



Metanephrines in human serum

Illustration of analytical performance for metanephrines 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 metanephrines 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 metanephrines' reagents set (<https://www.diagnostix.com/en/lcms/metanephrines>) according to the manufacturer's specifications. A 500 µL serum sample spiked using the set of calibrators was used for the procedure.

Liquid chromatography conditions: Chromatographic separation was achieved using a Phenomenex Biozen 2.6 µm Glycan column. Mobile phases A and B from the reagent set were used. The total run time was 5 minutes at a flow rate of 500 µL/min. The injection volume was 20 µL.

Mass spectrometry conditions: Mass spectrometry analysis was performed using the Citrine Triple Quad MS/MS system, operating in positive 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. Chromatograms of the compounds evaluated utilizing the described method are shown in Figure 1. Calibration curves over the defined concentration ranges for each compound are illustrated in Figure 2.

Table 1. Performance statistics for the analysis of metanephrines in human serum. The range [nmol/L], % accuracy, %CV, S/N ratio and linearity were calculated using a peak-to-peak algorithm. Values for the lowest calibrator and over the measured range were used, as appropriate.

Compound	Range [µg/mL]	% Accuracy	%CV	S/N*	Linearity [r^2]
Metanephrine	0.15-5.31	100	5.4	44.9:1*	0.99881
Normetanephrine	0.18-5.98	100	4.5	17.5:1*	0.99797
3-MT	0.14-5.96	98.21	3.6	37.5:1*	0.99595

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

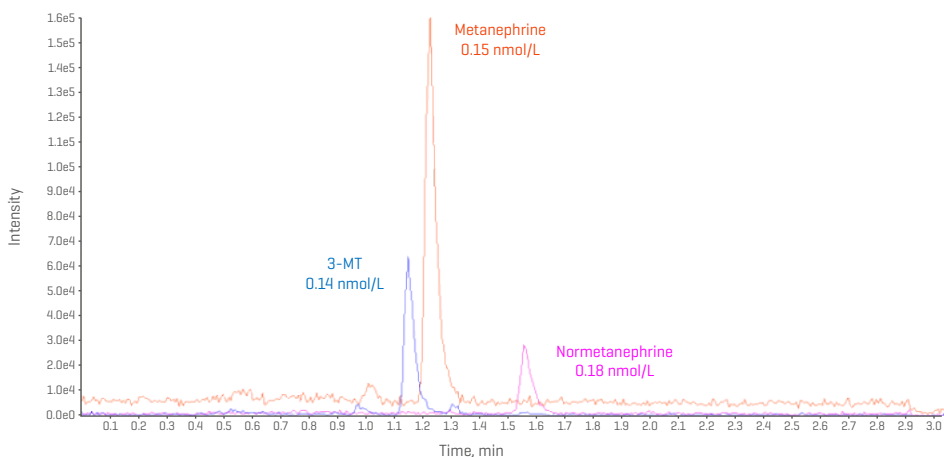


Figure 1. Chromatogram of metanephrine [orange], normetanephrine [purple] and 3-MT [blue] extracted from serum matrix. Chromatogram of calibration standards in matrix for metanephrine at 0.15 nmol/L, normetanephrine at 0.18 nmol/L and 3-MT at 0.14 nmol/L shows a S/N of 44.9:1 for metanephrine, 17.5:1 for normetanephrine and 37.5:1 for 3-MT based on a peak-to-peak algorithm.

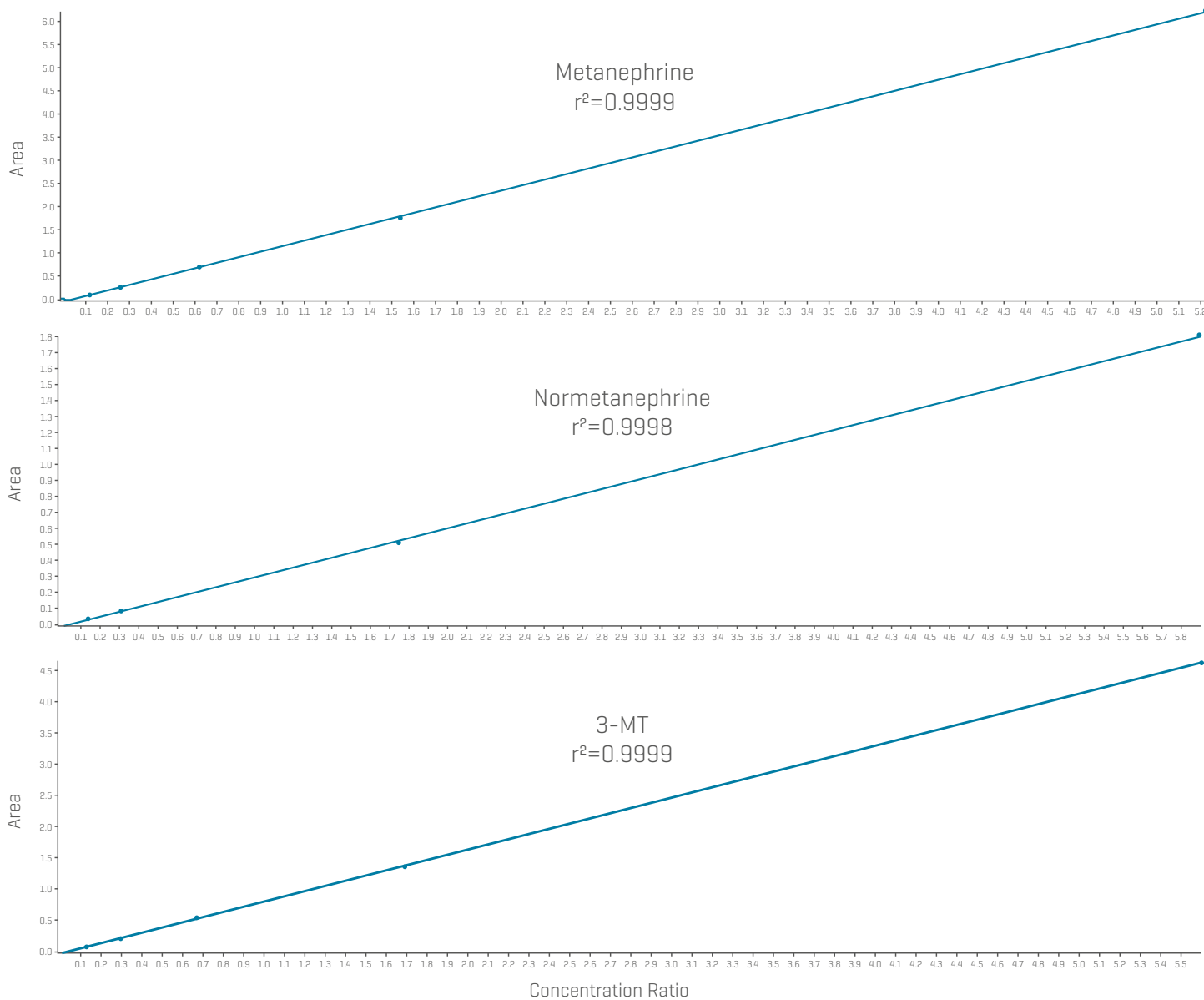
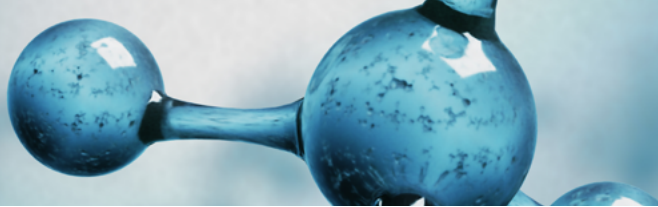
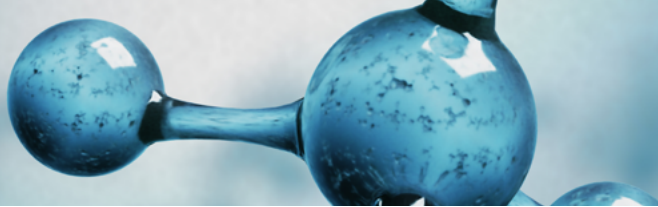


Figure 2. Linear calibration curves for metanephrine [top], normetanephrine [middle] and 3-MT [bottom] extracted from serum matrix. The calibration curves were run across the following concentration ranges [0.15-5.31 nmol/L for metanephrine, 0.18-5.98 nmol/L for normetanephrine and 0.14-5.96 nmol/L for 3-MT]. The curves were generated using linear regression and 1/x weighting for all three compounds, resulting in r² values of 0.9999 for metanephrine, 0.9998 for normetanephrine and 0.9999 for 3-MT, respectively.



Conclusions

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

Sensitivity: Analytical sensitivity was investigated with a series of calibration standards prepared as described and showed a S/N of 44.9:1 for metanephrine, 17.5:1 for normetanephrine and 37.5:1 for 3-MT, at the lowest matrix calibrator measured (0.15 nmol/L for metanephrine, 0.18 nmol/L for normetanephrine and 0.14 nmol/L for 3-MT), calculated using a peak-to-peak algorithm.

Assay linearity: Linearity was assessed in matrix over the following concentration ranges: 0.15-5.31 nmol/L for metanephrine, 0.18-5.98 nmol/L for normetanephrine and 0.14-5.96 nmol/L for 3-MT. The r^2 values were 0.9999, 0.9998, and 0.9999, respectively.

Accuracy: At the lowest matrix calibrators measured (0.15 nmol/L for metanephrine, 0.18 nmol/L for normetanephrine and 0.14 nmol/L for 3-MT), the % accuracy was 100% for metanephrine, 100% for normetanephrine and 98.21% for 3-MT, determined by 4 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

Reproducibility: At the lowest matrix calibrators measured (0.15 nmol/L for metanephrine, 0.18 nmol/L for normetanephrine and 0.14 nmol/L for 3-MT), the precision [%CV] was 5.4% for metanephrine, 4.5% for normetanephrine and 3.6% for 3-MT, 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 metanephrines in serum matrix.

Acknowledgements

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