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

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Introduction

- Many pharmaceuticals require optimal concentration levels in biological matrices for proper function.
- LC-MS/MS is the gold standard to monitor these concentrations. But with run times of ~5 min for each blank, calibrator, QC, and patient sample, the 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/plasma with still minor sample preparation.
- DART: fast analysis in < 30 sec/sample, 95% reduction of solvents and consumables, no ion source contamination, no HPLC troubleshooting
- Shown here: quantification of multiple panels of drugs like antiepileptics, MPA, antimycotics for clinical research purposes.

Methods

Fig. 1 describes the liquid-liquid extraction (LLE) sample preparation. For some analyses, 50 µL of a salt solution was added to assist the phase separation.

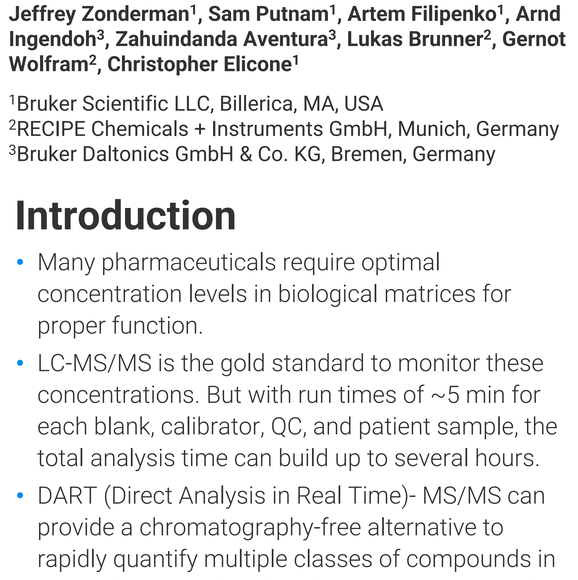

Fig. 1 Workflow for sample preparation and the chromatography-free analysis

- 5 µL of the upper layer were spotted onto an HTS-96 DART plate and dried for < 5 min on the bench.
- MS/MS analysis on the EVOQ® DART-TQ+ 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.

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
	Voriconazole	linear, 1/x	0.9954	0.259 – 11.7
Antiepileptics Panel A	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
	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

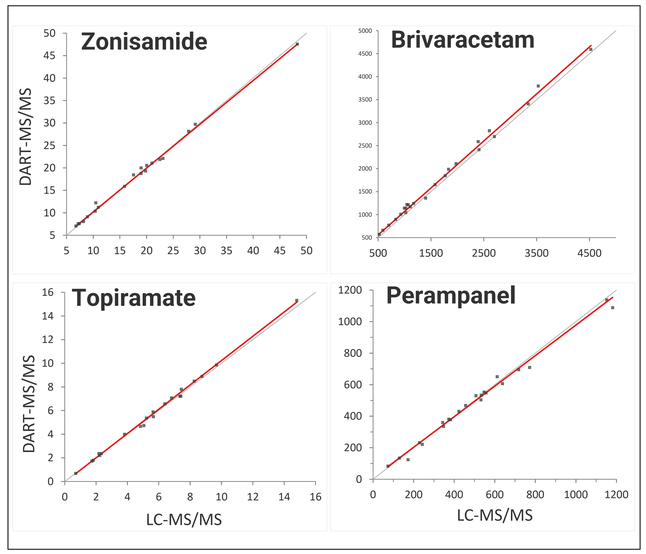

Fig. 3: Example cross-correlations between LC/MS and DART

Summary

The study data indicates the suitability of DART-MS/MS for the quantification of various compound classes like antibiotics, antiepileptics, antimycotics or tricyclic antidepressants. The methods' high throughput and simple sample preparations prove the potential of an advantageous alternative to traditional LC-MS/MS methods which require expensive HPLC columns, buffers, and long analysis times. It reduces operational costs and supports green chemistry. Next is the expansion to further compound classes such as TCAs, neuroleptics, benzodiazepines, vitamins, and antibiotics.

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

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