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series	cap color	membrane	pore size	part #
eXtreme|FV®		PVDF	0.2µm	85531

Quick and Easy Sample Preparation of Urine for the Analysis of Psychoactive Drugs using the eXtreme|FV® by LC-MS/MS

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Presented at MSACL 2016

Introduction

This improved sample preparation method allows for the quantitative measurement of Benzodiazepines in urine. Benzodiazepines are psychoactive drugs that enhance the effect of the neurotransmitter GABA at the GABAA receptor. The urine samples were prepared using the eXtreme|FV®, followed by LC-MS/MS analysis. The most critical aspects of reliable urine analysis are the reduction of interferences from the sample matrix and analyte recovery. eXtreme|FV®, were compared to SPE for sample preparation to reduce the sample matrix causing interference prior to analysis. SPE is time consuming, adversely impacts recovery, uses large amounts of solvent and are expensive. The improved sample preparation method using the Thomson eXtreme|FV® allows for the analysis of 9 Benzodiazepines.

Equipment

- Thomson eXtreme|FV® 0.2µm PVDF (P/N 85531)
- Thomson 48 position Vial Filter Press (P/N 35015)
- Eppendorf MixMate®
- Vortex Mixer
- Dry Block Heater set at 55°C ± 2°C
- Microcentrifuge
- AB Sciex 4500 Mass Spectrometer
- Shimadzu Prominence HPLC
  - Column: Restek Ultra Biphenyl Columns (5µm, 50 x 2.1 mm)
    - Mobile Phases:
      - A: 0.1% Formic Acid in HPLC Water
      - B: 0.1% Formic Acid in Methanol
    - Flow Rate: 0.5 mL/min
    - Run Time: 8.5 minutes
    - Injection Volume: 15µL

Analytes

Table 1. Drugs analyzed in this Benzodiazpine Panel

7-Aminoclonazepam (7AMINO)	Nordiazepam (NDIAZ)	Oxazepam (OXAZ)
α-hydroxy-Alprazolam (OH-AL)	Lorazepam (LOR)	Temazepam (TEM)
Hydroxy-Midazolam (OH-MID)	Zolpidem (ZOLP)	Diazepam (DIAZ)

Improved Sample Preparation

1. Add 300 µL of 40% Methanol to each Thomson Vial.
2. Add 50 µL of hydrolyzed urine sample to its respective Thomson Vial (see htslabs.com for hydrolysis method used).
3. Place Thomson Filter Plunger on top of Thomson Vial.
4. Press filter plunger down approximately ¼ of the way into each of the Thomson Vials.
5. Vortex for 2 minutes at 1750 rpm using the Eppendorf Mix Mate.
6. Slowly press the filter plunger the rest of the way down using the Thomson 48 position press.
7. Samples are now ready for LC-MS/MS analysis

Results

The improved method utilizes the Thomson eXtreme|FV®s for sample clean-up significantly reducing the cost and time of per sample analysis. This method was validated for all the analytes in Table 1. Mass spectrum of all the analytes in Table 1 can be seen in Fig. 1. Table 2 shows the validated concentrations used to generate a 6 point calibration curve. Linearity of the assay for the drugs listed in Table 1. Unextracted standards (neats) were run along with 3 different negative patient samples, extracted and spiked with standard and internal standard post extraction at the cutoff concentration to access ion suppression and drug recovery. To calculate drug recovery, the mean area counts of the extracted samples was compared to the mean area counts of the unextracted samples. To determine ion suppression, the mean concentration of the extracted samples was compared to the mean concentration of the post-extracted samples.

Table 2. Final concentrations for the various analytes

Levels	Final Concentration All other analytes (ng/mL)	Final Zolpidem Concentration (ng/mL)
Level 1	75	75
Level 2	300	300
Level 3	1000	500
Level 4	5000	2500
Level 5	10000	5000

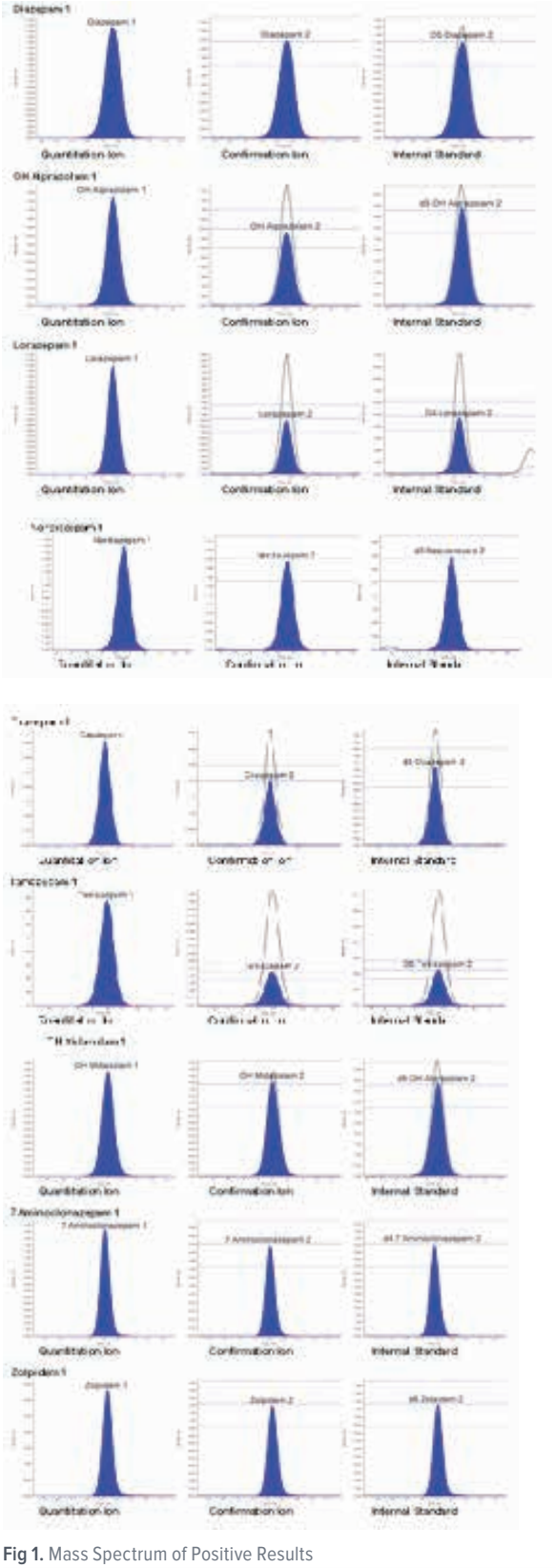

Fig 1. Mass Spectrum of Positive Results

Conclusion

Alleviate the need to use and dispose of Hexane, Glacial Acetic Acid, Potassium Hydroxide.