Psychotherapeutic drug compounds in human serum

Illustration of analytical performance for a panel of 11 psychotherapeutic drug compounds 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 amitriptyline, nortriptyline, Z-10-OH-nortryptiline, E-10-OH-nortyptiline, clozapine, norclozapine, clomipramine, norclomipramine, imipramine, desipramine and zuclopentixol 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 1.6.3. Serum calibrators, controls and samples were processed using the following conditions:

Sample preparation: Sample preparation was performed using Diagnotix's psychotropic medication 1 reagent set [https://www.diagnotix.com/en/products/psychofarmaca-1] according to the manufacturer's specifications. A 25 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 reagent set were used. The total run time was 7.5 minutes at a flow rate of 400 µL/min. The injection volume was 3 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. An example chromatogram of the compounds evaluated utilizing the described method is shown in Figure 1. Calibration curves over the defined concentration ranges for the compounds run in positive and negative mode are illustrated in Figure 2 and 3, respectively.

Table 1. Performance statistics for the analysis of the psychotherapeutic drug compounds in human serum. Measured range (pg/mL), % accuracy, %CV and S/N ratio and linearity for the psychotherapeutic drug compounds. Values for the lowest calibrator and over the measured range were used, as appropriate.

Compound	Range (pg/mL)	% Accuracy	%CV	S/N*	Linearity (r^2)
Zuclopentixol	7.63-100	98.6	11.1	81.4*	0.9994
Clozapine	68.6-950	99.0	6.3	303.8*	0.9999
Clomipramine	20.9-487	99.0	3.9	316.4*	0.9999
Norclozapine	63.7-762	107.5	6.3	103.2*	0.9998
Norclomipramine	22.9-536	105.3	8.7	298.5*	0.9994
Imipramine	18.4-417	101.5	9.2	231.3*	0.9967
E-OH-Nortiptyline	34.5-314	104.7	12.6	195.4*	0.9988
Amitriptyline	16.5-394	98.2	3.2	103.6*	0.9999
Desipramine	28.1-420	104.6	4.6	307.3*	0.9999
Nortriptyline	19-349	104.5	6.0	115.3*	0.9999
Z-OH-Nortiptyline	10.4-133	104.1	9.6	75.8*	0.9998

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

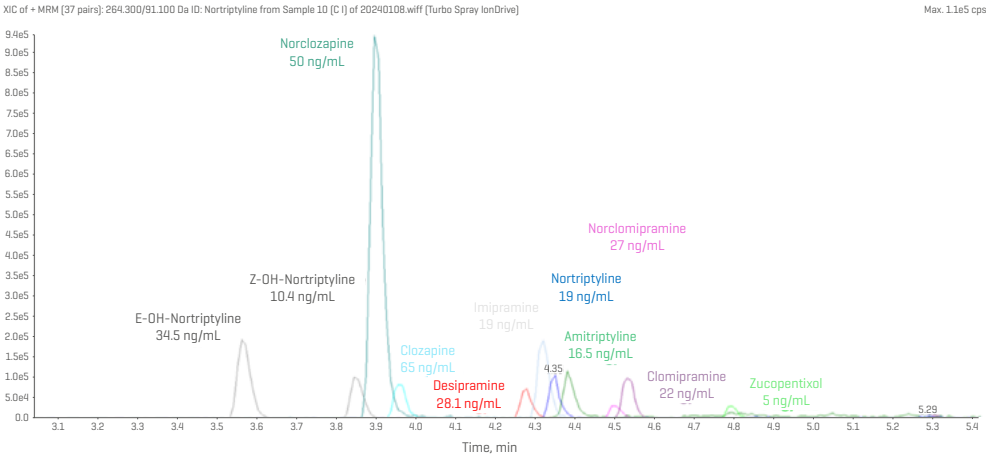

Figure 1. Chromatogram of the 11 psychotherapeutic drug compounds at 1 ng/mL extracted from serum matrix. Chromatogram of calibration standard for the panel of 11 psychotherapeutic drug compounds acquired with positive ESI mode and an optimized scheduled MRM algorithm, using the Citrine MS/MS system following the sample preparation and LC-MS/MS conditions.

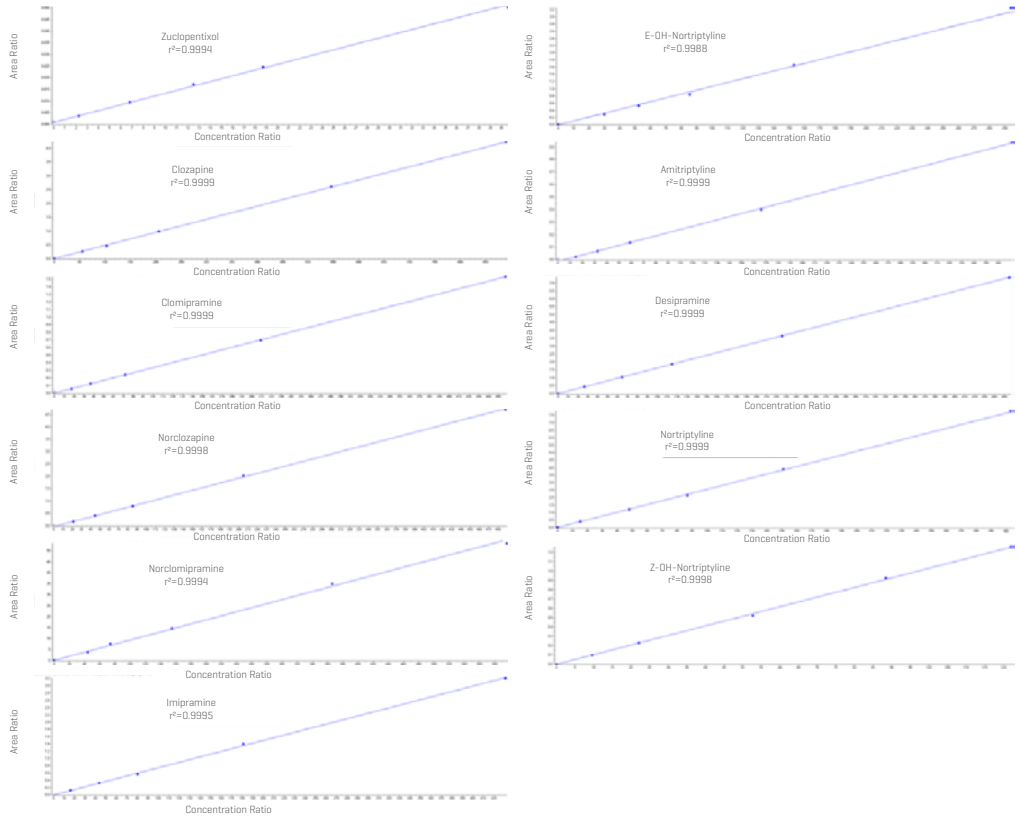

Figure 2. Linear calibration curves for the 11 psychotherapeutic drug compounds analyzed positive mode extracted from serum matrix. The calibration curves were run in triplicate across the measured ranges shown in Table 1. The curves were generated using a linear regression and 1/x weighting, resulting in r^2 vales >0.99 for all the compounds analyzed in serum matrix.

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 S/N values at the lowest matrix calibrator measured as shown in Table 1.

Assay linearity: Linearity was assessed in matrix over the measured ranges shown in Table 1. Psychotherapeutic drug compounds in the panel demonstrated r^2 value >0.99.

Accuracy: At the lowest matrix calibrators, the % accuracy was between 98.2% and 107.5.0% for the compounds analyzed in serum, determined by 4 replicates in matrix. Data evaluated is based on calculated concentration with internal standard.

Reproducibility: At the lowest matrix calibrators, the precision [%CV] was between 3.2% and 12.6% for all compounds analyzed in serum with a mean of 7.4% and a median of 6.3%, 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 accurate, sensitive and reproducible analytical performance for the quantitation of a panel of 11 psychotherapeutic drug compounds in serum matrix.

Acknowledgements

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