In-Well Hydrolysis Using UCT's Refine™ Ultra-Filtration Technology

UCT Part Numbers

RFNUCT96
96 Well Ultra-Filtration Plate

OR

RFNSPE1
1 mL Ultra-Filtration Column

OR

RFNSPE3
3 mL Ultra-Filtration Column

UASBETA-GLUC-10
Abalonase™ Ultra purified Beta-glucuronidase

Summary:

Various biological sample types commonly contain proteins that complicate downstream analysis if not effectively removed. While sample preparation techniques, such as solid phase extraction (SPE), can successfully remove these unwanted matrix components, complex and lengthy extraction procedures are not ideal for high through-put laboratories. With the advancement of LC-MS/MS technology, many laboratories have implemented dilute and shoot procedures in order to reduce sample preparation time, reduce consumable spending and reduce overall turnaround time. The caveat to this quick protocol is the toll that it ultimately takes on the HPLC column and mass spec source.

UCT's Refine™ Ultra-Filtration Technology allows for sample precipitation and filtration to occur simultaneously in the individual column/well. A novel, hydrophobically treated, submicron frit combination facilitates the removal of sample proteins without a complicated extraction. This application note outlines a suggested procedure for in-well hydrolysis of urine samples. Using the Refine™ Ultra-Filtration Technology reduces column backpressure while also reducing analyte suppression, when compared to a standard dilute and shoot protocol.

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Extraction Procedure:

1. Place an appropriate collection container under the Refine™ Ultra-Filtration Plate or Column
2. Add 250 µL of sample and 250 µL Abalonase™ Ultra Enzyme working solution to each respective well.
3. Hydrolyze for 30 minutes at 55°C.
4. Add 500 µL D.I. H_2O to each respective well.
5. Mix the sample. This can be executed via vortexting at maximum speed or multiple pipette aspirations and dispenses.
6. Filter the sample using one of the following techniques:
    a. Centrifuge: For 5 minutes at 500 g or until filtrate is collected.
    b. Vacuum: Apply vacuum at ~20" of Hg for up to 5 minutes or until filtrate is collected.
    c. Positive Pressure: Apply 2-5 psi using positive pressure for up to 5 minutes or until filtrate is collected.

Results – Analyte Suppression:

Upon passing urine samples containing hydrolysis enzyme through the Refine™ Ultra-Filtration plate, analyte suppression was significantly reduced across the board for the monitored benzodiazepine and opiate panels ranging from 7-71% improvement.

Table 1. Percent Reduction in Analyte Suppression Following Use of Refine™ Plate Compared to Dilute and Shoot

	Concentration (ng/mL)				
Analyte	**25**	**50**	**100**	**250**	**500**
Alprazolam	18%	26%	21%	23%	15%
Clonazepam	12%	16%	18%	11%	10%
Diazepam	37%	29%	34%	26%	21%
Lorazepam	10%	14%	19%	12%	13%
Midazolam	19%	28%	23%	16%	21%
Nordiazepam	18%	14%	31%	13%	12%
Oxazepam	20%	24%	27%	16%	13%
Codeine	34%	15%	19%	23%	29%
Hydrocodone	50%	27%	34%	30%	24%
Hydromorphone	54%	28%	38%	40%	27%
Morphine	71%	35%	45%	65%	36%
Oxycodone	10%	12%	19%	26%	21%
Oxymorphone	33%	10%	16%	30%	24%
6-MAM	30%	14%	21%	13%	10%

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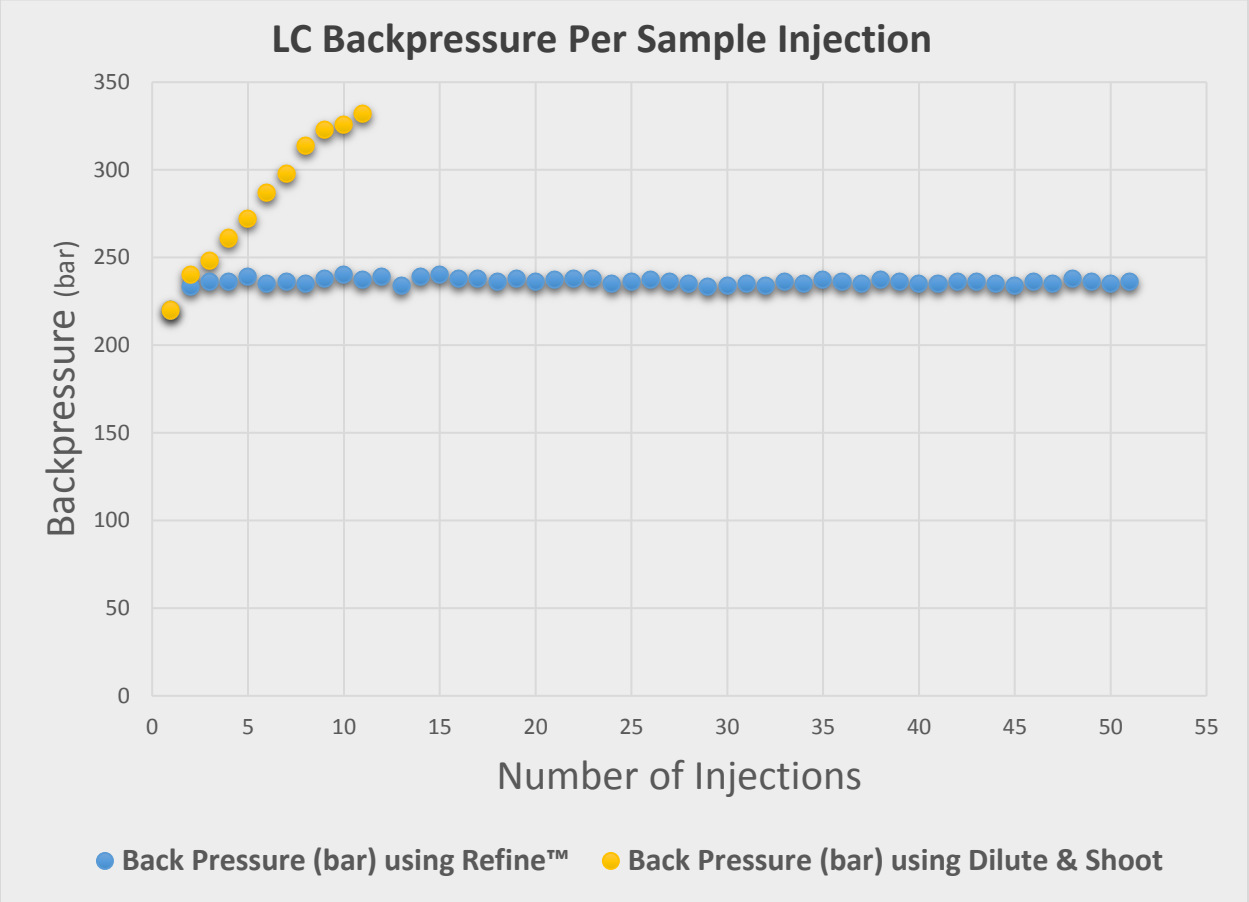
Results – Backpressure Stabilization:

Backpressure per sample injection was also assessed when comparing the utilization of Refine™ Ultra-Filtration clean-up technology versus a Dilute & Shoot preparation for hydrolyzed urine samples. After 50+ injections, the back pressure remained stable when utilizing the Refine™ plate as opposed to D&S, where a drastic increase was seen after as little as 10 injections.



Conclusion:

Using the above procedure, the Refine™ Ultra-Filtration technology allowed for a reduction in instrumental back pressure and removal of unwanted matrix components in urine samples. Overall, this allows for an end user to have enhanced analyte selectivity and an increase in HPLC/UPLC column life. The extraction procedure can be completed in approximately 20 minutes and can easily replace a dilute and shoot protocol with minimal additional investment in laboratory resources. The hydrophobically treated submicron frit combination allows for efficient in-well hydrolysis and is amenable to both forensic and clinical laboratory settings.

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