

Q&A questions from our recent webinar

DART® and mosquito: ultra-low volume deposition of samples improves target signal to noise and decreases matrix suppression with DART-MS

01 Do you need to use a mosquito for this or can we use any automated liquid handler?

- A** We used 3-5µl of sample for many years, mainly with volatile solvents that evaporated before analysis. Those volumes were too high for us to use with low vapor pressure solvents. Once we started using smaller volume samples the results were so encouraging contacted SPT and we have had it in our lab since then. Of course, with smaller volumes the high degree of precision and reproducibility of the drop placement when using mosquito with our QuickStrip was critical to the development of JumpShot.
- B** Additionally, most people are carrying out nanoscale chemistry in 1536 wells, primarily due to the geometric properties of the well wells, and hence you require a liquid handler that is able to directly aspirate from these 1536 reaction well in low volumes and transfer to the Quickstrip/mesh for DART analysis. mosquito is ideal for this.

02 Can you use the same instrument for nanoscale reaction set up as for loading the DART QuickStrips?

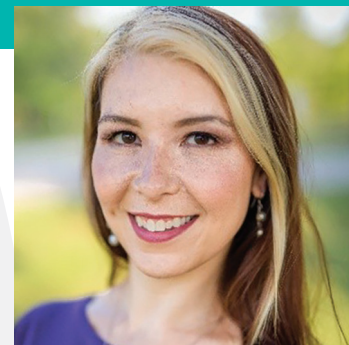
- A** Yes, absolutely, this is a completely standard mosquito LV, the exact same instrument that many people are using for nanoscale HTE (high throughput Experimentation) set up.

03 How is mosquito able to handle the volatile solvents used in some of these reactions?

- A** The mosquito uses disposable, positive-displacement pipette tips to aspirate and dispense liquids. These tips are made from inert and solvent resistant materials, stainless steel for the piston and HDPE for the barrel, and being positive displacement there is no issue with liquids dripping out of the end of the tips as you would expect with air displacement pipettes. mosquito is also very fast, and can even piece 384 well plate seals, so evaporation can be kept to a minimum.
- B** I would add that IonSense has used the mosquito for addition of internal standard to low volume samples. the increase accuracy of volume control in the microliter range can facilitate the quantitation with limited sample manipulation.

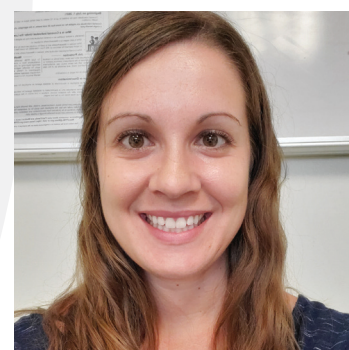
04 How does this set up enable nanoscale chemistry at higher throughputs?

- A** The final step in HTE is product detection and the community predominantly uses LC US/VIS and LC/FIMS methods which are time consuming. The JumpShot HTS can process a 96 well plate in under 6 minutes and 384 in under 22 minutes.



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mosquito spots the plates in a few minutes making the process very efficient. The potential for sampling at increasing time intervals is feasible with DART/MS utilizing mosquito to sample direct from the reaction plate without using quenching solvents. Its also more economical since sample processing is minimal.

- B From SPT's perspective the it helps in two ways, firstly it reduces the barrier to entry in terms of equipment needed to start on nanoscale synthesis, as most labs doing this already have, or plan to get a mosquito for reaction set up. Having this same instrument be used for the analysis steps represents a significantly improved ROI. Secondly, for labs that do not wish to make the significant investments in either high throughput MALDI, or Acoustic MS, then this represents a relatively high throughput analysis method, and an affordable price point, which eliminates one of the major bottle necks to wider adoption of this technique.

05 Are internal standards necessary for quantification with this method? How does the Analyzerpro software incorporate the internal standard processing?

Incorporating internal standards with this setup can certainly improve the quantitation. We have data demonstrating improved reproducibility of the analysis. Operationally, using the quantitation tab of analyzerpro, there is a column where you can insert the mass of the internal standard and the internal standard normalization. With those values set correction will automatically be calculated and reflected in the calibration curve and quantitation values.

06 Which mass spec(s) can be used with this setup?

The DART source is compatible with most major mass spec vendors, including Thermo, Agilent, Waters, JEOL, Bruker, Shimadzu, and Sciex. The AnalyzerPro software is compatible with data files from all of the vendors with the exception of a few Sciex models. Composition analysis is routinely completed using high resolution MS systems which quantitation requires MS/MS capable systems

07 Are there any contamination/carryover issues with this analysis?

In general carryover is not an issue especially with JumpShot and use of low volume samples. The rapid vaporization of 1 ul and sub-microliter samples coupled with the use of pulsed gas reduces the overall volume of chemicals being desorbed into the ionizing gas. It is possible to increase the volume of sample to where you are analyzing several microliters at a time, however your chances of carryover could go up. We utilized the mosquito specifically to enable more accurate volume control with these viscous, low vapor pressure solvents used in HTE. Larger volumes of sample diluted in volatile organic solvents can be analyzed, however the sample should be dried before desorption to avoid carryover.

08 Has the method been tested against other figures of merits apart from linearity?

Other figures of merit for this method vary depending on the target compounds and the mass spec. In general %RSD resides around 15-20% (without internal standard normalization), and LOD's and LLOQ's range from low to mid ppb range.

09 In the shown quantitation example, what was the approximate LLOQ?

In the specific quantitation example shown, the approximate LLOQ was 78 ppb. While LLOQ is both compound specific and dependent on the MS system, generally LLOQ's range from the low to mid ppb range with DART.

10 If I already have an older version of DART, Is it possible to upgrade to the pulsed part?

DART upgrades to JumpShot with the pulse gas capability are available. The DART controller must have the serial number of SVP20XXX or SVP21XXX or higher. DART-100 units are not compatible with the upgrade, however there is the possibility of a trade-in discount.



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