UCT CLEAN SCREEN FASt

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

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OVERVIEW / INTRODUCTION

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.

This poster describes a method 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 referred to as FASt, eliminates the timely centrifugation, reduces matrix suppression effects and removes particulates greater than ~ 1um. Samples can be diluted at a ratio as low as 1:1, which is useful for analytes at very low concentrations.

METHOD

I. <u>FASt Method – Opiates</u>

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

* 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 uL.
- Set up extraction manifold with FASt cartridges and auto-sampler collection vials.
- Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.
- Cap vials and put directly onto LC/MS for analysis.

II. <u>FASt Method – Benzodiazepines and Basic Compounds</u>

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

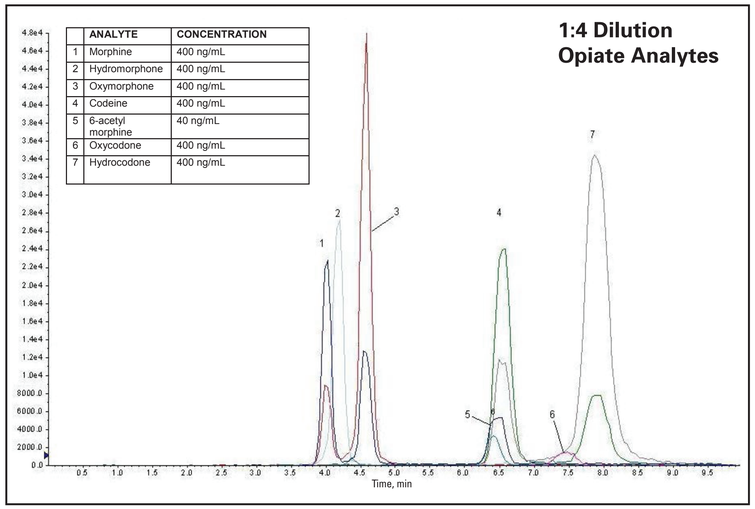
* If sample is hydrolyzed add appropriate aliquot volume after hydrolysis is complete.
** Diluent is 50:50 (Acetonitrile: Distilled Water)

- 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 uL.
- Set up extraction manifold with FASt cartridges and auto-sampler collection vials.
- Pour sample into FAST cartridge and elute sample directly into auto-sampler vials.
- Cap vials and put directly onto LC/MS for analysis.

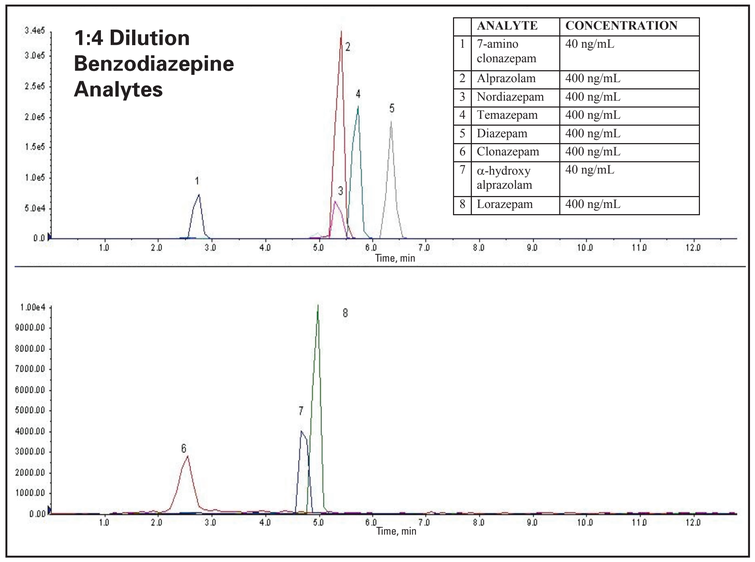
CONCLUSION

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.

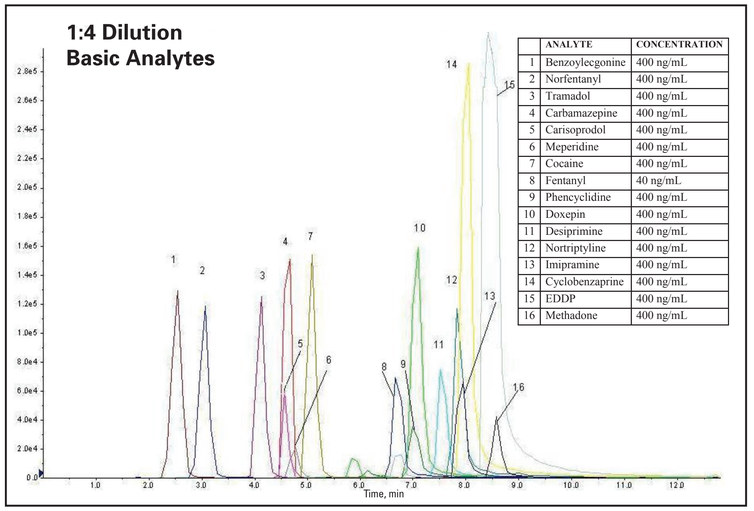
URINE SPECTRA



	ANALYTE	CONCENTRATION
1	Morphine	400 ng/mL
2	Hydromorphone	400 ng/mL
3	Oxymorphone	400 ng/mL
4	Codeine	400 ng/mL
5	6-acetyl morphine	40 ng/mL
6	Oxycodone	400 ng/mL
7	Hydrocodone	400 ng/mL

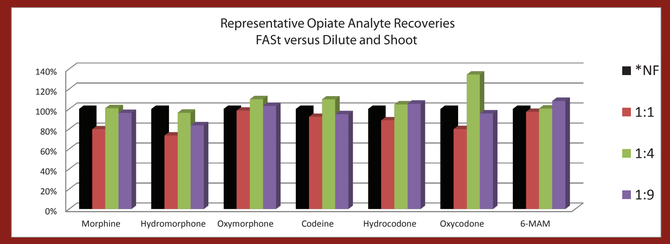


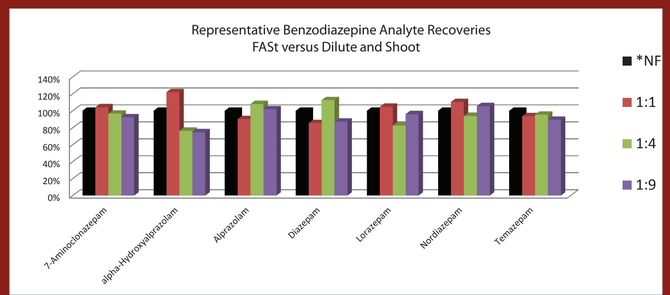
	ANALYTE	CONCENTRATION
1	7-amino clonazepam	40 ng/mL
2	Alprazolam	400 ng/mL
3	Nordiazepam	400 ng/mL
4	Temazepam	400 ng/mL
5	Diazepam	400 ng/mL
6	Clonazepam	400 ng/mL
7	α-hydroxy alprazolam	40 ng/mL
8	Lorazepam	400 ng/mL

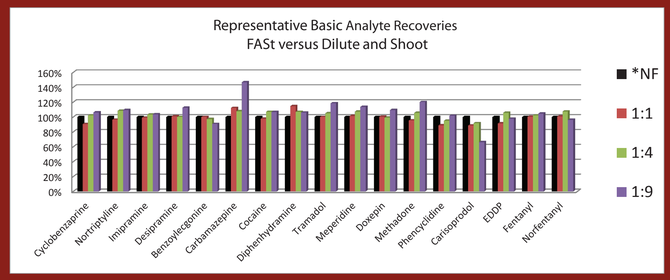


	ANALYTE	CONCENTRATION
1	Benzoylecgonine	400 ng/mL
2	Norfentanyl	400 ng/mL
3	Tramadol	400 ng/mL
4	Carbamazepine	400 ng/mL
5	Carisoprodol	400 ng/mL
6	Meperidine	400 ng/mL
7	Cocaine	400 ng/mL
8	Fentanyl	40 ng/mL
9	Phencyclidine	400 ng/mL
10	Doxepin	400 ng/mL
11	Desiprimine	400 ng/mL
12	Nortriptyline	400 ng/mL
13	Imipramine	400 ng/mL
14	Cyclobenzaprine	400 ng/mL
15	EDDP	400 ng/mL
16	Methadone	400 ng/mL

RESULTS







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

These charts represents 1:1, 1:4 and 1:9 dilution ratios and the % recovery compared to the *NF sample based on calculated peak areas of each compound.