

Cryogenic Milling: An Enabling Technology for High Throughput Residue Sample Preparation

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Introduction

The sample preparation processes in residue analytical methods have classically been the rate limiting step in the laboratory workflow. The superior sample comminution achieved in cryogenic milling has been used in our labs to dramatically improve sample preparation throughput across a range of products including glyphosate, acetochlor, dicamba and more. Sample homogeneity has been thoroughly characterized with excellent precision in replicates for sample sizes down to 75 mg.

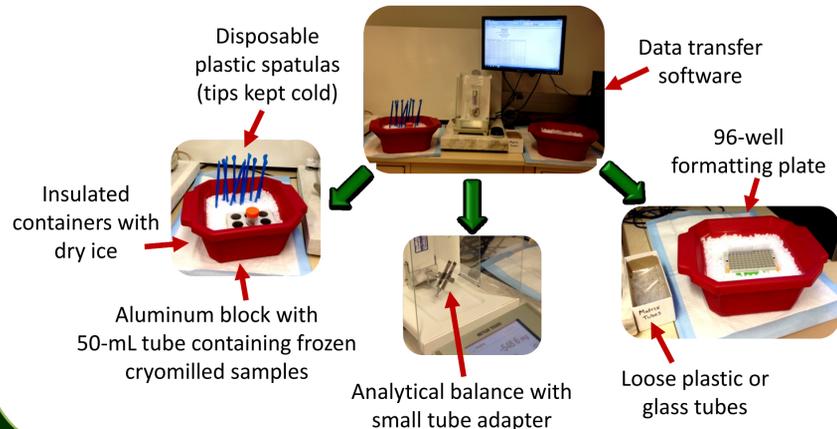
Why Most Residue Methods Have Limited Throughput

- Bulk sample processing (VCM with dry ice) has limitations with homogeneity
- Requires larger subsamples to be extracted to attain a “representative” and reproducible result
- These large samples (4+ g) force the use of high-volume and low-throughput manual extractions
- Subsequent processes often continue to maintain these large volumes further minimizing sample throughput downstream
- Centrifugation, filtration, LLE, SPE and evaporation are all limited by the size of the extraction container

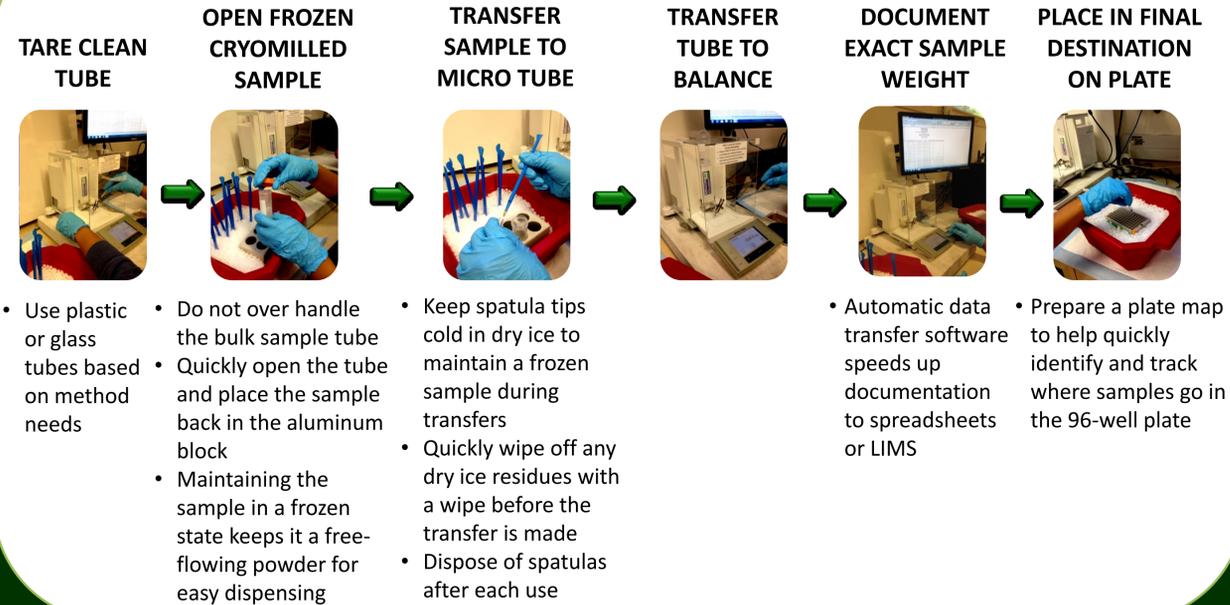
Advantages of Process Scale-Down

- Can process 70+ samples on a single plate
 - Process large numbers of samples 4x faster (or more!)
 - Multiple plates processed in a single day per analyst (method dependent)
 - Generate results more quickly for decisions and study turnaround
 - Steps like centrifugation, LLE, SPE, evaporation and others are performed on a single plate in parallel
 - Process lends itself to automation
- Improved quality
 - Fewer manipulations and less opportunity for error
 - Improved consistency across a plate
 - Less “art” and variability from analyst to analyst
 - Lower extraction volumes make internal standards feasible throughout the process
- Save cost on materials
 - Cheap consumables vs. time spent washing glassware
 - Dramatic reduction in solvent use, consumption and disposal
 - Improved safety and ergonomics
 - All equipment needed is commercially available - Only a few key pieces of relatively inexpensive equipment are required (beyond the analytical tools you already have)

Micro-Dispensing Station



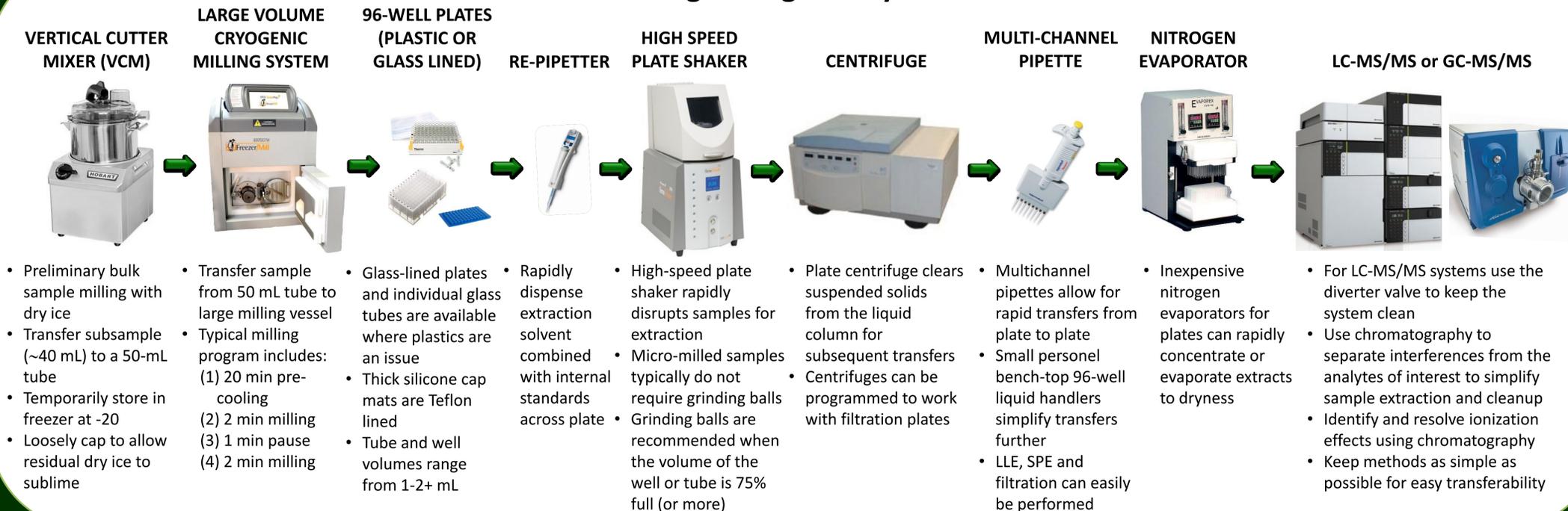
Micro-Dispensing Process



How Well Does it Work?

- Process has been successfully utilized for residue studies for glyphosate, dicamba, acetochlor and other proprietary compounds
 - Methods have precision and accuracy well within guideline requirements and equivalent or better than previous scaled-up versions
 - Hydrolysis for common chemophore methods can be performed in 96-well format
- Challenging matrices like undelinted cotton seed, corn stover, sugarbeets and soil can be micro-milled to a fine powder
- A recent round-robin was performed to demonstrate the transferability of the micro-milling process
 - Homogeneity was demonstrated through the precision of replicate subsamples
 - Combinations of glyphosate/AMPA (Monsanto) or Spinosyn D/Spinosyn A (Dow AgroSciences) were fortified at 100 ppb before cryomilling
 - Matrices including corn grain, corn forage, corn stover, soybean seed, undelinted cotton seed and oranges were tested
 - **% RSDs for six subsamples taken from each fortified and cryomilled sample ranged from 6-13%**
- Process is also a great way to prepare samples for long-term frozen stability studies
 - Prepare and dispense all samples at time 0
 - Pull replicate sample tubes at stability interval from freezer
 - Place in plate with standards and QCs – Process and Analyze!

Processing through Analysis



Conclusions

- Incorporation of cryogenic milling enables direct scaledown and dramatically increases subsequent sample throughput
- Adaptation requires only a few key pieces of relatively inexpensive, commercially available equipment
- Process can be set up to further maximize efficiency
- Method (and overall lab) safety can be greatly improved