

Thermo Scientific HyperSep SPE Applications

Covering Pharmaceutical, Biochemical and Environmental Techniques

Pharmaceutical

Cyclosporin from Blood

Compounds:	Cyclosporin
Part Number:	60108-304
Phase:	HyperSep™ C18
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	Mix 1 mL heparinised blood with 2 mL water/acetonitrile (7:3, v/v) Stir mixture and centrifuge after 5 min
Conditioning:	3 mL acetonitrile followed by 3 mL water/acetonitrile (8:2, v/v)
Application:	Force or aspirate the sample slowly through column
Washing:	0.5 M acetic acid/acetonitrile (8:2, v/v) followed by 0.5 M acetic acid/acetonitrile (6:4, v/v)
Elution:	Acetonitrile

Antineoplastic Agents from Plasma

Compounds:	Bisantrene, mitoxantrone
Part Number:	60108-302
Phase:	HyperSep C18
Volume:	1 mL
Bed Weight:	100 mg
Conditioning:	2 mL methanol followed by 2 mL distilled water
Application:	Force or aspirate 1-2 mL plasma slowly through column
Washing:	2 mL distilled water
Elution:	2 x 200 µL volumes 0.5 M methanolic HCl

Antiarrhythmic Drug Flecainide from Plasma

Compounds:	Flecainide
Part Number:	60108-392
Phase:	HyperSep C8
Volume:	1 mL
Bed Weight:	100 mg
Sample Pretreatment:	Mix 1 mL plasma with 1 mL water and 200 µL 0.2M sodium carbonate solution
Conditioning:	2 mL methanol followed by 2 mL distilled water
Application:	Force or aspirate sample slowly through column
Washing:	2 mL distilled water followed by 2 x 1 mL volumes acetonitrile
Elution:	500 µL methanol, then elute from column after 1 min

Benzodiazepines from Serum

Compounds:	Benzodiazepines
Part Number:	60108-302
Phase:	HyperSep C18
Volume:	1 mL
Bed Weight:	100 mg
Sample Pretreatment:	Mix 500 µL serum with 100 µL 0.1 M sodium carbonate solution. Add internal standard if required
Conditioning:	2 mL methanol followed by 2 mL distilled water
Application:	Force or aspirate sample slowly through column
Washing:	2 mL distilled water followed by 50 µL methanol
Elution:	2 x 200 µL volumes methanol

Methadone in Urine

Compounds:	Methadone
Part Number:	60108-742
Phase:	HyperSep Verify-CX
Volume:	10 mL
Bed Weight:	200 mg
Sample Pretreatment:	To 2 mL urine add internal standard(s) and 1 mL 100 mM phosphate buffer (pH 6). Mix/vortex. Use 100 mM monobasic or dibasic sodium phosphate to ensure sample pH of 6.
Conditioning:	3 mL CH ₃ OH 3 mL deionized water 2 mL 100 mM phosphate buffer (pH 6) Aspirate at < 3 inches Hg
Application:	Load at 1 to 2 mL/min
Washing:	3 mL deionized water 1 mL 100 mM acetic acid 3 mL CH ₃ OH Dry column (5 min at >10 inches Hg)
Elution:	3 mL CH ₂ Cl ₂ /IPA/NH ₄ OH (78:20:2) Collect eluate at 1-2 mL/min Prepare elution solvent daily

Drugs from Blood and Urine

Compounds:	Amphetamines, barbiturates
Part Number:	60108-304
Phase:	HyperSep C18
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	Adjust 10 mL urine to desired pH value using HCl or ammonia and centrifuge Amphetamines and Opiates: pH 8-9 Active components: pH 7-8 Barbiturates: pH 7
Conditioning:	6 mL methanol followed by 6 mL Distilled water pH7
Application:	Force or aspirate sample slowly through column
Washing:	6 mL water Dry column under vacuum for 5 min
Elution:	Aspirate 750 µL eluent into column. Elute after 1 min and then flush with 750 µL eluent. Eluents: Cannabinoids: acetone. Barbiturates, active components, bases, amphetamines: acetone/chloroform (1:1)

Abused Drugs in Equine Urine

Compounds:	Aminocaproic acid (6-aminohexanoic acid)
Part Number:	60108-421
Phase:	HyperSep SCX
Volume:	1 mL
Bed Weight:	100 mg
Sample Pretreatment:	Mix 1 mL urine with 1 mL phosphoric acid (7 mM). Use concentrated H ₃ PO ₄ to adjust pH to 2
Conditioning:	1 mL methanol followed by 1 mL distilled water and 1 mL 7 mM H ₃ PO ₄
Application:	Force or aspirate sample slowly through column Dry column under vacuum for 30 sec
Washing:	1 mL 7 mM phosphoric acid 0.5 mL 0.1 M acetic acid 1 mL methanol Dry column under vacuum for 30 sec
Elution:	2 x 1 mL ammoniacal methanol (1%)

Key Words

- HyperSep SPE
- Sample Preparation
- Pharmaceutical Applications
- Biochemical Applications
- Environmental Applications

Pharmaceutical

Gabapentin in Serum, Plasma or Whole Blood

Compounds:	Gabapentin
Part Number:	60108-302
Phase:	HyperSep™ C18
Volume:	1 mL
Bed Weight:	100 mg
Sample Pretreatment:	500 µL sample, calibrator or control to be placed into a glass test tube. Add 25 µL internal standard (5.0 mg/L). Add 500 µL 20% acetic acid and vortex tube.
Conditioning:	3 mL CH ₃ OH followed by 3 mL deionised water and 1 mL 100 mM HCl
Application:	Load sample at 1-2 mL/min
Washing:	3 mL deionized water followed by 3 mL ethyl acetate and 3 mL hexane Dry column under vacuum for 30 sec
Elution:	1 mL 2% NH ₄ OH in CH ₃ OH Evaporate to dryness at < 40 °C

Carboxy ^Δ9 Tetrahydrocannabinol (THC) in Urine

Compounds:	Carboxy ^Δ 9 THC
Part Number:	60108-304
Phase:	HyperSep C18
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	To 5 mL urine add 0.5 mL 10N KOH Heat at 55 °C for 15 min and then cool. Add 1 mL glacial acetic acid.
Conditioning:	6 mL methanol followed by 6 mL 0.01N HCl
Application:	Force or aspirate sample slowly through column
Washing:	2 x 500 µL volumes acetonitrile/0.01N HCl (60:40, v/v). Evaporate to dryness under vacuum
Elution:	2 x 500 µL volumes n-heptane/ethyl acetate (85:15, v/v)

Amphetamines, Opiates and Phencyclidine in Oral Fluid

Compounds:	Amphetamines, opiates, phencyclidine
Part Number:	60108-741
Phase:	HyperSep Verify™-CX
Volume:	1 mL
Bed Weight:	50 mg
Sample Pretreatment:	Add 100-500 µL neat sample to a clean tube. Add internal standard and leave for 10 min at ambient temperature. Add 800 µL 100 mM phosphate buffer pH 6 Mix/vortex for 10 sec. Use 100 mM monobasic or dibasic sodium phosphate to ensure pH 6.
Conditioning:	200 µL CH ₃ OH 200 µL deionized water 200 µL 100 mM phosphate buffer pH 6
Application:	Do not exceed 1 mL/min
Washing:	500 µL deionized water 500 µL 100 mM acetic acid 500 µL CH ₃ OH. Dry column
Elution:	800 µL CH ₂ Cl ₂ /IPA/NH ₄ OH (70:26:4) Do not exceed 1 mL/min Prepare elution solvent daily

Amphetamines in Urine

Compounds:	Amphetamines
Part Number:	60108-742
Phase:	HyperSep Verify-CX
Volume:	10 mL
Bed Weight:	200 mg
Sample Pretreatment:	To 2 mL urine add internal standard(s), 1 mL 100 mM phosphate buffer pH 6 and 1 mL 0.35 M sodium periodate. Mix/vortex and incubate at ambient temperature for 20 min. Use 100 mM monobasic or dibasic sodium phosphate to ensure sample pH of 6.0 ± 0.5.
Conditioning:	3 mL CH ₃ OH followed by 3 mL deionised water and 1 mL 100 mM phosphate buffer (pH 6) Aspirate at < 3 inches Hg to prevent sorbent drying
Application:	Load at 1 to 2 mL/min
Washing:	3 mL deionized water followed by 1 mL 100 mM acetic acid and 3 mL CH ₃ OH Dry column (5 min at >10 inches Hg)
Elution:	3 mL CH ₂ Cl ₂ /IPA/NH ₄ OH (78:20:2) Collect eluate at 1 to 2 mL/min Prepare elution solvent daily Concentrate eluate by adding 30 µL silation grade DMF to eluate. Evaporate to 30 µL at < 40 °C

Therapeutic and Abused Drugs in Urine for Acid/Neutral and Basic Drugs

Compounds:	Barbiturates, ibuprofen, cotinine, amphetamine, codeine, ketamine
Part Number:	60108-742
Phase:	HyperSep Verify-CX
Volume:	10 mL
Bed Weight:	200 mg
Sample Pretreatment:	To 2 mL urine add internal standard(s) and 1 mL 100 mM phosphate buffer (pH 6). Mix/vortex. Use 100 mM monobasic or dibasic sodium phosphate to ensure sample pH of 6.
Conditioning:	3 mL CH ₃ OH 3 mL deionized water 2 mL 100 mM phosphate buffer (pH 6) Aspirate at < 3 inches Hg
Application:	Load at 1 to 2 mL/min
Washing:	3 mL deionized water 1 mL 100 mM acetic acid Dry column (5 min at >10 inches Hg) 2 mL hexane
Elution:	Acidic and Neutral: 3 mL hexane/ethyl acetate (50:50) Collect eluate at < 2 mL/min Evaporate to dryness at < 40 °C Basic: 3 mL CH ₂ Cl ₂ /IPA/NH ₄ OH (78:20:2). Collect eluate at 1-2 mL/min Evaporate to dryness at < 40 °C using evaporator

Antiepileptics from Serum

Compounds:	Carbamazepine, dilantin, phenobarbital, primidone
Part Number:	60108-302
Phase:	HyperSep C18
Volume:	1 mL
Bed Weight:	100 mg
Sample Pretreatment:	Mix 500 µL serum with 500 µL 4-methylprimidone in citrate buffer pH 4 (internal standard)
Conditioning:	2 mL methanol followed by 2 mL water
Application:	Force or aspirate sample slowly through column
Washing:	2 column volumes distilled water
Elution:	2 x 100 µL volumes acetone

Biochemical

Catecholamine Metabolites from Urine

Compounds:	Vanillylmandelic acid, homovanillic acid
Part Number:	60108-521
Phase:	HyperSep™ SAX
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	Collect 24h urine (preserved with 0.1 M HCl). Store at 4 °C or -20 °C resp. Dilute sample prior to extraction 1:1 with water. Use 0.5 M NaOH to adjust pH to 7.5.
Conditioning:	6 mL methanol followed by 6 mL distilled water
Application:	Force or aspirate pretreated sample through column
Washing:	6 mL distilled water
Elution:	6mL 1.5M Sodium Hydroxide solution

Aflatoxin M1 Mycotoxin from Milk

Compounds:	Aflatoxin M1
Part Number:	60108-305
Phase:	HyperSep C18
Volume:	6 mL
Bed Weight:	500 mg
Sample Pretreatment:	Dilute 20 mL milk with 30 mL distilled water
Conditioning:	10 mL methanol followed by 10 mL distilled water
Application:	Force or aspirate sample slowly through column
Washing:	10 mL distilled water followed by 10 mL n-hexane – then dry column at 50 °C for 10-20 min or at ambient temp overnight
Elution:	3 mL dichloromethane/acetone (4:1, v/v)

Water Soluble Vitamins from Aqueous Solutions

Compounds:	Niacinamide, pyridoxine, riboflavin, thiamine
Part Number:	60108-304
Phase:	HyperSep C18
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	Use an amber glass bottle. Mix 50 mL sample with riboflavin content < 6 mg with 0.5 mL acetic acid and 0.1 g heptane-1-sulphonic acid sodium salt. Flush bottle with nitrogen, heat to 55 °C, shake. Cool down rapidly.
Conditioning:	3 mL Methanol followed by 3 mL of a solution of 0.5 mL acetic acid and 0.1 g heptane-1-sulfonic acid sodium salt in 50 mL water (to be prepared daily)
Application:	2 mL sample solution to be forced or aspirated through column
Washing:	2 x 250 µL of a solution of 0.5 mL acetic acid and 0.1 g heptane-1-sulphonic acid sodium salt in 50 mL water
Elution:	3 x 500 µL methanol – Immediate analysis required

Anthocyan Dyes from Red Wine

Compounds:	Anthrocyan dye
Part Number:	60108-309
Phase:	HyperSep C8
Volume:	3 mL
Bed Weight:	500 mg
Conditioning:	3 mL methanol followed by 3 mL distilled water
Application:	Force or aspirate wine sample slowly through column
Washing:	1.5 mL distilled water
Elution:	Small volume methanolic HCl

Folic Acid from Food

Compounds:	Folic acid
Part Number:	60108-521
Phase:	HyperSep SAX
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	Homogenize 10 g food sample in 100 mL 0.01 M phosphate buffer pH 7.4. Filter
Conditioning:	6 mL n-hexane followed by 6 mL methanol and 6 mL distilled water
Application:	Force or aspirate 10 mL filtrate through column
Washing:	6 mL distilled water
Elution:	5 mL 10% NaCl in 0.1 M sodium acetate buffer

Ketamine in Urine

Compounds:	Diazepam, cocaine, 6-MAM
Part Number:	60108-742
Phase:	HyperSep Verify™-CX
Volume:	10 mL
Bed Weight:	200 mg
Sample Pretreatment:	To 2 mL urine add internal standard and 1 mL 100 mM phosphate buffer (pH 6). Mix/vortex. Use 100 mM monobasic or dibasic sodium phosphate to ensure sample pH of 6
Conditioning:	3 mL CH ₃ OH followed by 3 mL deionized water and 1 mL 100 mM phosphate buffer (pH 6)
Application:	Load at 1 mL/min
Washing:	3 mL deionized water 1 mL 100 mM acetic acid 3 mL CH ₃ OH Dry column (5 min at > 10 inches Hg)
Elution:	3 mL dichloromethane/isopropanol/ammonium hydroxide (78:20:2) – collect eluents at 1-2 mL/min using minimal vacuum Evaporate to dryness at < 40 °C

Environmental

Cyanuric Acid in Drinking Water

Compounds:	Cyanuric Acid
Part Number:	60106-402
Phase:	HyperSep™ Hypercarb
Volume:	6 mL
Bed Weight:	500 mg
Sample Pretreatment:	Adjust water sample to pH 3
Conditioning:	Wash column with 10 mL methanol Condition column with 10 mL LC-grade water
Application:	Force/aspirate 250-500 mL into column at rate of 5 mL/min
Washing:	Dry column under vacuum
Elution:	20 mL methanol – evaporate to dryness at 50 °C under nitrogen
Source:	Marie Claire Hennion, ESPCI, Paris

Organochlorine Insecticides from Water – Step 2

Compounds:	Aldrin, p,p'-DDE, o,p'-DDE, o,p'-DDT, p,p'-DDT, dieldrin, endosulfan I, endosulfan II, endrin, heptachlor, heptachlor epoxide, lindane, p,p'-methoxychlor
Part Number:	60108-426
Phase:	HyperSep Silica
Volume:	6 mL
Bed Weight:	1g
Conditioning:	200 µL water followed by 10 mL n-hexane
Application:	Mix concentrated C18 eluate with 1 mL n-hexane. Force/aspirate through column and collect eluate.
Elution:	10 mL n-hexane, combine eluates Elute a second part with benzene/hexane (6:4, v/v) Analyse both parts separately

Pesticides and PAHs from Water

Compounds:	Pesticides, PAHs
Part Number:	60108-302
Phase:	HyperSep C18
Volume:	1 mL
Bed Weight:	100 mg
Conditioning:	1 mL methanol followed by 1 mL distilled water
Application:	Force or aspirate 50-100 mL water through column
Washing:	Dry column under vacuum
Elution:	Pour 500 µL ethyl acetate into column. Allow to percolate without vacuum. Collect 100 µL eluate

Organochlorine Insecticides from Water

Compounds:	Aldrin, p,p'-DDE, o,p'-DDE, o,p'-DDT, p,p'-DDT, dieldrin, endosulfan I, endosulfan II, endrin, heptachlor, heptachlor epoxide, lindane, p,p'-methoxychlor
Part Number:	60108-305
Phase:	HyperSep C18
Volume:	6 mL
Bed Weight:	500 mg
Sample Pretreatment:	Filter sample if required
Conditioning:	12 mL ethyl acetate followed by 6 mL methanol and 6 mL distilled water
Application:	Force or aspirate sample slowly through column.
Washing:	6 mL distilled water – dry column under vacuum for 15 min
Elution:	2 x 500 µL ethyl acetate Concentrate eluate to 250 µL in stream of nitrogen at 40 °C. Refer to Step 2 if second extraction step necessary

Trace Metal Elements from Water

Compounds:	Bi, Cd, Co, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Ti
Part Number:	60108-388
Phase:	HyperSep Phenyl
Volume:	3 mL
Bed Weight:	500 mg
Sample Pretreatment:	Adjust 500 mL water to pH 8-9 Add 1 mL 0.1% aqueous sodium diethyl dithiocarbamate solution
Conditioning:	3 mL methanol followed by 3 mL water
Application:	Force or aspirate sample through column at rate of 3-4 mL/min
Washing:	2 mL distilled water. Dry column under vacuum for 3-4 min.
Elution:	6 mL Methanol

Explosives from Water

Compounds:	1,3-dinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, nitrobenzene, RDX (hexahydro-1,3,5-trinitro-s-triazine), tetryl (N-methyl-N,2,4,6-tetranitroaniline), 1,3,5-trinitrobenzene, 2,4,6-trinitrotoluene
Part Number:	60108-305
Phase:	HyperSep C18
Volume:	6 mL
Bed Weight:	500 mg
Sample Pretreatment:	Adjust 500 mL water sample to pH 6 Dissolve 150 g NaCl in the sample and filter
Conditioning:	12 mL methanol followed by 12 mL water
Application:	Force or aspirate sample slowly through column
Washing:	1 mL distilled water. Dry column under vacuum for 5 min
Elution:	2 x 1 mL volumes methanol

Extraction of Tear Gas

Compounds:	Chloroacetophenone (cs), o-chlorobenzylidenemalonitrile (cn), trans-8-methyl-n-vanillyl-6-nonenamide (oc)
Part Number:	60108-742
Phase:	HyperSep Verify™-CX
Volume:	10 mL
Bed Weight:	200 mg
Sample Pretreatment:	Clothing: Cut out portion of sprayed area and a 'negative' control sample. Extract each into hexane Canisters: Spray onto a Kimwipe™ and extract sprayed area and a negative control sample into hexane.
Conditioning:	3 mL CH ₃ OH followed by 3 mL deionized water and 1 mL 100 mM phosphate buffer (pH 6)
Application:	Load at 1 mL/min
Washing:	3 mL deionized water and 3 mL hexane. Dry column for 5 min at >10 inches Hg
Elution:	1 mL CH ₃ OH Evaporate to dryness at < 40 °C

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