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

Improved Sample Preparation Methods for Athlete Doping Analysis of Common Compounds in Urine by LCMS

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Abstract

Anti-doping testing by urine analysis requires fast and robust screening methods with repeatable sample preparation. Since, every sample has to be screened, methods are designed to be sufficiently sensitive and specific to identify all suspect samples. One must be careful to minimize false suspects. Ensuring samples are spiked with internal standards accordingly will help verify that samples are being extracted and tested correctly and with accurate uniformity.

The Australian Sports Drug Testing Laboratory, our collaborators, have invested time in determining a limited number of comprehensive screening methods. These methods, using Thomson's eXtreme|FV®s , comply with the World Anti-Doping Agency's (WADA) Prohibited List.

In exploring new methods labs have looked at both detection and sample prep as routes to quicker and more accurate analysis. Liquid chromatography coupled with mass spectrometry detection is prevalent, superseding many of the gas chromatographic coupled with mass spectrometry methods because of the simpler sample preparation. Specifically, the anti-doping testing shown below consisted of sample preparation without the initial use of cumbersome traditional SPE methods, and instead consisted of the comparison of filtration techniques. Filter plates versus Thomson eXtreme|FV®s were tested to determine which product allowed for a method of simple and quick urine analysis while complying with the WADA's guidelines.

Experiment

The experiments were performed at the National Measurement Institute (Australia) in the Sports Drug Testing Laboratory.

The 11.8 minute run time for the instrumental analysis meets the requirements of the WADA Technical Document- Minimum Required Performance Level (TD2013MRPL). This document details the analysis of a large number of analytes from the classes on the WADA Prohibited List, while meeting sensitivity requirements. The analytes included compounds in the following classes anabolic agents, B2-agonists, hormone antagonists and modulators, diuretics, stimulants, narcotics, glucocorticoids, B-blockers, etc.

Full Method

A comparison between sample preparation using filter plates sourced from several different manufactures, and Thomson eXtreme|FV®s PVDF 0.2µm (85531-500) was conducted. The preparation with the Thomson eXtreme|FV®s were automated using a Tecan robotics platform for liquid dispensing in the Thomson 48 position rack (#35010-RACK), and 48 position press (#35015).

Direct Urine Preparation

1. Label each eXtreme|FV® with sample/quality control sample information.
2. Pipette 200 µL of each sample into labeled eXtreme|FV®.
3. Add 200 µL of the Mefruside Internal Standard (300 ng/mL in 0.5% formic acid) to each filter vial cup.
4. Place the eXtreme|FV® tops onto each vial and press shut.

LCHRMS System

UPLC coupled to High Resolution Mass Spectrometry with an electrospray source in full scan mode. Data acquisition in both positive and negative polarity modes within a single 11.8 min chromatographic run.

- Column: C18, 2.1mm × 50mm, 1.7µm
- Column Temperature: 30 °C
- Flow rate: 300µL/min
- Mobile Phase:
  - A: 0.3% aqueous Formic Acid in Water
  - B: 0.3% Formic Acid in Acetonitrile
- Gradient:

Time	A%	B%
0.00	95	5
0.50	95	5
3.50	80	20
5.50	75	25
7.00	43	57
8.00	10	90
8.60	10	90
8.80	95	5

- Injection volume: 10µL
- Sample tray temperature: 18°C
- Column Temperature: 30°C
- Method run time: 11.8 minutes
- Gas: UHP Nitrogen

Conclusions

The Thomson eXtreme|FV®s PVDF 0.2 µm (85531-500) performed the best in compound extraction and identification while allowing the end user to follow the WADA validated method. The elimination of SPE steps from laboratory methods is a large time saver, and enables urine-direct-injection solely using Thomson eXtreme|FV®s for filtration. Together the Thomson 48 position Filter Vial Press and automation enabled 48 position rack equaled timing of filter plate methodology but provided the best extraction and identification of all filter types. A total of 180 compounds can be identified through the screening analysis with the Thomson eXtreme|FV®s PVDF 0.2µm (85531-500).

The method presented is being used for the analysis of athlete's urine samples for banned substances at the Australian Sports Drug Testing Laboratory.

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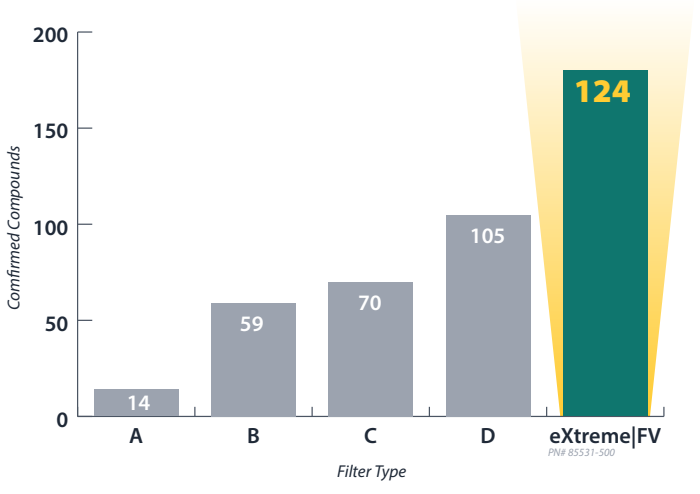
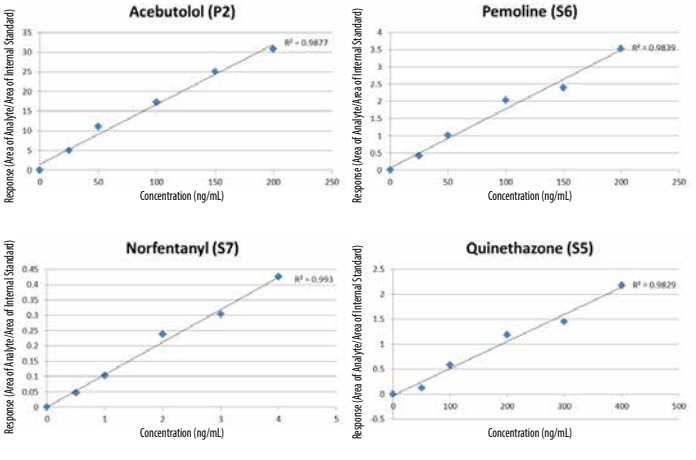
Comparison of Filter Types



Linearity of The Analysis Method Was Assessed Over a Range From 25% To 200% Of MRPL With R2 Generally Being Greater Than 0.98



124 Confermed Compounds

5-Hydroxyindapamide
Bisdesmethylsibutramine
Desmethylsibutramine
Exemestane
Mefruside (+)
Mefruside (-)
D3-epitestosterone glucuronide
D3-epitestosteronea
AICAR
GW1516
Atenolol
Bisoprolol
Esmolol
Metipranolol
Nadolol
Nadoxolol
Oxprenolol
Clenbuterol
Gestrinone
Methyldienolone
Methyltrienolone
Metribolone
Tetrahydrogestrinone
Tibolone
Zilpaterol
3'-Hydroxystanozolol
4'-Hydroxystanozolol
Bambuterol
Formoterol
Salbutamol
Salmeterol
Terbutaline
Andarine
Exemestane metabolite
Aminoglutethimide
Raloxifene
Fulvestrant
GW1516 (501516)
Methazolamide
Piretanide
Quinethazone
Spironolactone
Trichlormethiazide
Acetazolamide
Althiazide
Amiloride
Bendroflumethiazide
Benzthiazide
Bumetanide
Canrenone
Chlorexolone
Chlorothiazide
Chlorthalidone
Clopamide
Probenecid
Cyclopenthiazide
Cyclothiazide
Dichlorphenamide
Epitizide
Eplenerone
Etacrynic acid (frag?)
Furosemide
Hydrochlorothiazide
Mefruside metabolite 2
Indapamide
Metolazone
Polythiazide
Torasemide
Triamterene
Xipamide
Caffeine
Cis-4-Methylaminorex
Cotinine (Nicotine metab)
MBDB
Methoxyamphetamine
Methylenedioxyethylamphetamine
Adrafinil
Amiphenazole
Amphetamine
Benzoylecgonine
Benzylpiperazine
Carphedon
Cathine
Crotethamide
Cyclazodone
Ephedrine
Phenylpropanolamine
Pseudoepherine
Etamivan
Etilefrine
Fenetylline
Hydroxy mesocarb
Isometheptene
MDA
MDMA
Methylphenidate
Modafinil
Modafinil Acid (metabolite)
Nikethamide
Oxilofrine
Pemoline
Pentetrazol
Phenmetrazine
Pholedrine
p-Hydroxy amphetamine
Ritalinic Acid
nor-Selegiline
Methylecgonine
Codeine
Hydromorphone
Morphine
JWH018 N-(5-hydroxypentyl) metabolite
JWH073 N-butanoic acid metabolite
Budesonide
Cortisol
Cortisone
Flumethasone
Fluticasone propionate metabolite
Methylprednisolone
16a-OH-Prednisolone
Prednisolone
Sildenafil
Tadalafil
Vardenafil

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