

CLEAN SCREEN FASt® Filter and Shoot



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Since the introduction and implementation of LC/MS as a staple analytical tool in forensic laboratories, there have been new approaches to sample preparation. The higher sensitivity of LC/MS and the ability to inject 'aqueous' containing samples directly into the instrument has opened new options for conventional sample preparations. The need for rapid turnaround time for a larger list of drugs has also put pressure on laboratories to find alternatives to traditional methods. The usual liquid -liquid and solid phase extraction processes have seen a growth of 'crash and shoot' or 'dilute and shoot' sample preparation methods. Although these latter methods work most of the time for certain applications (i.e. primarily urine samples), these alternatives have also introduced new shortcomings.

LC/MS analysis is very prone to matrix suppression phenomenon. The 'crash' or 'dilute' methods no longer remove matrix and concentrate samples but instead dilute the final eluate. These methods can raise the LOD and by definition, lower the sensitivity of the method. The diluted samples will still contain unwanted matrix that when introduced into the system can contaminate the instrumentation. In addition, these methods usually require a 10-15 minute centrifugation of the samples prior to injection. This step is done to eliminate any particulates that might get caught in either the guard column or more expensive LC columns. Most LC column packing particle sizes are not greater than 5um and can therefore be subject to clogging by certain samples.

CLEAN SCREEN FASt[®] employs a process that uses positive pressure and a solid phase sorbent bed built with small pore frits to quickly and efficiently prepare samples for LC/ MS analysis. This method eliminates the timely centrifugation, reduces matrix suppression effects and removes particulates greater than ~ 1µm. Samples can be diluted at a ratio as low as 1:1, which is useful for analytes at very low concentrations.

A FASter AND CLEANER SPE ALTERNATIVE TO 'DILUTE AND SHOOT'

PART #: CSFAS203 CLEAN SCREEN FASt® 200mg/3mL ZSFAS020 CLEAN SCREEN FASt® 200mg/10mL WSH96FAS11-10LD 96 Deep Well Plate 100mg

I. FASt Method – Opiates

Sample Dilution Ratio	Sample* Volume	Dilution** Volume
1:1	500 μL	500 μL
1:4	200 µL	800 µL
1:9	100 µL	900 μL

* If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

** Diluent is 50:50 (Methanol: Distilled Water)

- Sample and diluents are added in an appropriately labeled tube. Add appropriate volume internal standard(s). It is recommended to use an internal standard volume of no more than 200 μL.
- 2. Set up extraction manifold with FASt cartridges and auto-sampler collection vials.
- 3. Pour sample into FASt cartridge and elute sample directly into auto-sampler vials.
- 4. Cap vials and put directly onto LC/MS for analysis.

II. FASt Method – Benzodiazepines and Basic Compounds

Sample Dilution Ratio	Sample* Volume	Dilution** Volume
1:1	500 μL	500 μL
1:4	200 μL	800 µL
1:9	100 μL	900 µL

* If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

** Diluent is 50:50 (Acetonitrile: Distilled Water)

- 1. Sample and diluents are added in an appropriately labeled tube. Add appropriate volume internal standard(s). It is recommended to use an internal standard volume of no more than 200 µL.
- 2. Set up extraction manifold with FASt cartridges and auto-sampler collection vials.
- 3. Pour sample into FASt cartridge and elute sample directly into auto-sampler vials.
- 4. Cap vials and put directly onto LC/MS for analysis.





LC Column: Restek Ultra Biphenyl 5 um 100x2.1mm - Catalog#: 9109512

Opiate LC Method

% B 25 50 50 25 25	Time (min) 0.00 3.00 6.00 6.01 9.00	Flow Rate 0.4 mL/min	Run Time: 9.00 m Injection Volume Column Oven Ten	:10µL	Mobile	Phase A: 0.1% Formic Acid H20 Phase B: 0.1% Formic Acid MeOH
	3,445 3,045 2,045 2,045 1,545	1:4 Dilution Benzodiazepine Analytes		ANALYTE 1 7-amino clonazepam 2 Alprazolam 3 Nordiazepam 4 Temazepam 5 Diazepam 6 Clonazepam	CONCENTRATION 40 ng/mL 400 ng/mL 400 ng/mL 400 ng/mL 400 ng/mL	



Benzodiazepine Method

Isocratic Flow at 0.4 mL/min

0.15% B

Run Time: 8.50 min

Injection Volume: 10µL

0.15% A

Column Oven Temperature: 40°C

Mobile Phase A: 0.2% Formic Acid / 2mM NH4 Formate in H20 Mobile Phase B: 0.2% Formic Acid / 2mM NH4 Formate in ACN

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Basic Method

<u>%B</u>	<u>Time (min)</u>	Flow Rate	Run Time: 13.00 min	Mobile Phase A: 0.2% Formic Acid / 2mM NH4 Formate in H20
25	0.00	0.4 mL/min	Injection Volume: 10µL	Mobile Phase B: 0.2% Formic Acid / 2mM NH4 Formate in ACN
90 90 25		10.50 11.00 11.01	Column Oven Temperature : 40°C	







*NF refers to the 'dilute and shoot' recovery as a normalized referenced (e.g. 100%) based on calculated peak areas.

This chart represents 1:1, 1:4 and 1:9 signify the dilution ratios and the % recovery compared to the *NF sample based on calculated peak areas of each compound sampled in duplicate.

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THC-COOH IN URINE CLEAN SCREEN FASt® THC

PART#: CSFASTH203 CLEAN SCREEN FASt® THC 200mg/3mL ZSFASTH020 CLEAN SCREEN FASt® THC 200mg/10mL WSH96FASTH11-10LD 96 Deep Well Plate 100mg

I. Hydrolysis of Urine Sample for THC-delta-9-COOH:

- 1. To 2 mL urine add appropriate internal standards prepared.
- 2. Add 50 µL of 10 N NaOH. Heat for 15 minutes at 60-70 °C
- 3. Add 50 µL 1:1 acetic acid: DI water. (pH should be 7.0+1.0)
- 4. Add 200 µL pH 7.0 0.1M Phosphate buffer (The sample is ready to be filtered).

II. Load Sample:

SAMPLE DILUTE RATIO:

*No Centrifugation required prior to loading

Sample Dilution Ratio	Sample* Volume	Dilution** Volume	
Dilution Ratio	Urine	Diluent**	
1:1	500 μL	500 μL	
1:4	200 µL	800 μL	
1:9	100 µL	900 μL	

* If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.

** Diluent is 50:50 (ACN: Distilled Water)

- 1. Sample and diluents are added directly to 96 Well FASt Plate/Columns.
- Add appropriate volume of internal standard(s). It is recommended to use an internal standard volume of no more than 200 μL.

III. Filtration and Collection:

- 1. Set up extraction manifold with FASt well plates/columns and auto-sampler collection plates.
- 2. Pour sample into FASt well plate/columns and elute sample directly into auto-sampler collection vials.

IV. Analysis:

1. Place auto-sampler well plate/vials directly onto LC/MS for analysis.



Below are calibration curves, actual sample, and control data from THC-COOH positive urine samples. FASt THC (CSFASTH203) was used with the method found on page 6.

The indicated numbers in red parathenses are results from the same samples run with CSXCE2103 (CLEAN SCREEN XCEL[®] 2). This shows the accuracy and precision of this technique for THC-COOH analysis.



The FASt method outlined is a novel approach to improved sample preparation for LC/MS analysis. The method outlines a simple procedure to prepare urine samples for analysis of multiple drugs and metabolites, by quickly and efficiently reducing the amount of unwanted matrix (through sorbent adsorption) and particulates (filtering through special frits) in the final sample, the analysis can proceed with less chance of matrix suppression and LC column clogging. The FASt method can lengthen the amount of time an LC column can be used for analysis and lower the amount of down time for instrument maintenance. These benefits along with the ability to eliminate the centrifuge and sample transfer steps can lower costs by decreasing turn-around time and reducing instrument and LC column maintenance.









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