy of ±0.1 Da (or 100 ppm). Additionally, mass error may also arise from inherent systemic bias, further increasing the complexity of accurate mass identification.[2] However, postacquisition software can improve mass accuracy in unit-level instruments and reduce mass error.[3] Cerno Bioscience MassWorks software uses spectral calibration methodologies to significantly enhance the mass accuracy of MS data.[4,5] This application note evaluates the robustness and mass accuracy of the Agilent InfinityLab Pro iQ Plus single quadrupole mass detector through a series of sequential injections of a standard medicinal small-molecule mixture. The results demonstrate minimal signal drift in ion intensities and mass accuracy.

Experimental

Chemicals and standards

All reagents, solvents, and chemicals were of HPLC or LC/MS grade. Agilent LC/MS toxicology checkout mix (part number 5190-0556) was used as the internal standard. Agilent InfinityLab Methanol for LC/MS (part number 5191-5111-001) was used for mobile phase B. Agilent 5 M ammonium formate solution (part number G1946-85021) and Fisher Scientific Optima formic acid (Waltham, MA) were used as mobile phase additives. Ultrapure water produced using a Milli-Q Advantage A10 equipped with a Millipak Express 40 filter (MilliporeSigma, MGP04001) was used as mobile phase A. Agilent ESI-L LC/MS tuning solution (part number G1969-85000) was used for calibration in the MassWorks software.

Sample preparation

For injection of standard solutions, the toxicology checkout mix was diluted into a solvent blank (60:40 water:MeOH). Two solutions were made at concentrations of 100 pg/µL and 10 pg/µL. For mobile phase A, 5 M ammonium formate solution was diluted to 2 mM in water. In addition, 0.1% v/v formic acid was added to create a buffer solution of 2 mM ammonium formate and 0.1% formic acid. For mobile phase B, 0.1% v/v formic acid was added to MeOH.

Equipment

Chromatographic separation was accomplished using an Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 × 50 mm, 1.8 µm) coupled to an Agilent 1260 Infinity II LC system Clinical Edition (K2571-65350). This system was comprised of an Agilent 1260 Infinity II multicolumn thermostat Clinical Edition (K7116A), an Agilent 1260 Infinity II multisampler Clinical Edition (K7167A), and Agilent 1260 Infinity II binary pump Clinical Edition (K7112B). For compound detection, the LC system was coupled to an Agilent InfinityLab Pro iQ Plus (G6170A). Injections were ionized using an Agilent Jet Stream ion source (AJS; part number G1958B). LC/MS data were collected using Agilent OpenLab CDS (version 2.8). The LC parameters are shown in Table 1 and MS parameters are shown in Table 2.

Methods

Data processing: LC/MS data were obtained using Agilent OpenLab CDS software (version 2.8), followed by analysis using Agilent MassHunter Workstation Quantitative Analysis (Quant-My-Way software; version 12.0). Mass correction was accomplished using Cerno Bioscience MassWorks software (version 7.0.0.3). Spectral and mass accuracy corrections were achieved using Agilent ESI-L LC/MS tuning solution and applied across a mass range of *m/z* 100–1,000.

Cerno MassWorks mass accuracy calibration: To accomplish mass accuracy and spectral corrections, Cerno Bioscience MassWorks software was used. After 50 repeated low-concentration injections (10 pg/µL), a single 100 pg/µL standard solution was injected. After all standards from the high-concentration injection had eluted, ESI-L tuning solution was sprayed for 0.5 minutes before the end of the run. In MassWorks, 100 pg injections were loaded, and the infusion time for the tune mix was selected and averaged. After retention time selection and scan averaging, four tuning ions were selected and used as calibration ions: *m/z* 118.0863, 322.1527, 622.0290, and 922.0098 ($C_5H_{12}NO_2$, $C_6H_{19}N_3O_6P_3$, $C_{12}H_{19}F_{12}N_3O_6P_3$, and $C_{18}H_{19}F_{24}N_3O_6P_3$, respectively). Mass data were calibrated using the default calibration parameter set. After calibration, Δ mDa (difference between observed mass and theoretical mass) was obtained for the selected tuning ions. This calibration was saved and applied to its corresponding data file. Calibration and mass correction were accomplished with 23 calibration injections, throughout a total of 1,150 injections across the study. Calibration infusion parameters are shown in Table 3.

Liquid chromatography

Table 1. Agilent 1260 Infinity II LC Clinical Edition method.

1260 Infinity II Bio LC System	
Column	Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 × 50 mm, 1.8 µm)
Sampler Temperature	6 °C
Needle Wash	1 (70% MeOH in H_2O)
Mobile Phase A	H_2O with 2 mM ammonium formate and 0.1% formic acid
Mobile Phase B	MeOH and 0.1% formic acid
Flow Rate	0.500 mL/min
Injection Volume	1 µL
Column Temperature	60 °C
Gradient Program	Time (min) %B 0.00 10 0.50 50 3.00 50 4.00 85 5.00 90 5.50 90 6.00 10 Postrun: 1.5 min

Mass spectrometry

Table 2. LC/MS parameters.

Pro iQ Plus LC/MS System	
Ion Source	AJS
Polarity	Positive
Gas Temperature	300 °C
Drying Gas Flow	13 L/min
Nebulizer	35 psi
Sheath Gas Temperature	250 °C
Capillary Voltage	4,000 V
Capillary Current	2 nA
Nozzle Voltage	1,000 V
Scan Type	Full scan + selected ion monitoring (SIM)
Detector Gain Factor (+)	1

Table 3. Calibrant infusion parameters.

Start Time	Type	Value
0 min	Diverter	To MS
6 min	Calibrant	On
6.5 min	Calibrant	Off

Acquisition parameters

Table 4. MS acquisition parameters.

Scan Type	Polarity	Compound	*m/z*	Dwell Time (ms)	Gain	Fragmentor
Scan	Positive	–	100–1,000	350	1	Ramp
Scan	Negative	–	100–1,000	350	1	Ramp
SIM	Positive	Phentermine	150.1	5	1	90
SIM	Positive	Methamphetamine	150.1	5	1	90
SIM	Positive	MDMA	194.1	5	1	90
SIM	Positive	Benzoylecgonine	209.1	5	1	90
SIM	Positive	Phencyclidine	244.2	5	1	90
SIM	Positive	Carisoprodol	261.2	5	1	90
SIM	Positive	Diazepam	285.1	5	1	90
SIM	Positive	Hydrocodone	300.2	5	1	90
SIM	Positive	Trazodone	372.2	5	1	90

Results and discussion

Prior to the complete robustness tests, acquisition method parameters were optimized for the separation and detection of the Agilent LC/MS toxicology checkout mix. Using a ZORBAX RRHD Eclipse Plus C18 chromatographic column, separation of the standard mix components was achieved in a short six-minute run time. In total, nine compounds were monitored through selected ion monitoring (SIM), in addition to a full scan from *m/z* 100–1,000 in both positive and negative ion modes (Figure 1). It should be noted that of the initial nine compounds, two exist as structural isomers and cannot be distinguished by mass alone or without comparable standards. Therefore, both phentermine and methamphetamine were consolidated to just phentermine. Acquisition parameters are shown in Table 4. Additionally, the instrument was retuned prior to final robustness testing.

For this robustness analysis, more than 1,150 injections of low concentration (10 pg/µL) were performed. Additionally, for every set of 50 low-concentration injections, one injection of higher concentration (100 pg/µL) standard mixture was injected. In the high-concentration injections, a direct infusion of ESI-L tuning solution was achieved using the integrated calibrant infuser. To enable this, after all expected peaks had eluted at six minutes, LC flow was automatically diverted to waste, while calibrant was diverted to the MS. This postchromatographic infusion approach allows tracking of mass accuracy throughout the series of injections.

Prior to assessing mass accuracy across the 1,150 injections, the peak area of each toxicology mix component was evaluated. Figure 2 shows the peak area assignments of each mixture component after a set of 50 low-concentration injections. Notably, eight of the nine components displayed responses within 20% of their respective mean values. The perceived decrease in peak areas may be associated with a decrease in column performance over its lifetime.

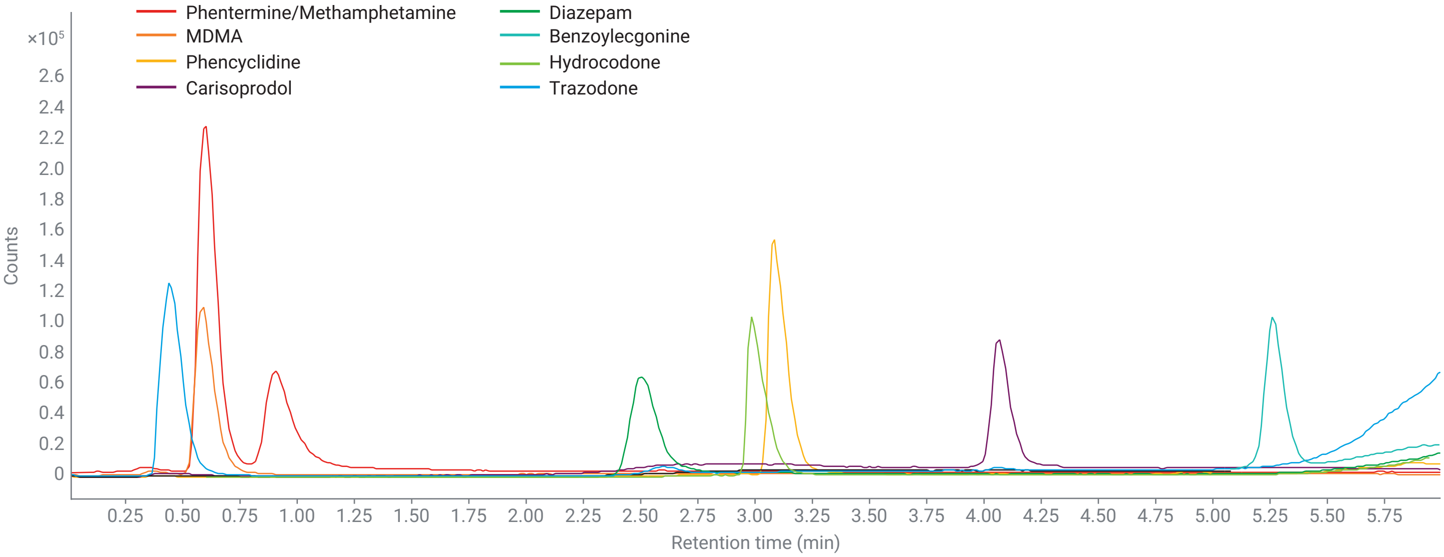
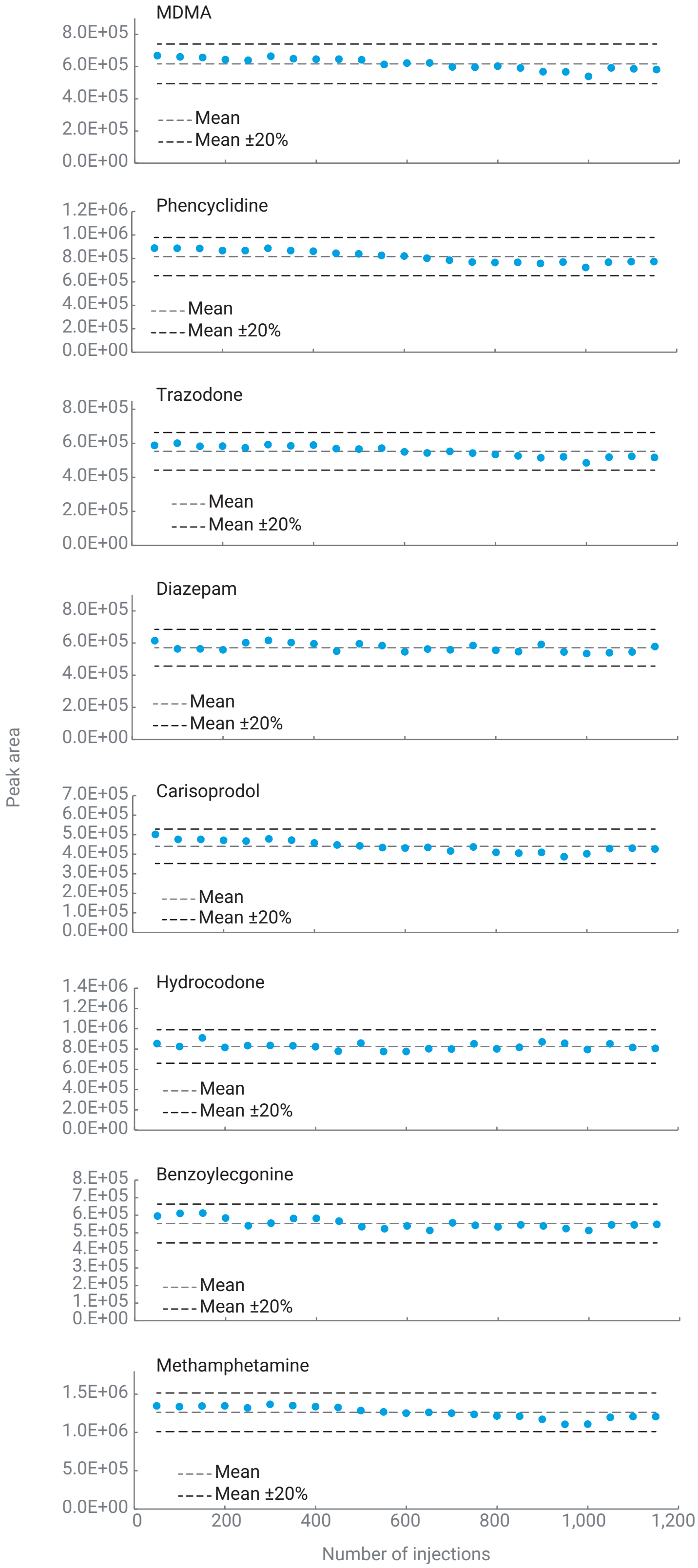


Figure 1. Representative SIM chromatogram acquired using method optimized for the compound mixture.



Average peak area: 616,826
Standard deviation: 35,830
%RSD: 5.81%

Average peak area: 815,875
Standard deviation: 52,027
%RSD: 6.38%

Average peak area: 552,915
Standard deviation: 31,761
%RSD: 5.74%

Average peak area: 570,910
Standard deviation: 25,376
%RSD: 4.44%

Average peak area: 440,670
Standard deviation: 29,579
%RSD: 6.71%

Average peak area: 824,752
Standard deviation: 33,192
%RSD: 4.02%

Average peak area: 552,942
Standard deviation: 28,393
%RSD: 5.14%

Average peak area: 1,262,633
Standard deviation: 77,172
%RSD: 6.11%

Figure 2. Chromatographic peak area of toxicology mixture components across 1,150 injections.

External calibration was accomplished using Cerno Bioscience MassWorks software and selected ions from the calibration tune mix. Mass accuracy was evaluated by obtaining the mass error of calibrants, with calibrant ions *m/z* 622.0290 and *m/z* 922.0098 displaying an average Δ mDa of 14.60 (23.47 ppm) and 16.21 (26.06 ppm), respectively. Percent relative standard deviations (%RSDs) fell below 20%, with a %RSD of 9.41% for *m/z* 622 and 11.19% for *m/z* 922 (Figure 3). These results indicate that the Pro iQ Plus exhibits no significant decrease in mass accuracy across the 1,150+ injections, indicating consistent instrument performance.

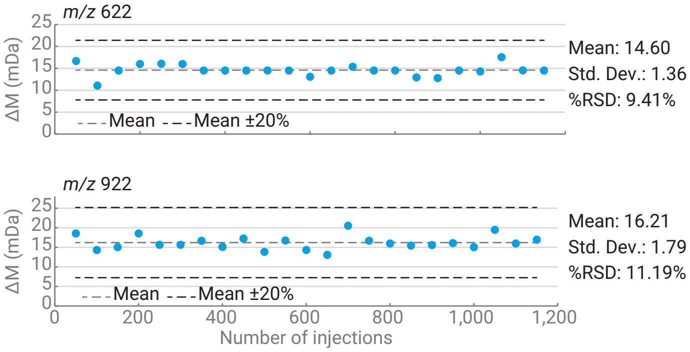


Figure 3. Mass accuracy of tune solution calibrant ions prior to calibration.

These calibrations were applied to each of their corresponding injections. Figure 4 shows the absolute difference between corrected mass values and expected theoretical mass. These data demonstrate that calibration using MassWorks software (blue) brings masses much closer to their expected values compared to raw mass measurements (green). This calibration achieves a sub-10 mDa error of 8.39 mDa in diazepam (*m/z* 285.08), down from a raw uncorrected mass error of 42.3 mDa—an approximately 5-fold improvement.

In other components, correction using MassWorks calibration further enhanced mass accuracy, yielding an average sub-25 mDa mass error—an approximately 3-fold improvement over the raw data. Similar to mass accuracy for calibrant ions, Pro iQ Plus performance does not show an increase in mass error across injections, indicating little to no performance loss.

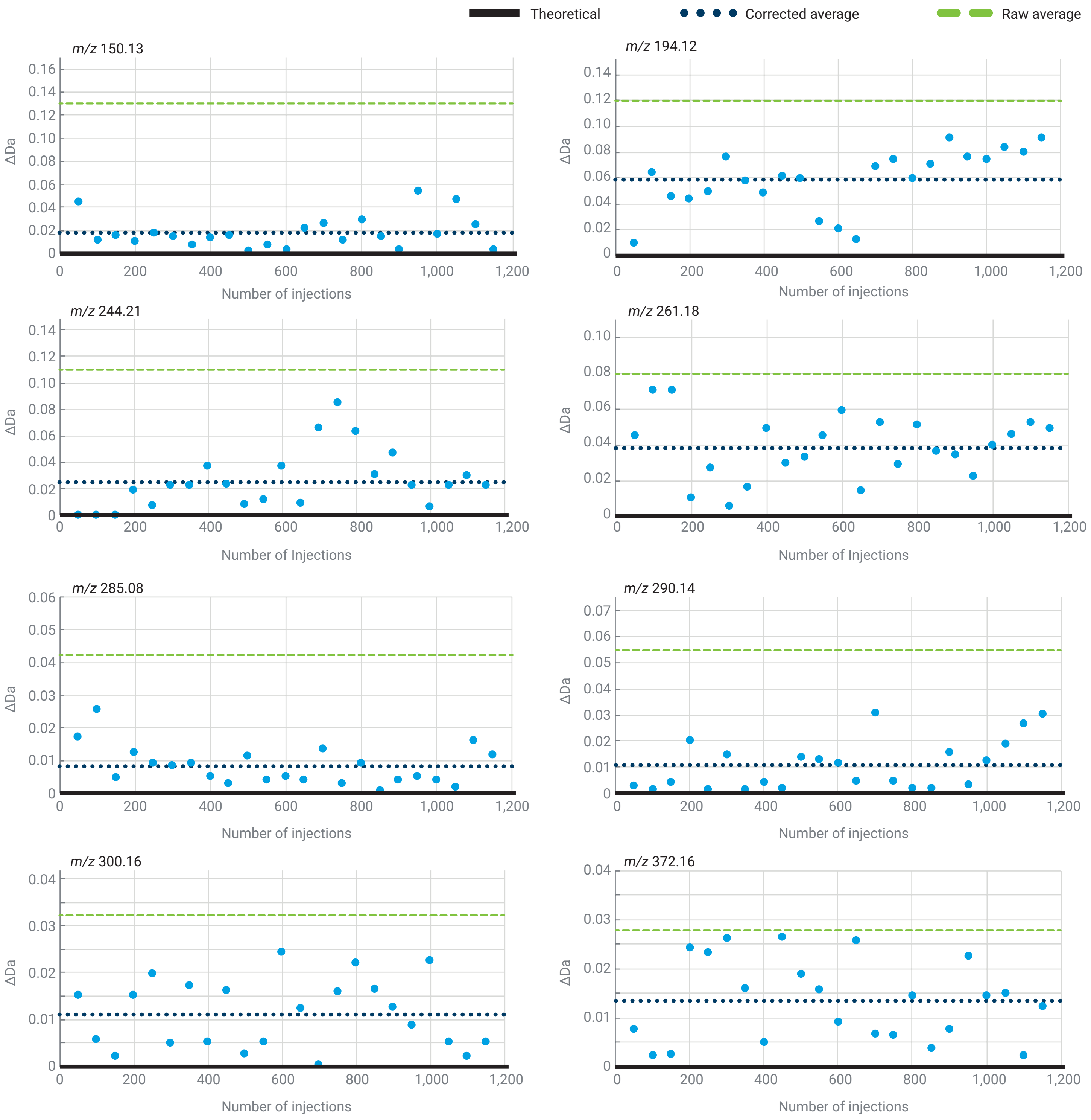


Figure 4. Mass error of toxicology mix components before (green) and after correction (blue) using MassWorks software.

Conclusion

In this study, the robust performance of the Agilent InfinityLab Pro iQ Plus single quadrupole mass detector was demonstrated across 1,150 sample injections. Through this set of analyses, system performance remained stable, with minimal changes in component peak area and consistent mass error. Additionally, the application of Cerno Bioscience MassWorks TrueCal showed that mass error can be further reduced through postacquisition calibration.

References

1. Tan, J.; Rylski, A.; Bergqvist, A.; Stephanson, N. N. Automated and Fully Validated High-Throughput LC-MS/MS Assay for Analyzing Multiple Drugs of Abuse in Oral Fluids Using Novel Features in Sample Preparation and Chromatographic Conditions. *J. Mass Spectrom.* **2025**, *60(5)*, e5132. DOI: 10.1002/jms.5132.
2. Gibbons, B. C.; Chambers, M. C.; Monroe, M. E.; Tabb, D. L.; Payne, S. H. Correcting Systematic Bias and Instrument Measurement Drift with mzRefinery. *Bioinformatics* **2015**, *31(23)*, 3838–3840. DOI: 10.1093/bioinformatics/btv437.
3. Olsen, J. V.; de Godoy, L. M. F.; Li, G.; Macek, B.; Mortensen, P.; Pesch, R.; Makarov, A.; Lange, O.; Horning, S.; Mann, M. Parts per Million Mass Accuracy on an Orbitrap Mass Spectrometer via Lock Mass Injection into a C-Trap. *Mol. Cell. Proteomics* **2005**, *4(12)*, 2010–2021. DOI: 10.1074/mcp.T500030-MCP200.
4. Abel, R. J. Identification of Unknown Compounds from Quadrupole GC-MS Data Using Cerno Bioscience MassWork. *Can. Soc. Forensic Sci. J.* **2014**, *47(2)*, 74–98. DOI: 10.1080/00085030.2014.904605.
5. Wang, Y.; Gu, M. The Concept of Spectral Accuracy for MS. *Anal. Chem.* **2010**, *82(17)*, 7055–7062. DOI: 10.1021/ac100888b.

www.agilent.com

DE-013148

Printed in the USA, March 31, 2026
5994-9061EN

Agilent
Trusted Answers