

Simultaneous Quantification of Methotrexate and Its Metabolites 7-Hydroxy Methotrexate and DAMPA in Serum via Coated Blade Spray-tandem MS



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Background

Measurement of methotrexate (MTX) in patients receiving high doses of MTX (HDMTX) is important due to the risk of toxicity and wide variations in clearance between different patients receiving the same dose. Coated Blade Spray (CBS) is a sample preparation technology that can be directly interfaced with MS instrumentation for rapid screening and quantitation. As a proof-of-concept, we have developed a CBS method for the quantitation of MTX and its metabolites 7-Hydroxy Methotrexate (7OH-MTX) and DAMPA in human serum via tandem mass spectrometry. Our preliminary results revealed that CBS could deliver performance comparable, or better, than those typically achieved with technologies generally used in the clinical laboratory such as immunoassay and liquid chromatography-MS.

Purpose



Objective

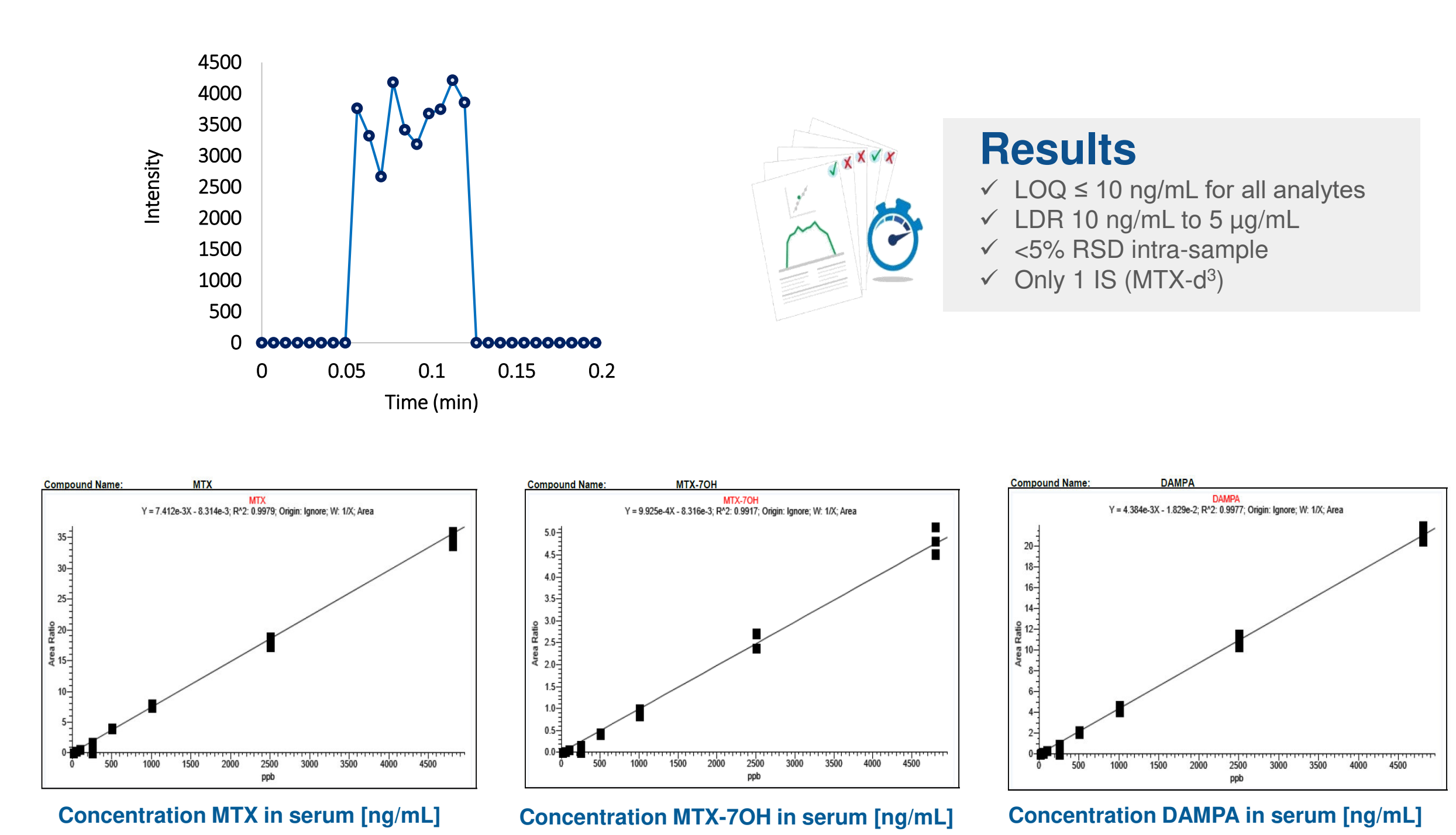
Rapid quantitation of MTX and metabolites via CBS-MS/MS and successful comparison against LC-MS/MS assay

Methods

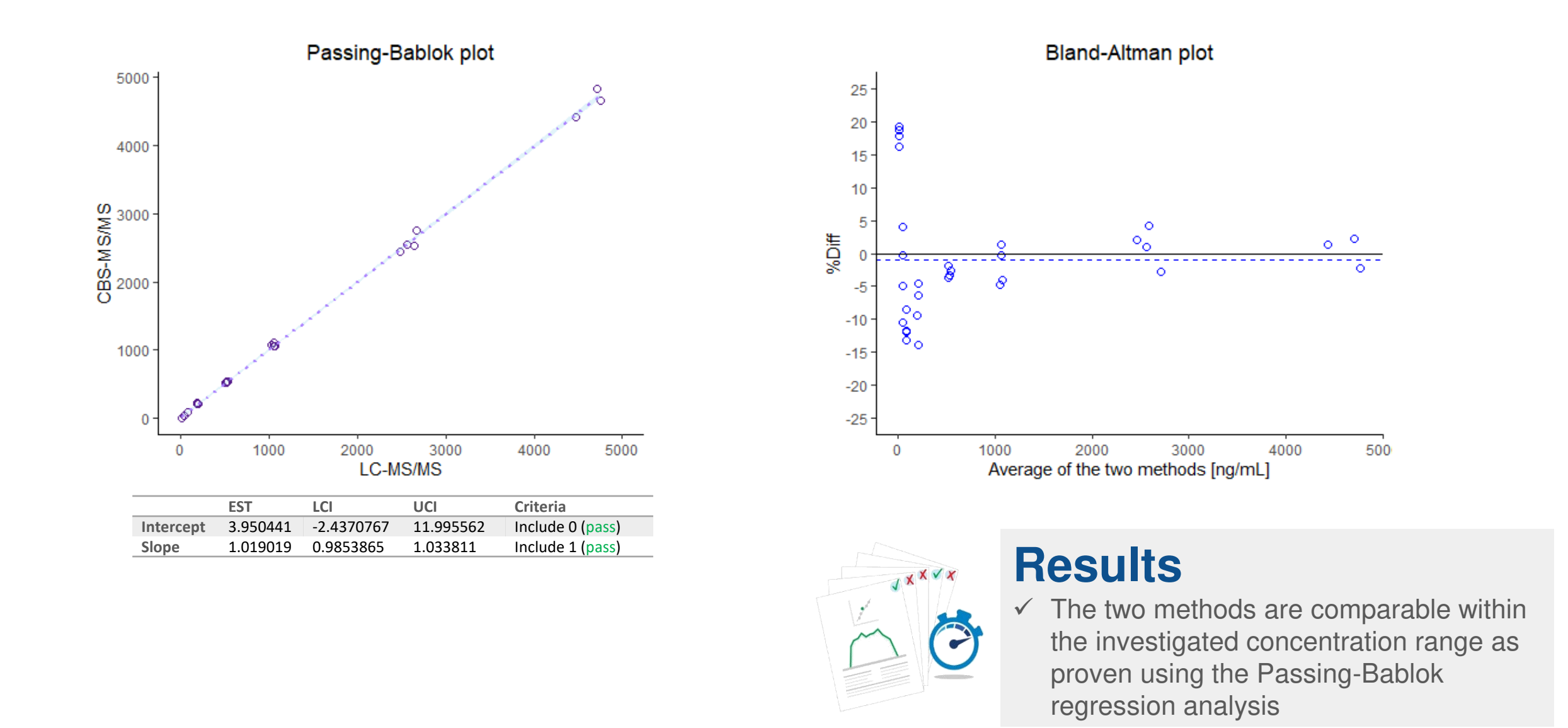
CBS devices coated with HLB particles were purchased from Restek Corporation. Instrumental analysis via tandem mass spectrometry were performed on a Thermo TSQ Altis. A fully automated interface for CBS compatible with Thermo instruments developed by Restek Corporation was used in this study. The optimized analytical workflow via CBS-MS comprises three simple steps. First, analyte collection on the device by immersing the blade in a well-plate containing the sample for 5 min. Then, rinsing any matrix components potentially lingering at the surface of the coating for 10s. Finally, applying 7.5µL of elution/ionization solvent on the coated area of the blade and, after 2 seconds, applying 2.75 kV to the non-coated area generating an electrospray from the tip of the blade.

Results

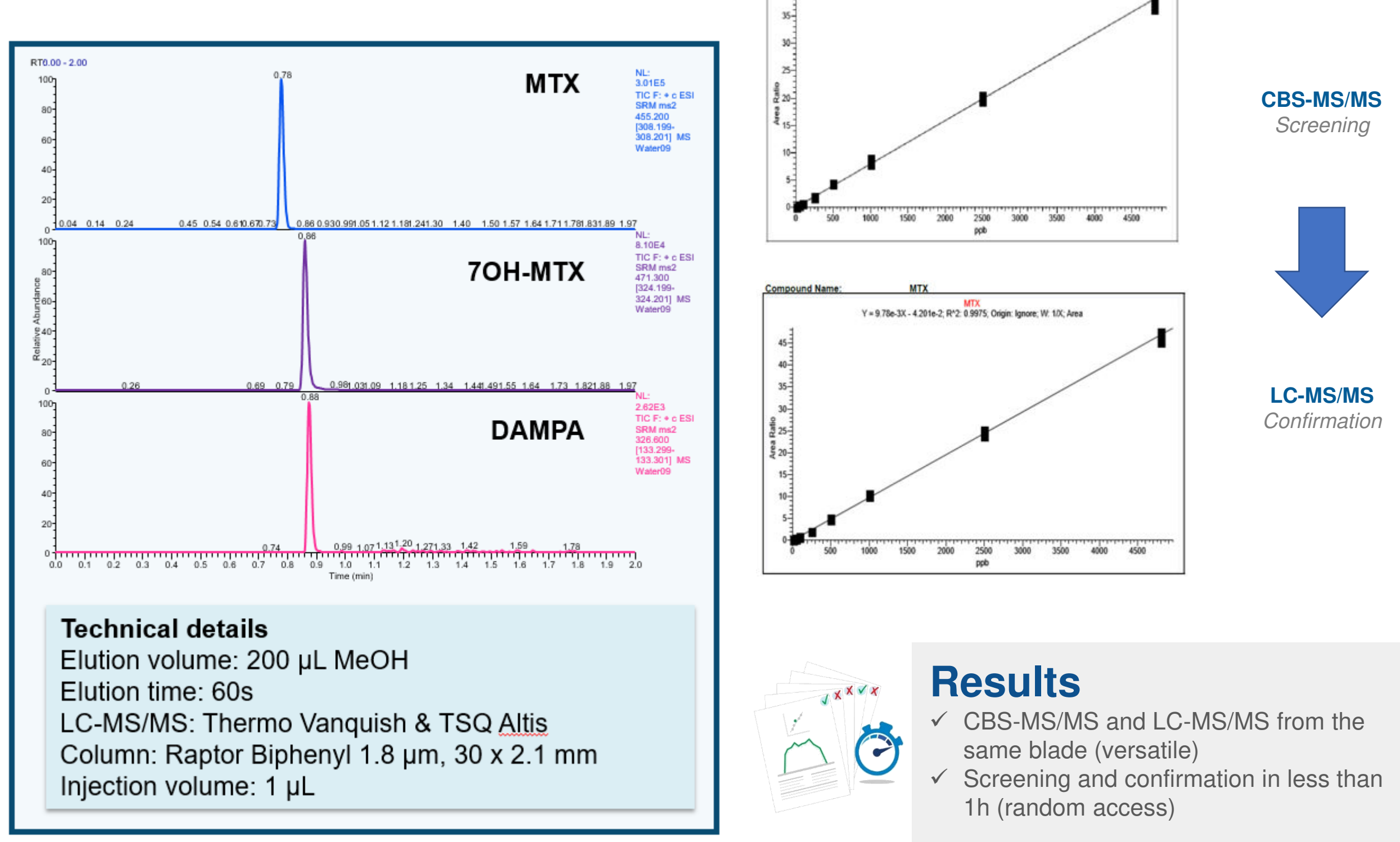
1. Coated Blade Spray delivers a comparable sensitivity to LC-MS/MS



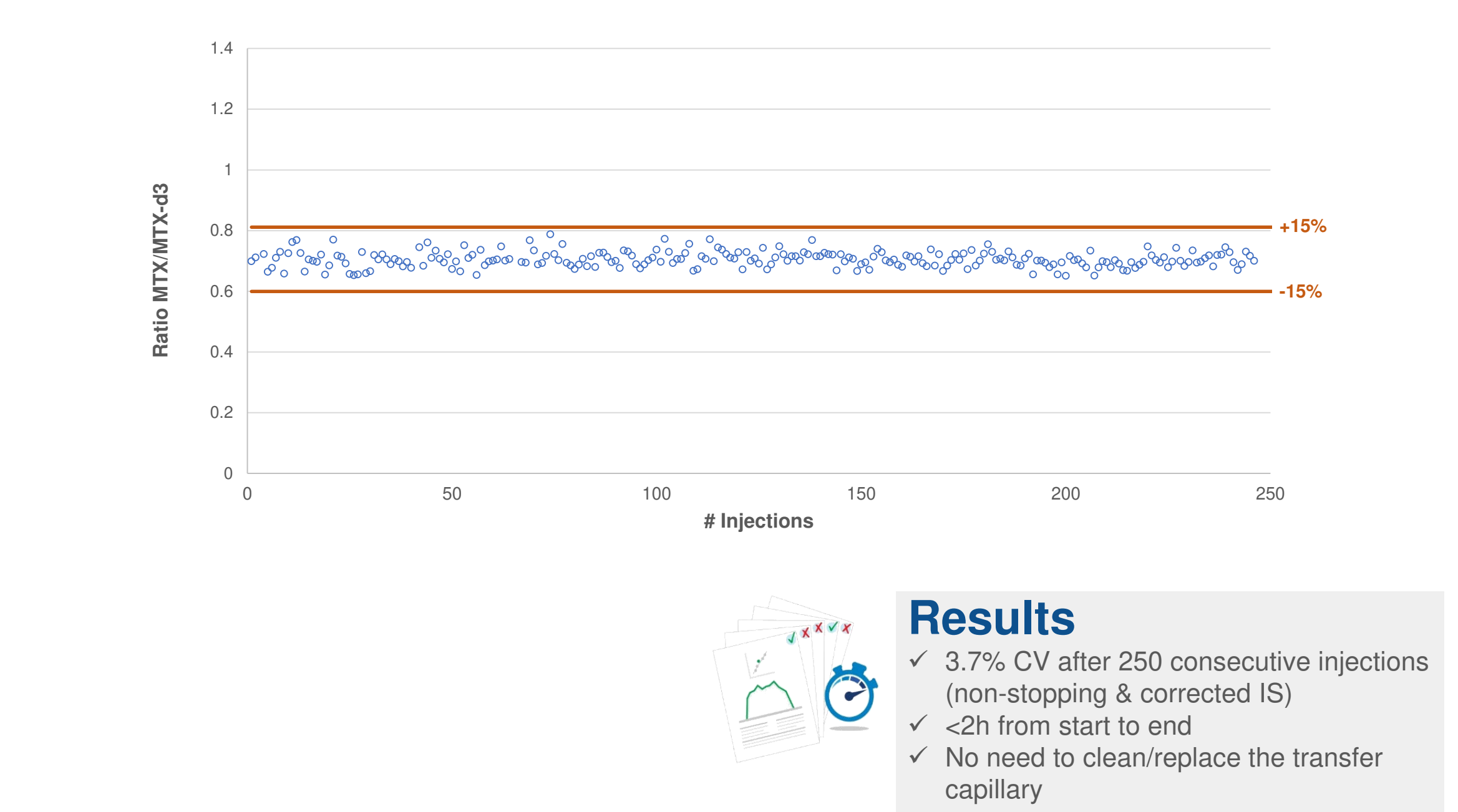
2. Coated Blade Spray delivers a comparable selectivity to LC-MS/MS



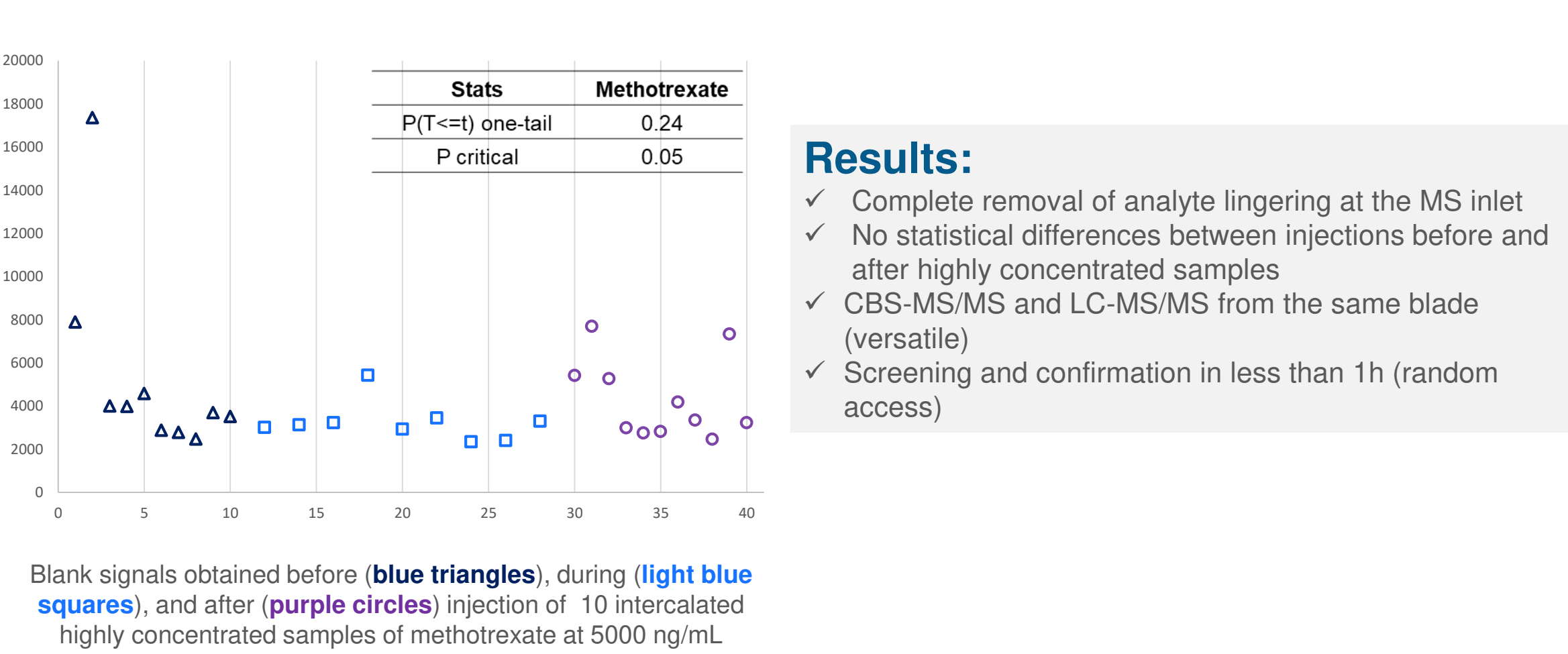
3. Coated Blade Spray is amenable for Screening & Confirmation assays



4. Coated Blade Spray is as reliable as LC-MS/MS approach



5. Total CBS solution minimizes instrumental carry-over



Conclusion

CBS was used successfully in the analysis of MTX and its metabolites in serum. Results reveal that the optimized CBS-tandem MS method may deliver an LLOQ of 1 ng/mL for MTX and metabolites based on a mean CV and bias of <20% at this concentration. Linearity experiments suggested the assay was linear to approximately 5 µg/mL. A method comparison of the CBS-MS/MS to a LC-MS/MS one was performed using 64 different spiked samples across the range of 25 ng/mL to 4.8 µg/mL and a linear relationship was demonstrated between both methods per the Passing-Bablok analysis.

Outlook

Coated Blade Spray is an easy-to-use sample preparation tool that couples directly to a mass spectrometer for qualitative or semi-quantitative analysis, providing truly rapid screening without the need of chromatography.

References

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Winston-McPherson, G. N.; Schmeling, M.; Hoofnagle, A. N. *Methods Mol. Biol.* **2019**, *1872*, 101–110.
Hoffman, M. A.; Schmeling, M.; Dahlin, J. L.; Bevins, N. J.; Cooper, D. P.; Jarolim, P.; Fitzgerald, R. L.; Hoofnagle, A. N. *Clin. Chem.* **2020**, *66*, 474–482

