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

Diego A. Lopez, Ryan Micklitsch, Shane Stevens, German Gomez-Rios, Thomas Kane
Restek Corporation, Bellefonte, PA, USA
Email: Diego.Lopez@restek.com; HPLC 2022 – San Diego, CA

Best Poster Award Participant

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

What if a Simple Sample Preparation Device...

...Could Go Directly to Analysis via Mass Spectrometry...

...To Provide Truly Rapid Screening…

...Without Needing Chromatography for Every Sample?

Objective

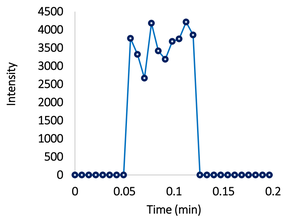
Rapid quantitation of MTX and metabolites via CBS-MS/MS and successful comparison against LC-MS/MS assay

Methods

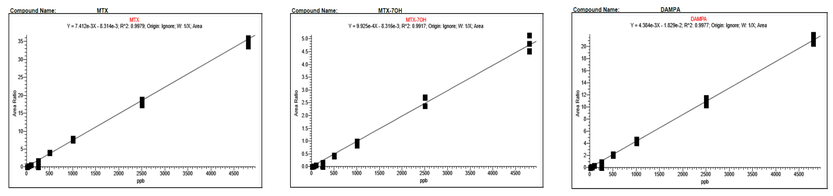
CBS devices coated with HLB particles were purchased from Restek Corporation. Instrumental analysis via tandem mass spectrometry were performed on a Thermo TSQ Altis. A fully automated interface for CBS compatible with Thermo instruments developed by Restek Corporation was used in this study. The optimized 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

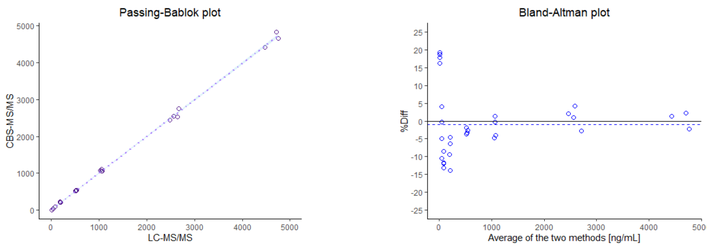


Results
- ✓ LOQ ≤ 10 ng/mL for all analytes
- ✓ LDR 10 ng/mL to 5 µg/mL
- ✓ <5% RSD intra-sample
- ✓ Only 1 IS (MTX-d_3)



Concentration MTX in serum [ng/mL] | Concentration MTX-7OH in serum [ng/mL] | Concentration DAMPA in serum [ng/mL]

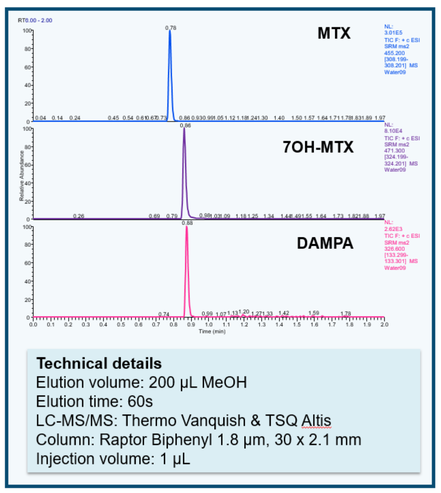
2. Coated Blade Spray delivers a comparable selectivity to LC-MS/MS



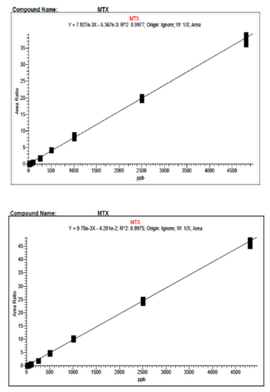
	EST	LCI	UCI	Criteria
Intercept	3.950441	-2.4370767	11.995562	Include 0 (pass)
Slope	1.019019	0.9853865	1.033811	Include 1 (pass)

Results
- ✓ The two methods are comparable within the investigated concentration range as proven using the Passing-Bablok regression analysis

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



Technical details
Elution volume: 200 µL MeOH
Elution time: 60s
LC-MS/MS: Thermo Vanquish & TSQ Altis
Column: Raptor Biphenyl 1.8 µm, 30 x 2.1 mm
Injection volume: 1 µL

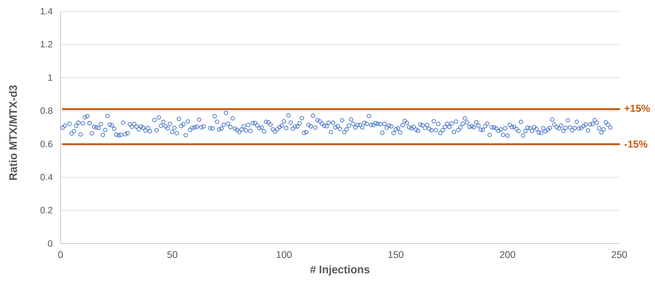


CBS-MS/MS *Screening*

LC-MS/MS *Confirmation*

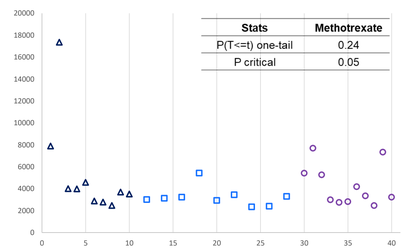
Results
- ✓ CBS-MS/MS and LC-MS/MS from the same blade (versatile)
- ✓ Screening and confirmation in less than 1h (random access)

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



Results
- ✓ 3.7% CV after 250 consecutive injections (non-stopping & corrected IS)
- ✓ <2h from start to end
- ✓ No need to clean/replace the transfer capillary

5. Total CBS solution minimizes instrumental carry-over



Stats	Methotrexate
P(T<=t) one-tail	0.24
P critical	0.05

Blank signals obtained before (**blue triangles**), during (**light blue squares**), and after (**purple circles**) injection of 10 intercalated highly concentrated samples of methotrexate at 5000 ng/mL

Results:
- ✓ Complete removal of analyte lingering at the MS inlet
- ✓ No statistical differences between injections before and after highly concentrated samples
- ✓ CBS-MS/MS and LC-MS/MS from the same blade (versatile)
- ✓ Screening and confirmation in less than 1h (random access)

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

Gómez-Ríos, G. A.; Pawliszyn, J. *Angew. Chem. Int. Ed.* **2014,** *53,* 14503–7

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