

APPLICATION

Extraction of 21 Drugs of Abuse from Urine using Novum™ Simplified Liquid Extraction (SLE)

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Overview

In this application we use the Novum SLE MAX 96-well plate to extract 21 different drugs of abuse from urine and compare the results across three different extraction solvents. As expected, each extraction solvent resulted in slightly different results. 6-MAM had the lowest recovery in all cases, but gave acceptable % CV's for both the MTBE and DCM extraction. In addition only Flurazepam and Norbuprenorphine showed % CV's that were outside the acceptable range when extracting with ethyl acetate. MTBE proved to be the most consistent extraction solvent as all 21 drugs had a % CV of less than 14 and showed an average recovery of 83 %.

Sample Pre-treatment Step

- Dilute 200 µL of urine with 200 µL of 0.5 M Ammonium hydroxide (NH₄OH). Vortex briefly (3-5 seconds).

Recommended Hydrolysis

When working with urine samples it is recommended to perform a hydrolysis step in place of the above pre-treatment. We recommend a β-glucuronidase hydrolysis.

- To 200 µL urine, add 175 µL 100 mM Ammonium acetate (pH 4) and 25 µL β-glucuronidase solution (100,000 units/mL, www.campbellscience.com, DR2100).
- Mix and vortex for 30 seconds. Incubate at 37-40 °C for 60 minutes (with gentle shaking).
- Bring samples to room temperature and add 20 µL of 5 M Ammonium hydroxide. Vortex thoroughly.

Extraction Procedure

Sample Loading

- Load the sample from pre-treatment step above onto the Novum SLE MAX 96-well plate (part no. 8E-S138-5GA) and apply a short and gentle pulse of vacuum (~ 5" of Hg) for 5-10 seconds or until the sample has completely entered the media.
- Wait for 5 minutes.

Elution

Ethyl Acetate (EtOAc)

- Dispense 900 µL of Ethyl Acetate onto the SLE media and allow the solvent to elute by gravity (~ 5 minute elution time) and collect the eluant in a collection plate.
- Repeat with another 900 µL Ethyl Acetate and collect the eluant in the collection plate, pooling the two fractions.
- Apply vacuum at 5" of Hg for 20-30 seconds to complete the extraction.

Dichloromethane (DCM)

- Dispense 900 µL of DCM onto the SLE media and allow the solvent to elute by gravity (~ 5 minute elution time) and collect the eluant in a collection plate.
- Repeat with another 900 µL DCM and collect the eluant in the collection plate, pooling the two fractions.
- Apply vacuum at 5" of Hg for 20-30 seconds to complete the extraction.

Methyl Tert-Butyl Ether (MTBE)

- Dispense 900 µL of MTBE onto the SLE media and allow the solvent to elute by gravity (~ 5 minute elution time) and collect the eluant in a collection plate.
- Repeat with another 900 µL MTBE and collect the eluant in the collection plate, pooling the two fractions.
- Apply vacuum at 5" of Hg for 20-30 seconds to complete the extraction.

NOTE: Prolonged application of vacuum will result in elution of urine out of the Novum SLE media and into the final extracted solvent.

Dry Down

- Evaporate the final extract to complete dryness under slow stream of N₂ at 40 °C for about 60 minutes.
- Reconstitute in mobile phase.

Table 1. Extraction solvent comparisons

Analyte	Ethyl Acetate		Dichloromethane		Methyl Tert-Butyl Ether	
	Average Recovery %	% CV	Average Recovery %	% CV	Average Recovery %	% CV
6-MAM	54	16	65	8	49	7
Amphetamine	107	3	108	1	88	5
Codeine	100	10	107	10	91	1
MDMA	83	4	92	6	74	7
Methamphetamine	102	12	109	9	89	12
Norbuprenorphine	109	15	108	8	73	12
PCP	100	2	84	8	102	5
Propoxyphene	82	9	93	10	83	8
Sufentanil	83	11	89	10	85	8
Buprenorphine	87	5	90	2	77	7
Fentanyl	100	6	105	4	89	6
Flurazepam	83	18	87	17	86	10
MDEA	85	4	91	5	79	5
Meperidine	102	9	113	6	86	7
Meprobamate	85	7	93	7	72	10
Methadone	96	2	92	3	82	6
Nordiazepam	106	1	109	1	92	5
Norfentanyl	82	3	92	6	69	2
Normeperidine	101	4	102	6	89	3
Temazepam	95	2	94	12	87	9
Tramadol	97	9	114	9	99	14



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Ordering Information

Novum™ Simplified Liquid Extraction (SLE)

Part No.	Description	Unit/Box
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/Box

Accessories

Collection Plates (deep well, polypropylene)		Unit
AH0-7192	96-Well Collection Plate, 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate, 1 mL/well	50/pk
AH0-7194	96-Well Collection Plate, 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk

Sealing Mats

AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AH0-7362	Sealing Tape Pad	10/pk

Vacuum Manifold

AH0-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea
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