



Methylmalonic acid (MMA) in human serum

Illustration of analytical performance for methylmalonic acid (MMA) in human serum.

The SCIEX Citrine MS/MS system is intended to identify inorganic or organic compounds in human specimens. All laboratory-developed tests must be developed, verified and validated in accordance with applicable laws and regulations prior to their use for clinical diagnostic purposes.

This document describes a test of the analytical performance of the SCIEX Citrine MS/MS system to analyze methylmalonic acid (MMA) in human serum matrix.

The analytical performance data presented here is for illustrative purposes only to demonstrate the potential capabilities of the system. Performance in individual laboratories may differ due to a number of factors, including system configuration, laboratory methods, and operator technique. This document does not constitute a warranty of merchantability or fitness for any particular purpose, express or implied, including for the testing of the compounds analyzed in this experiment.

Materials and methods

The Citrine MS/MS system was controlled, and data processed using Analyst MD software, version 1.6.3. Serum calibrators, controls and samples were processed using the following conditions:

Sample preparation: Sample preparation was performed using Diagnostix's methylmalonic acid reagents set (<https://www.diagnostix.com/en/products/methylmalonic-acid-reagents-set>) according to the manufacturer's specifications. A 100 µL serum sample spiked using the set of calibrators was used for the procedure.

Liquid chromatography conditions: Chromatographic separation was achieved using a Phenomenex Kinetex C18 column. Mobile phases A and B from the reagents set were used. The total run time was 6 minutes at a flow rate of 400 µL/min. The injection volume was 15 µL.

Mass spectrometry conditions: Mass spectrometry analysis was performed using the Citrine Triple Quad MS/MS system, operating in negative electrospray mode. Compound-dependent parameters were optimized by infusion.

Results

Analytical performance statistics including the concentration range evaluated, accuracy and precision (n=4 replicates), as well as signal-to-noise ratio (S/N) and linearity (r^2) are shown in Table 1. A chromatogram of the compound evaluated with the described method is shown in Figure 1. A calibration curve over the defined concentration ranges for the compound is illustrated in Figure 2.

Table 1. Performance statistics for the analysis of MMA in human serum. Measured range (nmol/L), % accuracy, %CV, S/N ratio and linearity for MMA. Values for the lowest calibrator (50 nmol/L) and over the measured range (50-1695 nmol/L) were used, as appropriate.

Compound	Range [pg/mL]	% Accuracy	%CV	S/N*	Linearity (r^2)
MMA	50-1695	89.21	7.5	12.8:1*	0.9995

*S/N ratio calculated using a peak-to-peak algorithm for lowest matrix calibrator measured.

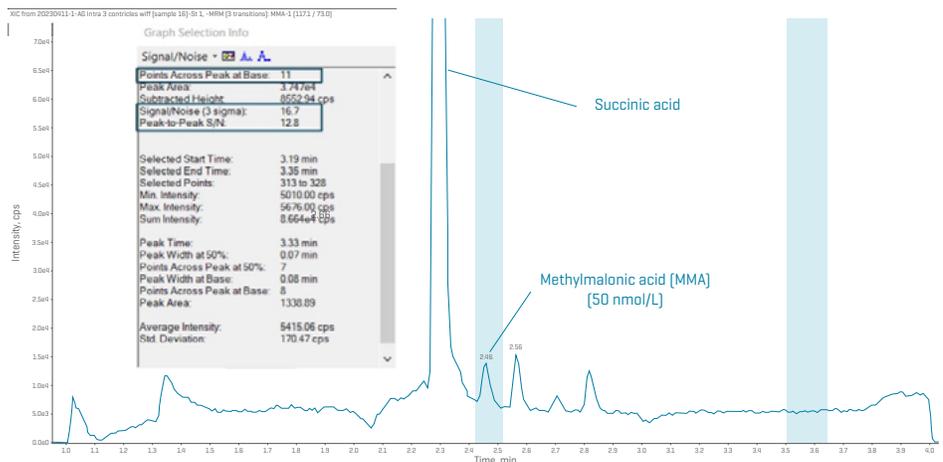


Figure 1. Analytical sensitivity of 50 nmol/L MMA standard extracted from serum matrix. Chromatogram of calibration standards for MMA at 50 nmol/L shows a S/N of 12.8:1 based on a peak-to-peak algorithm.

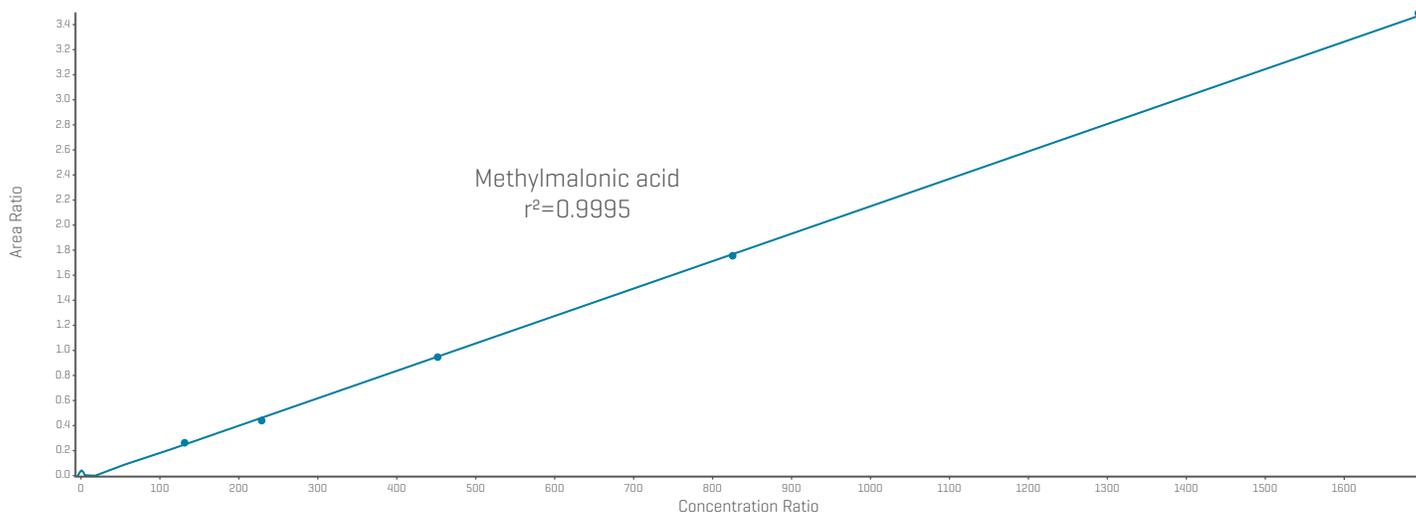


Figure 2. Linear calibration curve for MMA extracted from serum matrix using the primary MRM transition. The calibration curve using the matrix calibrators was analyzed across the concentration range [50-1695 nmol/L]. The curve was generated using linear regression and 1/x weighting, resulting in a r^2 value of 0.9995.

Conclusions

Based on the above performance testing, the following results were obtained:

Sensitivity: Analytical sensitivity was investigated with a series of calibration standards and QC samples prepared in matrix and processed as described and showed a S/N of 12.8:1 at the lowest matrix calibrator measured [50 nmol/L], calculated using a peak-to-peak algorithm.

Assay linearity: Linearity was assessed over a concentration range of 50-1695 nmol/L MMA in matrix, with 4 replicates at each concentration. The r^2 value was 0.9995.

Accuracy: At the lowest measured calibrator [50 nmol/L], the % accuracy was 89.21% for MMA, determined by 4 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

Reproducibility: At the lowest measured calibrator [50 nmol/L], the precision [%CV] was 7.5% for MMA, determined by 4 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

In these experiments, the Citrine MS/MS system exhibited the capability to deliver sensitive and reproducible analytical performance for the quantitation of MMA in serum matrix.

Acknowledgements

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