

Eliminating Method Development, Sample Preparation and Chromatographic Separations in High-Throughput Bioanalysis Using DART[®] on an Enhanced Resolution Triple-Quadrupole MS

Elizabeth Crawford¹, Shaoxia Yu², Brian Musselman¹ and Jing-Tao Wu²

¹IonSense, Inc., Saugus, MA, USA; ²Millennium Pharmaceuticals, Inc., Cambridge, MA, USA

Overview

Emerging Trends and Challenges for Quantitative Analysis in Biological Matrices (Bioanalysis)

- **Diverged trends in bioanalysis**
 - Routine bioanalysis is becoming a commodity
 - Throughput, turnaround or cost driven (Off-shore outsourcing)
 - Responsibility is being transferred from professional analytical chemists to trained personnel
 - The drive for increased understanding of pharmacology and toxicology demands new information from bioanalysis
- Biomarker quantitation
- Free drug concentration at the site of action
- Metabolite quantitation in the absence of standards
- Intracellular measurement
- **New technologies need to be developed to meet the challenges associated with these trends**
 - Faster, easier, and cheaper...
 - More precise sampling, more sensitive, more specific, more equi-molar response, less interference...

Introduction

In our previous work, we have demonstrated the use of ambient desorption ionization with metastable atoms (DART[®]) coupled to an MS/MS system for high-throughput bioanalysis without sample preparation and chromatographic separations [1,2]. While this system eliminated the bottleneck of sample preparation and chromatographic separation, each compound still needs to be tuned for optimal MRM transition channels, which is a relatively low throughput activity. The potential to achieve even higher sample throughput by eliminating MRM method development provides an attractive reprieve for the stressed bioanalytical labs in drug discovery. The combination of DART with an enhanced resolution triple-quadrupole mass spectrometer provides the potential to eliminate MRM method development in high-throughput bioanalysis by using the selected ion monitoring mode (SIM).

Why DART for Bioanalysis?

- Direct ionization methods offer potential advantages for bioanalysis, including speed, throughput, ease of sample preparation, etc.
- The fact that DART only works for a relatively low mass range offers the advantage for small molecule analysis in biological matrices
- DART is commercially available and more amenable for routine use
- Our previous data [1,2] have demonstrated good reproducibility and manageable matrix effects in directly analyzing samples in biological matrices

Methods

A Thermo Quantum Ultra[™] triple-quadrupole mass spectrometer with enhanced resolution capability was coupled with a DART ionization source. Each compound was analyzed to determine the relative sensitivity and mass value for subsequent SIM analysis using enhanced resolution. Plasma samples containing small molecule drug compounds were analyzed by using a CTC Analytics HTS PAL programmed to pick up and dip a glass capillary sampling tube directly into the plasma sample then pass the tube through the desorption ionization region to effect sampling. The elimination of chromatographic separation methods and simple entry of the mass of the protonated or deprotonated molecule of interest for the SIM acquisition file represents a significant reduction in the typical bioanalytical work flow.



Figure 1. DART-ET ionization source mounted with a Vapur Interface on a Thermo Quantum Ultra triple quad MS.

Potential Niches of DART for Bioanalysis

- High-throughput in vitro ADME samples
 - Metabolic stability/intrinsic clearance in microsome/S9
- Real-time estimate of drug levels
 - Determine dilution factors for high-concentration PK/TK samples
- AQL samples are a frequent cause of delay
- Dilution factor is often difficult to determine
 - Compare two oral formulations in a pilot study before an animal efficacy study
- Analytical tool of choice for sampling dried blood spots

DART Coupled to a Triple-Quadrupole with Enhanced Resolution

- Enhanced resolution provides added specificity in the absence of chromatographic separation
- For certain types of applications, enhanced resolution allows for the use of SIM/MIM mode for quantitation and thus eliminate the need for MRM method development
- SIM/MIM mode is a good match of the DART throughput

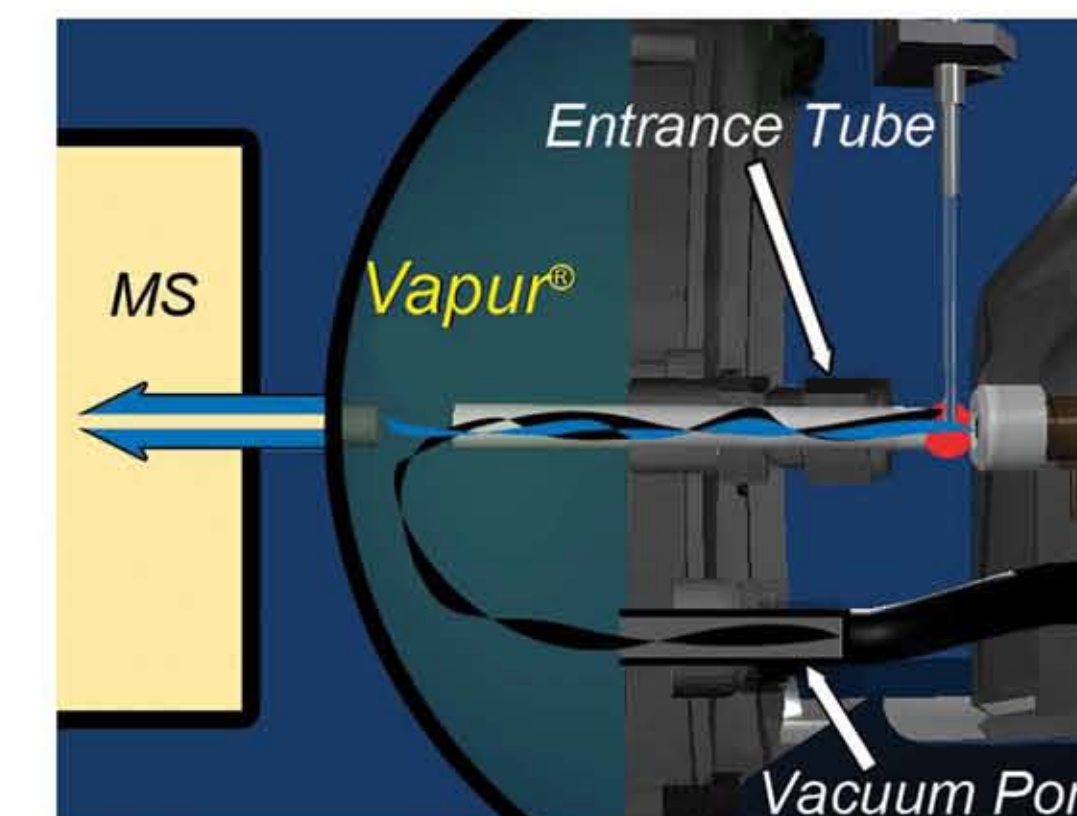


Figure 2. The Vapur Interface provides improved ion transmission into the MS API inlet, while removing the majority of the DART carrier gas.

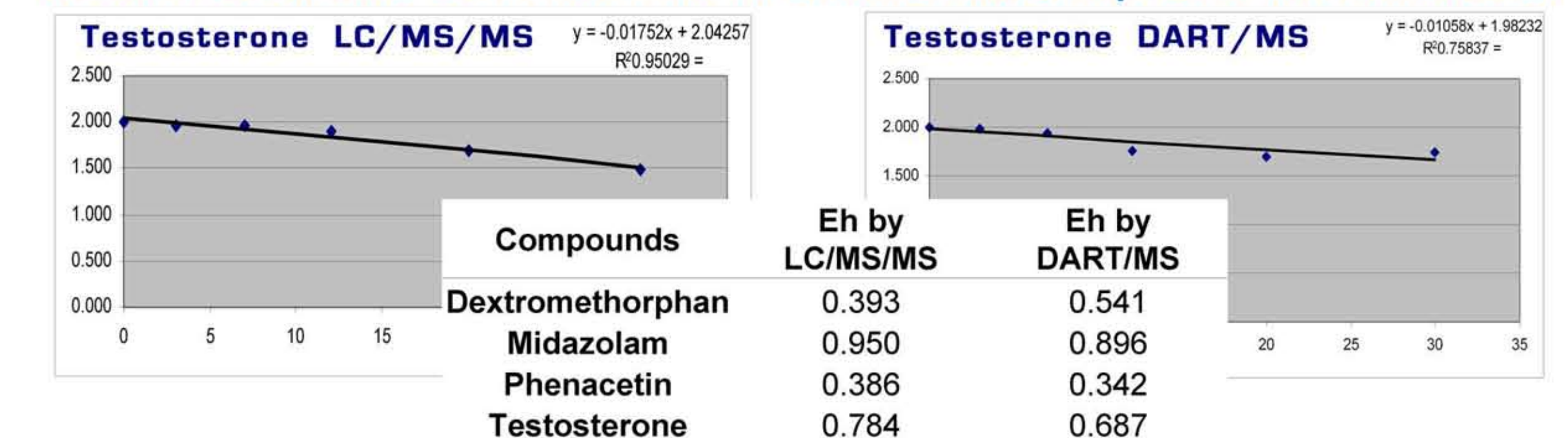
Dilution Factor Determination

- Samples may be out of the quantitation range due to the use of inappropriate dilution factors when...
 - There is no historical data for the compound in a specific species
 - Non linear pharmacokinetics
- These out-of-range samples cause delay and inefficient use of resources
- The use of DART helps determine appropriate dilution factors
 - Quickly estimate a ballpark concentration of PK/TK samples in early time points before sample analysis

Results

The combination of DART with an enhanced resolution mass spectrometer provides a viable approach for high-throughput and real-time screening of ADME properties of drug molecules.

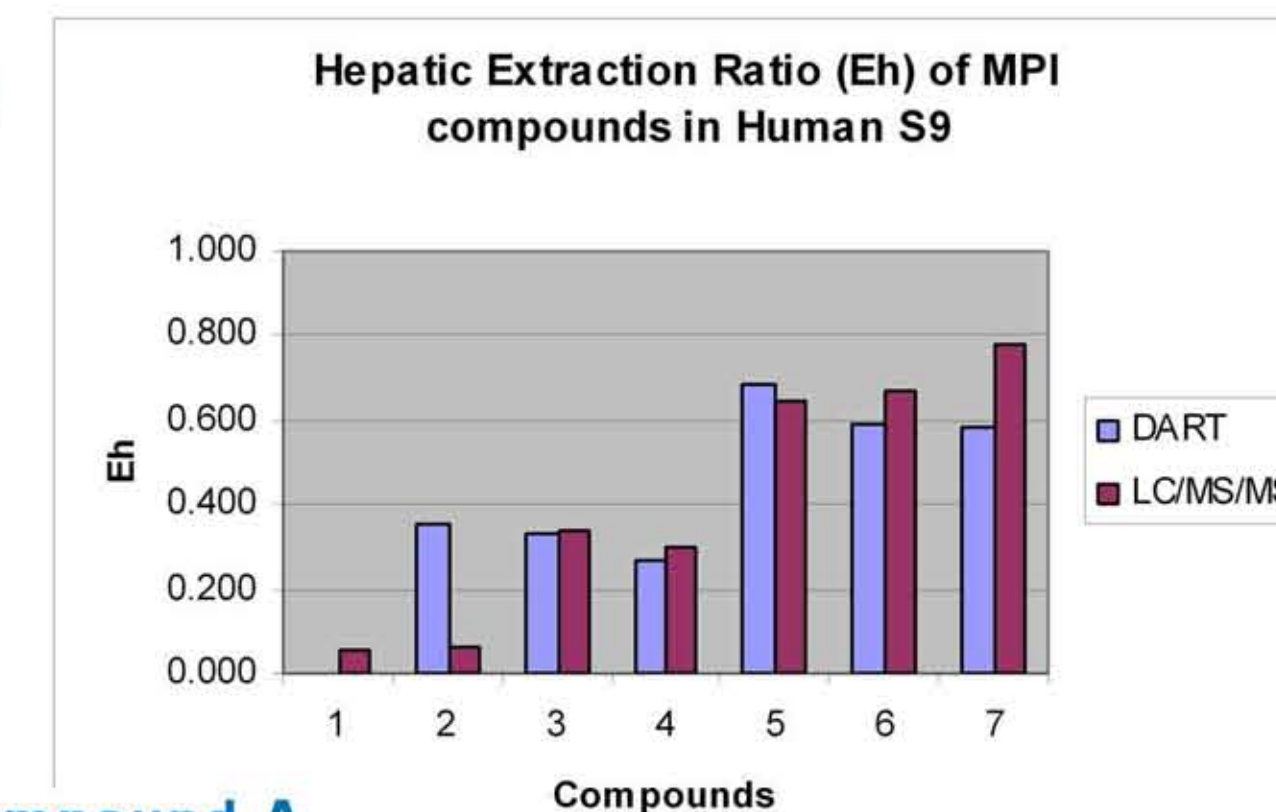
Intrinsic Clearance Determination of Commercial Compounds in Human Liver



Plasma Stability

% Remaining after 2hr Incubation at 37 °C

	LC/MS/MS	DART/MS
MPI 8		
Human Plasma	80.7 %	80.9 %
MPI 8		
Rat Plasma	89.2 %	89.9 %
MPI 9		
Human Plasma	76.4 %	85.7 %



Dilution Factor Prediction for Compound A 350 mg/kg PO dose in a rat tox study

Sample Name	Concentration Predicted With DART/MS (nM)	Dilution Factor	Concentration Obtained by LC/MS/MS (nM)	MPD%
Subject 1, 0.25 hr	21900	100	20200	5
Subject 1, 0.5 hr	39700	100	31300	21
Subject 1, 1 hr	38600	100	39300	-5
Subject 1, 4 hr	12200	100	12800	-8

Dynamic Range of 1 - 1,000 nM

Conclusions

- When combined with an enhanced resolution triplequadrupole instrument, DART offers the potential for:
 - high-throughput bioanalysis of in vitro ADME samples
 - "Real-time" analysis for certain types of in vivo samples
- In vivo PK samples may not be a niche application for DART at this point due to the compromises in sensitivity and specificity compared to conventional LC-MS/MS based methods
- The use of DART as a sampling tool for samples collected as dried blood spots may offer new advantages

References

- [1] Yu S et al. Proc. 56th ASMS Conf. Mass Spectrometry and Allied Topics, Denver, Colorado, 2008.
- [2] Yu S, Crawford E, Tice J, Musselman B, Wu JT. *Anal. Chem.* 2009; **81**: 193.