APPLICATIONS MANUAL

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IFOSFAMIDE IN BLOOD SERUM

CHROMPURE™ C18 SPE COLUMN (Cat# LBSC181001)

PREPARE SAMPLE

Solution 0.2g ifosfamide samples in 100ml volumetric flask with mobile phases to be 2mg/ml internal standard, add 12.5ul internal standard in 0.5ml serum samples.

CONDITION COLUMN

condition SPE column with 2ml Acetonitrile and 2mL Physiological saline.

PURIFICATION

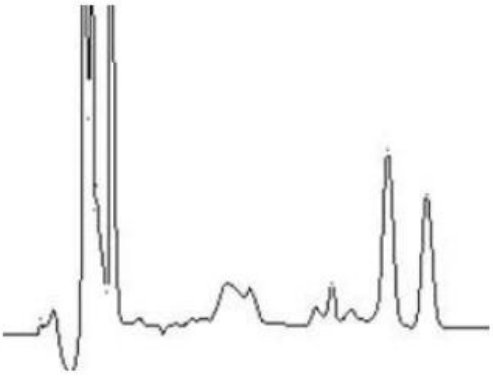
load the serum samples into SPE colums (chrompure C18)

Wash the SPE column with 1ml Physiological saline and followed by washing with 5% Acetonitrile, concentrate to dryness with N_2

Elute with 0.5ml Acetonitrile

CHROMATOGRAPHY

Inject 20 µl onto UV-HPLC, flow rate1.0ml/min, room temp , det. UV-Vis 200nm



Concentration(ug/ml)	Recoveries(%)
100	92.0
50	93.5
5	89.3

INGREDIENTS IN BLOOD SERUM

CHROMPURE™ PLS SPE COLUMN (Cat# LBSPLS5003)

PREPARE SAMPLE

To 1 mL of serum or plasma add internal standard* and 2 ml of 100 mM phosphate buffer (pH 6.0). Mix/vortex.

Centrifuge for 10 minutes at 2000 rpm and discard pellet. Sample pH should be 6.0 ± 0.5. Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

CONDITION COLUMN

condition SPE column with 3Ml Methanol and3mL H_2O

PURIFICATION

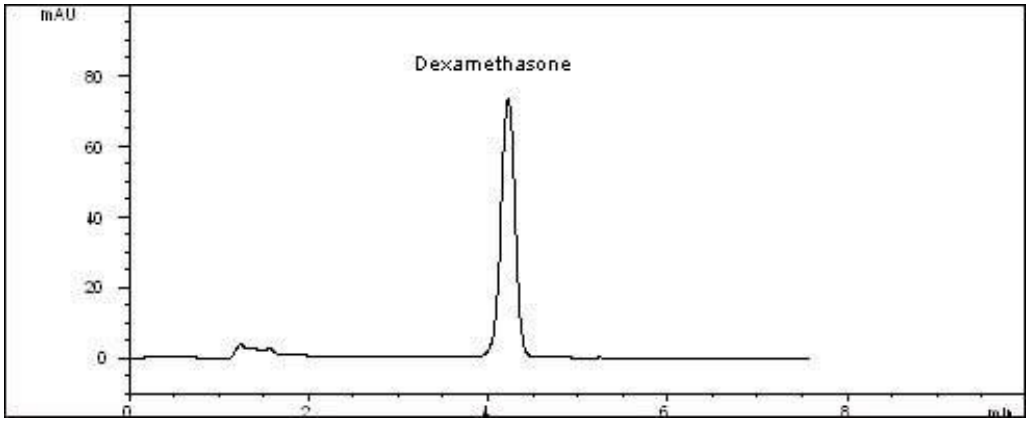
load the serum samples into SPE colums (chrompure PLS)

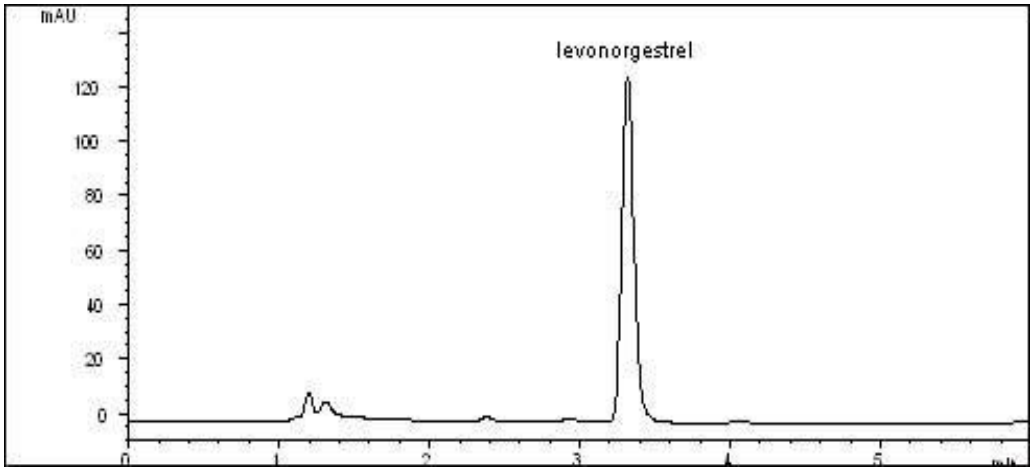
Wash the SPE column with 3ml H_2O or 5% Methanol

Elute with 3mL Methanol

CHROMATOGRAPHY

Inject 20 µl onto UV-HPLC, flow rate1.0ml/min, room temp .





Matter	Recoveries(%)
Dexamethasone	97.9
Ethinylestradiol	96.3
Hydrocortison μm	74.0
Triamcinolone	71.9
Levonorgestrel	93.9
Ganciclovir	54.1
Prednisone Acetate	98.6
Cefalexin	58.6
Cefradine	45.6

ORGANOPHOSPHORUS PESTICIDES IN TEA LEAF

CHROMPURE™ GCB SPE COLUMN (CAT#LBSGCB5006)

PREPARE SAMPLE

2g tea leaf add into water over night. Homogenize the tea leaf in 10ml(n-Hexan:acetone=1:1), repeat homogenize for 2-3 times. Unite all the liquid, homogenize, centrifuge and get the upper layer, Add proper anhydrous sodium sulfate and centrifuge , get the upper layer, evaporate supernatant to 1 mL under N2 at 35 ℃

CONDITION COLUMN

5 mL n-Hexan:acetone=1:1, GCB 500mg/6ml (Cat#LBSGCB5006)

PURIFICATION

Load 1ml sample onto GCB SPE column

elute with 20ml n-Hexan:acetone=1:1

Gather all samples and concentrate to dryness with N2 at 40 ℃, Reconstitute in 1 mL acetone:n-hexane (1:1)

CHROMATOGRAPHY

Inject 1.0 µl onto GC-FPD 50 ℃ (1min), 15 ℃ /min to 200 ℃ (2min), 5 ℃ /min ℃ to 260 ℃ (8min), FPD,250 ℃

SUDAN I II III IV IN CHILLI SAUCE

CHROMPURE™ Alumina-N SPE COLUMN (Cat# LBSALNB5003)

PREPARE SAMPLE

Homogenize 5g chilli sauce in 10ml(n-Hexan:acetone=3:1), ultrasonic 15min, centrifuge , get the acetone layer, ultrasonic the residual with 5ml n-Hexan for 2 time, gather the acetone layer, Add proper anhydrous sodium sulfate, filter by 0.45um membrane,concentrate to dryness with N_2 to 5ml

CONDITION COLUMN

5 mL n-Hexan, Al-N 500mg/3ml(Cat# LBSALNB5003)

PURIFICATION

Load the sample onto Al-N SPE column at speed 1 second/drop

Wash with 3*5ml n-Hexan to dry

Elute with 5ml n-Hexan(5% acetone)

Concentrate to dryness with N2 at 40 °C, fix to 5ml by methanol

CHROMATOGRAPHY

Inject 20 µl onto UV-HPLC, flow rate1.0ml/min, temp 35℃, det. UV-Vis 500nm

PAH IN WATER

CHROMPURE™ C18 SPE COLUMN (Cat# LBSC1810006)

PREPARE SAMPLE

6N HCL add to 250ml – 1L water adjust to PH<2

CONDITION COLUMN

6 mL dichloromethane,6ml methanol,6ml deionized water, C18 1000mg/6ml(Cat# LBSC1810006)

PURIFICATION

Load the water on into SPE tube 5ml/minute

Wash C18 column with 6ml deionized water, dry under vacuum pressure

Elute by 3*1ml dichloromethane

Gather all sample, concentrate to dryness with N2 at room temp to 0.5ml, add perylene-d12 to be internal standard

CHROMATOGRAPHY

Inject 1.0 μl onto GC-MSD, at 100℃ (1min), 6℃ /min to 300℃ (30min), MSD280℃, carrier gas: He 1.0ml/min, m/z 50-450

PHENOLS IN WATER

CHROMPURE™ PLS SPE COLUMN (Cat# LBSPLS1003)

PREPARE SAMPLE

100mL water spiked with phenols, if sample containing ine sediment may require prefiltration.

CONDITION COLUMN

6 mL methyl t-butyl ether, 6 mL methanol, 6 mL deionized water, PLS 100mg/3ml(Cat# LBSPLS1003)

PURIFICATION

Load 100 mL aqueous sample onto SPE tube in less than 5ml/min

Elute with 2*2.5mL methyl t-butyl ether and allow elution to proceed at a dropwise rate, add methyl t-butyl ether to 5ml

CHROMATOGRAPHY

Inject 1.0 µl onto GC-MSD, 200 ℃, splitless (45 sec delay), temp. at 65 ℃ to 185 ℃ at 10 ℃/min, hold 1 min, then to 275 ℃, at 20 ℃/min, hold 5 min. FID, 330 ℃, Carrier gas: Nitrogen, 1.0 mL/min

TRICYCLIC ANTIDEPRESSANTS IN SERUM AND PLASMA

CHROMPURE™ PXA SPE COLUMN (Cat# LBSPAX1003)

PREPARE SAMPLE

To 1 mL of serum or plasma add internal standard* and 2 ml of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Centrifuge for 10 minutes at 2000 rpm and discard pellet. Sample pH should be 6.0 ± 0.5. Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

CONDITION COLUMN

3 mL CH_3OH. 3 mL D.I. H_2O. 1 mL 100 mM phosphate buffer (pH 6.0). Aspirate at < 3 inches Hg to prevent sorbent drying. PAX 200mg/3ml(Cat# LBSPAX2003)

PURIFICATION

Load at 1 mL/minute. Wash with 3 mL D.I. H2O. 1 mL 100 mM acetic acid. 3 mL CH3OH. Dry column (5 minutes at > 10 inches Hg)

Elute with 3 mL CH2Cl2/IPA/NH4OH (78:20:2). Collect eluate at 1 mL/minute or use gravity flow. Evaporate to dryness at < 40 ℃.

CHROMATOGRAPHY

Inject 100 µL onto HPLC. Column temp = 30 ℃ Moblie phase- Acetonitrile/ Buffer/ Methanol (60:25:15), Buffer = 0.01 M K2HPO4 adjusted to pH 7.0 with H3PO4 Flow rate = 1.75 mL/min.

CLONAZEPAM & 7-AMINOCLONAZEPAM IN URINE

CHROMPURE™ PLS SPE COLUMN (Cat# LBSPLS0301)

PREPARE SAMPLE

To 2 mL of urine, add internal standard(s)* and 1 mL of ß-Glucuronidase solution. ß-Glucuronidase solution contains 5,000 F units/mL Patella vulgata in 100 mM acetate buffer (pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65 ℃. Cool before proceeding.

CONDITION COLUMN

3 mL CH3OH. 3 mL deionized water. 1 mL 100 mM phosphate buffer (pH 6.0).

PURIFICATION

Load samples at 1 to 2 mL/ minute.

Wash with 2 mL deionized water. 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0).Dry column (5 minutes at > 10 inches Hg). 2 mL hexane.

Elute with 3 mL ethyl acetate with 2% NH4OH: Collect eluate at 1 to 2 mL/minute.

Evaporate to dryness at < 40 ℃.

Add 50 µL ethyl acetate and 50 µL MTBSTFA(with 1% TBDMCS), Mix/vortex. React 20 minutes at 90 ℃. Remove from heat source to cool.

CHROMATOGRAPHY

Inject 1 to 2 µL sample

GABAPENTIN IN SERUM, PLASMA, OR WHOLE BLOOD
CHROMPURE™ C18 SPE COLUMN (Cat# LBSC181001)

PREPARE SAMPLE
500 µL of sample, calibrator, or control was placed into a disposable glass test tube and 25 µL of internal standard* (5.0 mg/L) was added. Vortex tube. Add 500 µL of 20% acetic acid and vortex tube again.

CONDITION SPE COLUMN
1 x 3 mL CH_3OH.
1 x 3 mL D.I. H_2O.
1 x 1 mL 100 mM HCL.

APPLY SAMPLE
Load at 1 to mL/minute.

WASH COLUMN
1 x 3 mL D.l. H_2O.
1 x 3 mL ethyl acetate.
1 x 3 mL hexane.
Dry column.
(5 minutes at > 10 inches Hg) or until column is dry.

ELUTION
1 x 1 mL 2% NH_4OH in CH_3OH.

DRY ELUATE
Evaporate to dryness at < 40 ℃
.

DERIVATIZATION
Add 50 µL of MTBSTFA + 1 % t-BDMCS** and 50 µl ethyl acetate.
Cap and heat at 70 ℃ for 30 minutes.
Remove and allow to cool.

QUANTITATE
Inject 1 to 2 µL onto gas chromatograph.

AMPHETAMINES IN URINE
CHROMPURE™ PCX SPE COLUMN (Cat# LBSPCX301)

SAMPLE PREPERATION
To 1 mL of urine add internal standard(s) and 1 mL 100mMphosphate buffer (pH 6.0).Mix/Vortex.

APPLY SAMPLE TO COLUMN
Load at a rate of 1 to 2 mL/min.

WASH COLUMN
1 x 1 mL DI H_2O.
1 x 1 mL 100mM acetic acid.
1 x 1 mL MeOH.
Dry column (3 mins at > 10 inches Hg).

ELUTE AMPHETAMINES
2 x 0.5 mL CH_2Cl_2 /IPA/NH_4OH (78/20/2), collect eluate at 1 to 2 mL/min.

CONCENTRATE ELUATE
Add 1 drop 1% HCl in MeOH to eluate before evaporating.
Evaporate to dryness at < 40 ℃.

DERIVATIZATION
Add 50 ul ethyl acetate and 50 ul TFA (Trifluoroacetic acid anhydride) then cap, mix/vortex.
Heat for 15 mins at 70 ℃, allow to cool, then evaporate to dryness at < 40 ℃.
Reconstitute with 100 µL ethyl acetate.

ANALYZE
Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

Analyte (TFA)	Target (Quantitation) Ion	Qualifier Ions
Amphetamine	140	91, 118
Amphetamine-d11*	144	98, 128
Methamphetamine	154	110, 118
Mehtamphetamine-d11*	160	113, 126

*Suggested internal standards

COCAINE/BENZOYLECGONINE IN URINE
CHROMPURE™ PCX SPE COLUMN (Cat# LBSPCX301)

SAMPLE PREPERATION
To 1 mL of urine add internal standard(s) and 300 µl 100mM HCl.
Mix/Vortex.

APPLY SAMPLE TO COLUMN
Load at a rate of 1 to 2 mL/min.

WASH COLUMN
1 x 1 mL DI H 2 O.
1 x 1 mL 100mM HCl.
1 x 1 mL MeOH.
Dry column (3 mins at > 10 inches Hg).

ELUTE COCAINE/BENZOYLECGONINE
2 x 0.5 mL CH_2Cl_2/IPA/NH_4OH (78/20/2), Collect eluate at 1 to 2 mL/min.

CONCENTRATE ELUATE
Evaporate to dryness at < 40 ℃.

DERIVATIZATION
Add 50 µL ethyl acetate and 50 µL BSTFA w/ 1% TMCS, then cap, mix/vortex.Heat for 20 mins at 70 ℃, allow to cool.

ANALYZE
Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

Analyte	Target (Quantitation) Ion	Qualifier Ions
Cocaine	182	198, 303
Cocaine-d3*	185	201, 306
Benzoylecgonine (TMS)	240	256, 361
Benzoylecgonine-d8 (TMS)*	243	259, 369

*Suggested internal standards

OPIATES IN URINE
CHROMPURE™ PCX SPE COLUMN (Cat# LBSPCX301)

SAMPLE PREPERATION

To 1 ml of urine add internal standard(s) and 1.0 ml ß-Glucuronidase solution. (ß-Glucuronidase solution contains 5000 Funits/mL Patella Vulgata in 100mM acetate buffer, pH 5.0). Hydrolyze for 3 hours at 60 ℃. Cool, then centrifuge for 10 minutes at high speed and discard pellet. Adjust pH to 6.0 ±0.5 with 1.0N NaOH. NOTE: For unconjugated (free) opiates; to 1 mL urine, add internal standard(s) and 1 mL 100mM phosphate buffer (pH 6.0). Proceed to Step #2.

APPLY SAMPLE TO COLUMN

Load at a rate of 1 to 2 mL/min.

WASH COLUMN

1 x 1 mL DI H 2 O.
1 x 1 mL 100mM acetate buffer (pH 4.5).
1 x 1 mL MeOH.
Dry column (3 mins at > 10 inches Hg).

ELUTE OPIATES

2 x 0.5 mL CH 2 Cl 2 /IPA/NH 4 OH (78/20/2), collect eluate at 1 to 2 mL/min. Evaporate eluate to dryness at < 40 ℃.

DERIVATIZATION

Add 50 uL ethyl acetate and 50 µL BSTFA w/ 1% TMCS, then cap, mix/vortex.
Heat for 20 mins at 70 ℃, allow to cool.

ANALYZE

Inject 1 to 2 µL onto gas chromatograph: For MSD monitor the following ions:

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions
Codeine	371	234, 343
Codeine-d6*	377	237, 349
Morphine	429	401,414
Morphine-d6*	435	404, 420
6-Acetylmorphine	399	287, 340

*Suggested internal standards

CARBOXY-THC IN URINE
CHROMPURE™ PCX SPE COLUMN (Cat# LBSPCX301)

SAMPLE PREPERATION
To 2 mL of urine add internal standard and 100 µL 10N NaOH. Mix/vortex. Hydrolyze for 20 mins at 60 ℃. Cool before proceeding. Adjust sample pH to 3.5 ± 0.5 with 1.0 mL glacial acetic acid.

APPLY SAMPLE TO COLUMN
Load at a rate of 1 to 2 mL/min.

WASH COLUMN
1 x 1 mL DI H 2 O.
1 x 1 mL 0.1M HCl/acetonitrile (70/30).
Dry column (3 mins at > 10 inches Hg).
1 x 200 µL hexane.

ELUTE CARBOXY-THC
2 x 0.5 mL hexane/ethyl acetate (50:50); Collect eluate at 1 to 2 mL/min.
Evaporate eluate to dryness at < 40 ℃.

DERIVATIZATION
Add 50 µL ethyl acetate and 50 µL BSTFA w/ 1% TMCS, then cap, mix/vortex.
Heat for 20 mins at 70 ℃, allow to cool.

ANALYZE
Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

Analyte (TMS)	Target (Quantitation) Ion	Qualifier Ions
Carboxy-THC	371	473, 488
Carboxy-THC-d3*	374	476, 491

*Suggested internal standards