

Chromatography-Free DART-MS/MS for the Rapid Quantification of Clinically Relevant Compound Classes



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Introduction

- Many pharmaceuticals require optimal concentration levels in biological matrix for proper function.
- LC-MS/MS is the standard to monitor concentrations. But with run times of ~5 min for each blank, calibrator, QC, and patient sample, the needed total analysis time can build up to several hours.
- DART (Direct Analysis in Real Time)-MS/MS can provide a chromatography-free alternative to rapidly quantify multiple classes of compounds in serum or plasma with still simple sample preparation.
- DART: fast analysis in < 30 sec/sample, reduction of solvent and consumable usage by > 95%, no source contamination, no HPLC
- Quantification of multiple panels of drugs: antiepileptics, MPA, antimycotics for clinical research purpose.

Methods

- The liquid-liquid extraction (LLE) sample preparation is described in Fig. 1. For some analyses, 50 µL of a salt solution was added to assist in the phase separation.
- 5 µL of the upper layer was spotted onto an HTS-96 DART plate and dried for 5 min on the bench.
- MS/MS analysis on the EVOQ TQ+ equipped with a DART source (Bruker). Mass transitions, collision energy, source temperature, spot size, cone gas, and grid voltage were optimized for each panel.
- Linearity and precision were tested for all analytes.
- High-throughput analysis was achieved with DART run times of less than 30 sec/sample, allowing for the analysis of 96 samples in under 48 min.

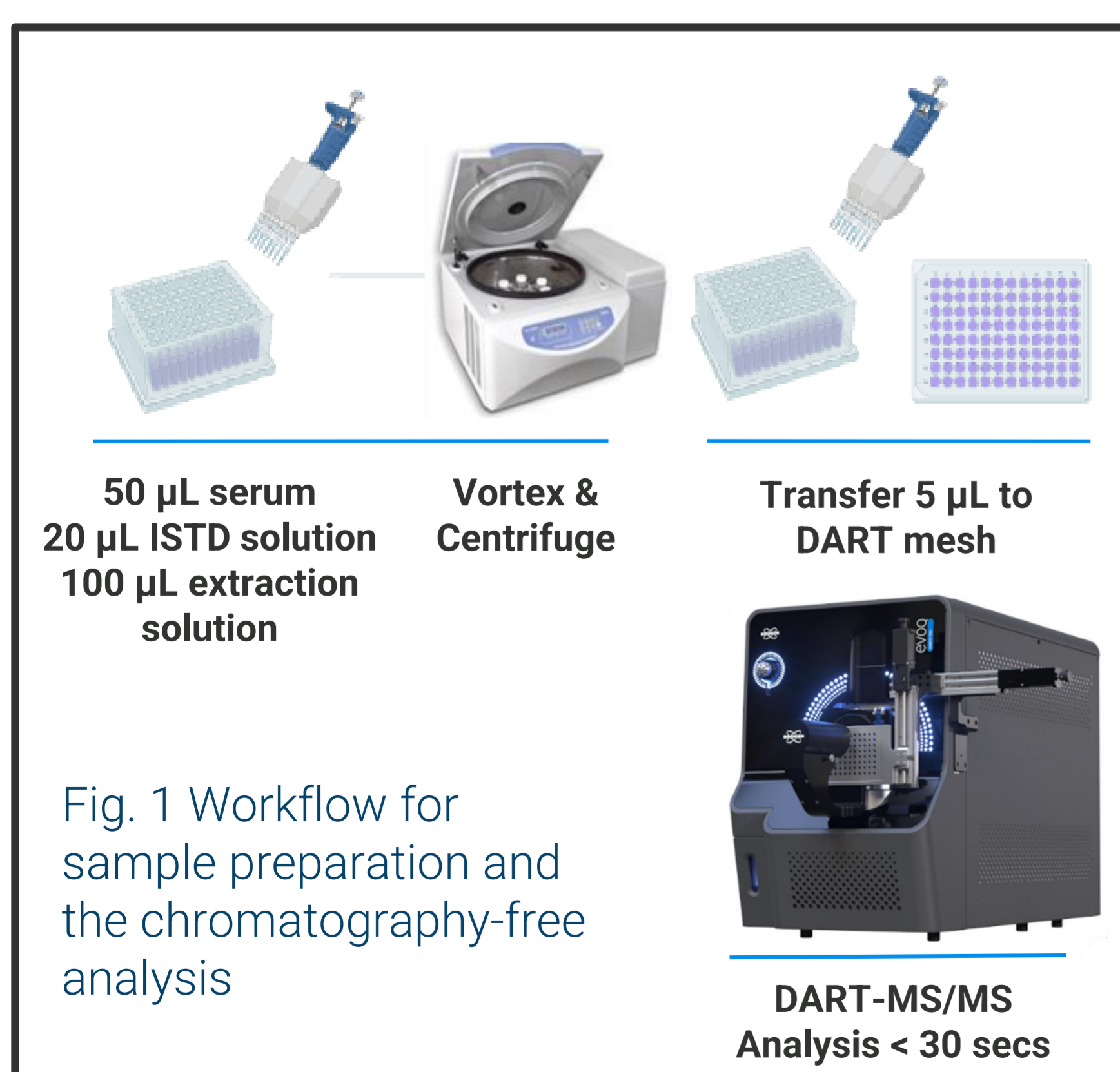


Fig. 1 Workflow for sample preparation and the chromatography-free analysis

Results

Linearity (Fig. 2):

- 5 replicate calibration series in serum at 12 concentration levels to cover the relevant concentration range for each compound
- Excellent linearity ($R^2 > 0.99$) with acceptable %CV at all levels.

Intra- and interday precision:

- Using matrix-matched QCs at 3 concentration levels, over 10 days with 4 timepoints/day.
- Precision was within required limits and trueness was 85% - 115% for all analytes.

Cross-correlation with LC-MS/MS (Fig. 3&4):

- Key compounds with high volume of patient samples: topiramate, zonisamide, rufinamide, perampanel, brivaracetam, lacosamide, mycophenolic acid, fluconazole, itraconazole, OH-itraconazole, isavuconazole, posaconazole, voriconazole.
- 223 samples. The comparison passed all criteria with an average bias of 3.1 – 13.2%.
- 105 spiked serum samples were also prepared and tested for all analytes across the analytical ranges.

Stability, LOQ, carry-over:

- The stability of extracted samples after spotting on the mesh was verified at room temperature over 7 h.
- No interference was found in these studies for 144 substances including neuroleptics, antidepressants, and benzodiazepines.
- LOD and LOQ values were highly sensitive to measure relevant concentrations for all compounds.
- Matrix effect and extraction efficiency experiments met criteria, confirming the method's reliability in serum and plasma.
- No carryover was observed between low and high samples and internal standard contribution at LLOQ was < 20% for all analytes.

Panel	Analyte	Fit type and weighting	R ²	Linear Range [mg/L]
Antimycotics	5-Fluorocytosine	linear, 1/x	0.9989	1.046 – 222.0
	Fluconazole	linear, 1/x	0.9982	0.134 – 28.2
	OH-Itraconazole	linear, 1/x	0.9977	0.036 – 7.82
	Isavuconazole	linear, 1/x	0.9990	0.229 – 20.4
	Itraconazole	linear, 1/x	0.9983	0.065 – 5.96
	Ketoconazole	linear, 1/x	0.9986	0.08 – 17.68
	Posaconazole	linear, 1/x	0.9979	0.116 – 10.84
Antiepileptics Panel A	Voriconazole	linear, 1/x	0.9954	0.259 – 11.7
	10-OH-Carbamazepine	linear, 1/x	0.9958	1.345 – 84.0
	Brivaracetam	linear, 1/x	0.9960	0.149 – 4.21
	Felbamate	linear, 1/x	0.9914	3.580 – 214
	Lacosamide	linear, 1/x	0.9936	0.176 – 27.2
	PEMA	linear, 1/x	0.9929	0.072 – 11.9
	Perampanel	linear, 1/x	0.9933	0.049 – 2.32
	Phenobarbital	linear, 1/x	0.9984	0.728 – 107.8
	Rufinamide	linear, 1/x	0.9979	0.54 – 84.2
	Stiripentol	linear, 1/x	0.9923	0.575 – 15.6
	Tiagabine	linear, 1/x	0.9973	0.012 – 0.76
MPA	Topiramate	linear, 1/x	0.9981	0.6 – 35.8
	Zonisamide	linear, 1/x	0.9983	1.555 – 88.2
MPA	Mycophenolic Acid	linear, 1/x	0.9934	0.038 – 15.6

Fig. 2 Linearity Results

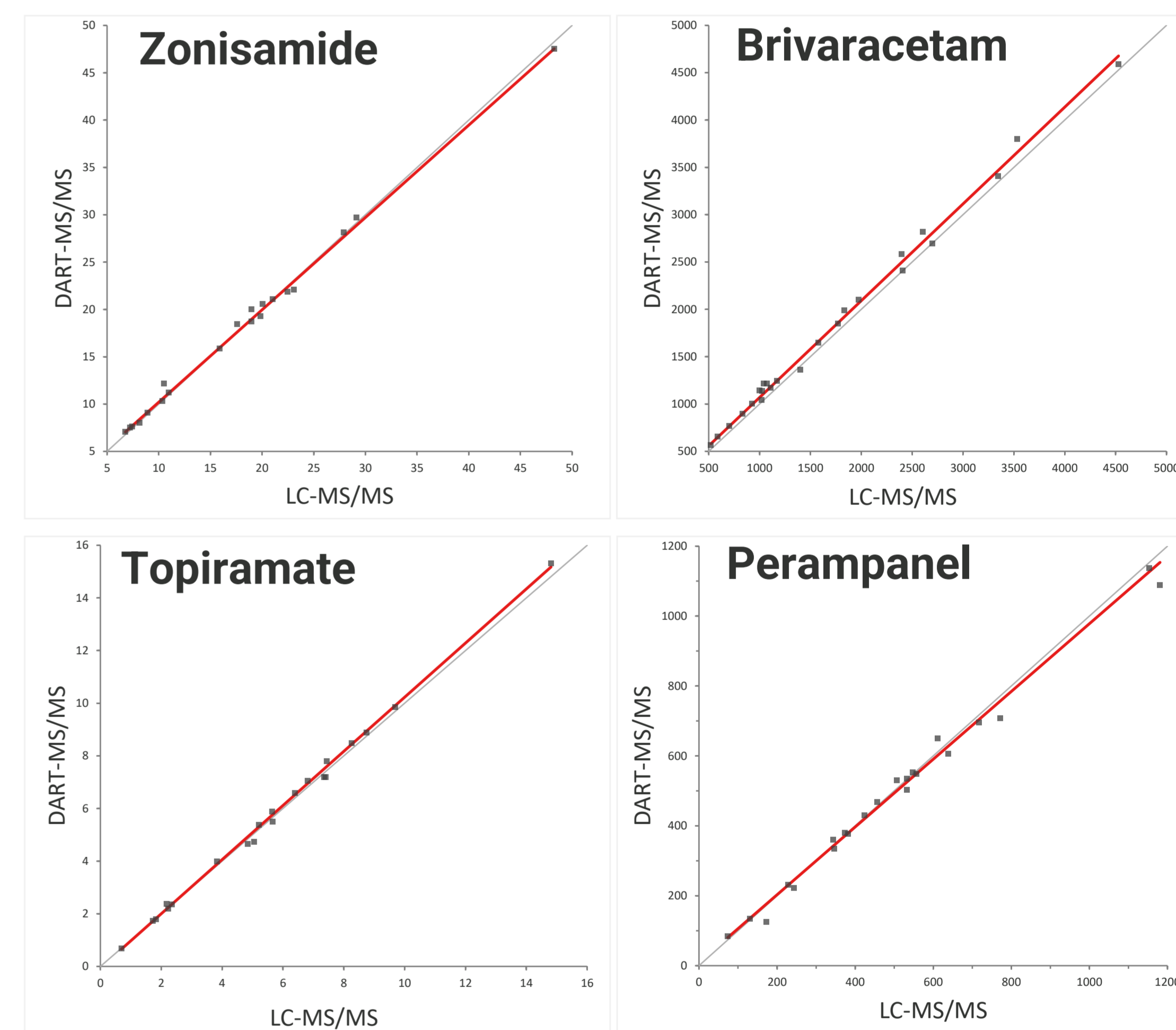


Fig. 3 Example cross-correlations between LC/MS and DART

Analyte	Slope	N	R ²	Average Bias
Fluconazole	1.091	7	0.9811	3.6%
OH-Itraconazole	0.958	6	0.9992	4.7%
Isavuconazole	0.872	18	0.9866	12.9%
Itraconazole	0.869	6	0.9979	9.9%
Posaconazole	1.218	17	0.9899	17.0%
Voriconazole	0.970	19	0.9941	9.9%
Brivaracetam	1.022	24	0.9955	6.9%
Lacosamide	1.026	27	0.9940	2.9%
Perampanel	0.969	22	0.9908	5.2%
Rufinamide	0.9113	6	0.9983	3.5%
Topiramate	1.028	21	0.9972	3.1%
Zonisamide	0.976	20	0.9964	3.1%
Mycophenolic Acid	1.052	30	0.9962	13.4%

Fig. 4: Summary of the cross-correlation slopes between LC-MS/MS and DART-MS/MS

Conclusion

- The studies demonstrate the suitability of DART-MS/MS to provide a chromatography-free alternative for rapidly quantifying various classes of pharmaceuticals in serum and plasma
- Maximized throughput with a total analysis time of less than 30 seconds per sample
- Minimal solvent and gas usage
- Highly reduced maintenance and troubleshooting
- Simple sample preparations
- Next: expansion to compound classes like TCAs, neuroleptics, benzodiazepines, vitamins, and antibiotics.

EVOQ DART-TQ+