Analgesics

SPE Extraction of Salicylic and Acetylsalicylic Acids from Synthetic Urine

CHROM 10642

Procedure using GracePure™ C18-Max, 100mg:

Sample Treatment – Spike 2mL synthetic urine with 100ppm salicylic acid and 100ppm acetylsalicylic acid.

Conditioning – Rinse device with 3mL methanol followed by 3mL DI water.

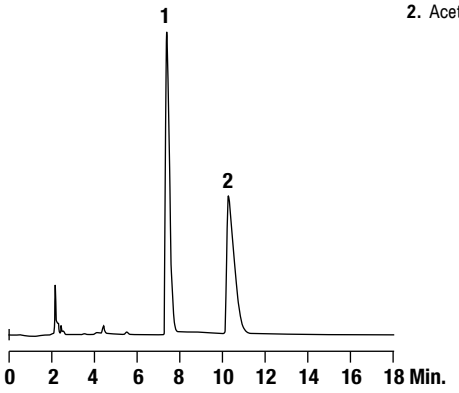
Sample Application – Apply 2mL spike urine.

Wash – Wash with 2mL 50mM phosphate buffer monobasic, pH 2.

Elution – Elute with 2mL methanol:water (50:50).

Column: Alltima™ HP C18 HiLoad, 5µm, 250 x 4.6mm (Part No. **87698**)
Mobile Phase: 5mM Sodium Acetate, pH5.2:Methanol (80:20)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

1. Salicylic Acid
2. Acetylsalicylic Acid



Sedatives

Benzodiazepines from Human Plasma

CHROM 10606 10608

Procedure using GracePure™ C18-Aq, 500mg:

Sample Treatment – Spike 500µL plasma with 10µL of a 0.1mg/mL standard solution. Combine with 100µL of 0.1M sodium carbonate buffer. Vortex and centrifuge for 10 minutes.

Conditioning – Rinse device 2mL water.

Sample Application – Apply entire sample.

Wash – Wash with 2mL water and then 50µL methanol.

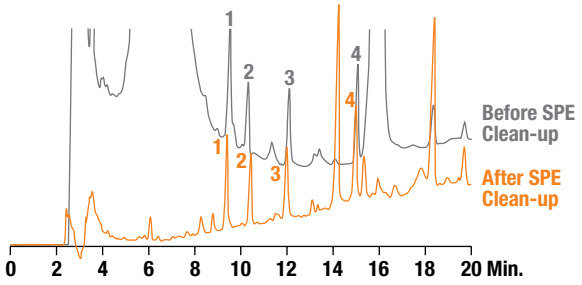
Elution – Elute with 600µL methanol.

Column: Alltima™ HP C18, 5µm, 250 x 4.6mm (Part No. 87680)
Mobile Phase: **A:** Water **B:** Acetonitrile
Gradient:

Time:	0	30
%B:	30	80

Flow Rate: 1mL/min
Detector: UV at 254nm

1. Oxazepam
2. Clonazepam
3. Temazepam
4. Diazepam



Diuretics

Diuretics from Urine

CHROM 10289 10290

Procedure using GracePure™ C18-Max, 1000mg:

Sample Treatment – Spike synthetic urine with 8 diuretics to a concentration of 1.25µg/mL each.

Conditioning – Rinse device 5mL methanol, followed by 5mL water.

Sample Application – Apply 15mL spiked urine sample at 1mL/min.

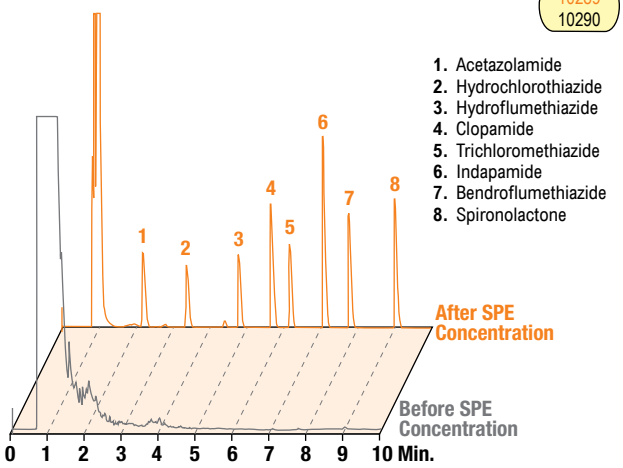
Wash – Wash with 5mL water.

Elution – Elute with 2mL methanol. Evaporate solvent and reconstitute in 250µL HPLC mobile phase.

Column: Alltima™ C18, 3µm, 100 x 4.6mm (Part No. **81382**)
Mobile Phase: **A:** 25mM Ammonium Acetate, 0.1% Trifluoroacetic Acid
B: Acetonitrile, 0.1% Trifluoroacetic Acid
Gradient:

Time:	0	10
%B:	20	90

Flow Rate: 1.0mL/min
Detector: ELSD



1. Acetazolamide
2. Hydrochlorothiazide
3. Hydroflumethiazide
4. Clopamide
5. Trichloromethiazide
6. Indapamide
7. Bendroflumethiazide
8. Spironolactone

St. John's Wort

Hypericins from St. John's Wort

CHROM 9107

Procedure Using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg St. John's Wort powder into five, 3mL aliquots of methanol:water (80:20). Combine and filter extracts to produce 15mL filtrate. Dilute to 30mL with water.

Conditioning – Rinse device with 5mL methanol followed by 5mL deionized water.

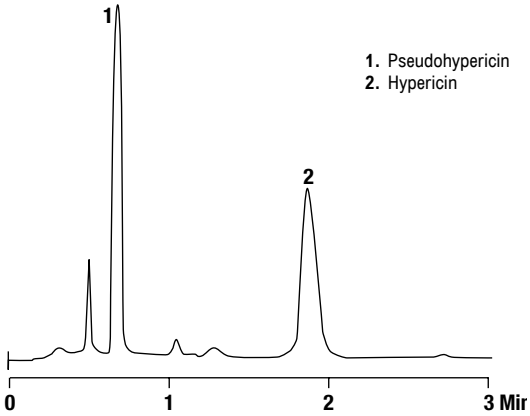
Sample Application – Apply 2mL filtrate to the top of the SPE device and draw through the C18 packed bed.

*Wash** – Wash device with 3mL of deionized water.

Elution – Elute with 2mL methanol.

*Repeat load and wash steps consecutively until filtrate is consumed.

Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ (Part No. **50605**)
Mobile Phase: Methanol:0.2% Phosphoric Acid (95:5)
Flow Rate: 4.0mL/min
Detector: VIS at 585nm



1. Pseudohypericin
2. Hypericin

Chamomile

Antioxidants from Chamomile

CHROM 9133

Procedure Using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 1g commercial chamomile tea grounds into 6mL dioxane:methanol (50:50). Filter extract and dilute to 20mL with water.

Conditioning – Rinse device with 5mL methanol followed by 5mL water.

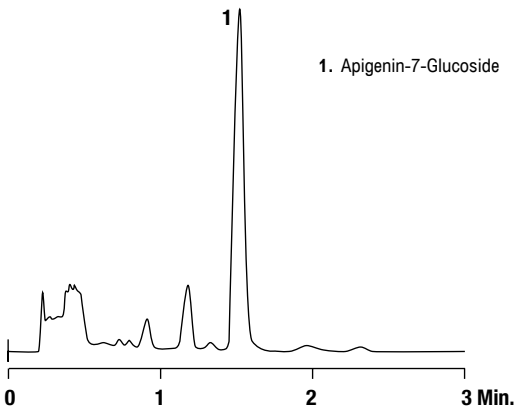
*Sample Application** – Apply 1mL filtrate.

*Wash** – Wash with 1mL water.

Elution – Elute first with 2mL methanol:water (50:50). Elute second, fraction and use for this analysis, with 2mL methanol.

*Before elution step, repeat load and wash steps until filtrate is consumed.

Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ (Part No. **50605**)
Mobile Phase: Acetonitrile:20mM K_2HPO_4, pH7.3 (65:35)
Flow Rate: 3mL/min
Detector: UV 340nm
Inj. Vol.: 5µL



1. Apigenin-7-Glucoside

Echinacea

Phenolic Acids from Echinacea

CHROM 9110

Procedure Using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg echinacea powder into five, 3mL aliquots of methanol. Combine and filter extracts to produce 15mL filtrate. Dilute to 30mL with water.

Conditioning – Rinse device with 5mL methanol followed by 5mL deionized water.

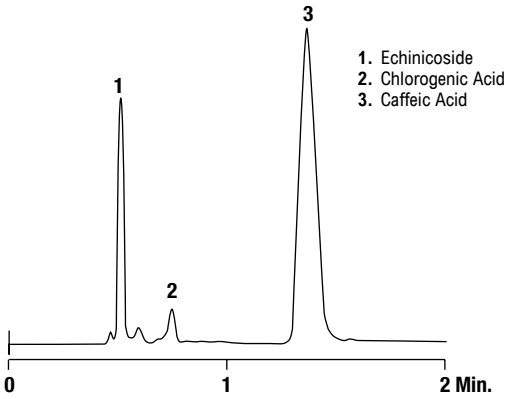
Sample Application – Apply 2mL filtrate to the top of the SPE device and draw through the C18 packed bed.

*Wash** – Wash device with 2mL of deionized water.

Elution – Elute with 5mL of methanol:water (50:50).

*Repeat load and wash steps consecutively until filtrate is consumed.

Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ (Part No. **50605**)
Mobile Phase: Acetonitrile:10mM K_2HPO_4, pH2.6 (20:80)
Flow Rate: 3.5mL/min
Detector: UV at 330nm



1. Echinicoside
2. Chlorogenic Acid
3. Caffeic Acid

Kava Kava

Sedatives from Kava Kava

CHROM 9125

Procedure using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg commercial kava kava root powder into four, 2mL aliquots of methanol:water (80:20). Combine and filter extracts to produce 8mL filtrate. Dilute to 10mL with water.

Conditioning – Rinse device with 5mL methanol followed by 5mL water.

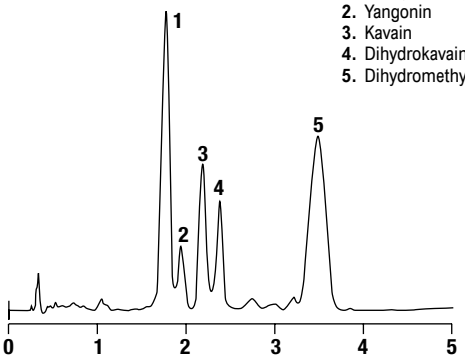
*Sample Application** – Apply 2mL filtrate.

*Wash** – Wash with 2mL water.

Elution – Elute with 3mL methanol.

*Before elution step, repeat load and wash steps until filtrate is consumed.

Column: Alltima™ C18, 3µm, 53 x 7mm Rocket™ (Part No. **50605**)
Mobile Phase: Acetonitrile:Isopropyl Alcohol:0.2% Acetic Acid (17:23:60)
Flow Rate: 3.5mL/min
Detector: UV 220nm
Inj. Vol.: 5µL



1. Methysticin
2. Yangonin
3. Kavain
4. Dihydrokavain
5. Dihydromethysticin

Dong Quai

Vasodilators from Dong Quai

CHROM 9132

Procedure using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg commercial kava kava root powder into four, 2mL aliquots of methanol:water (80:20). Combine and filter extracts to produce 8mL filtrate. Dilute to 10mL with water.

Conditioning – Rinse device with 5mL methanol followed by 5mL water.

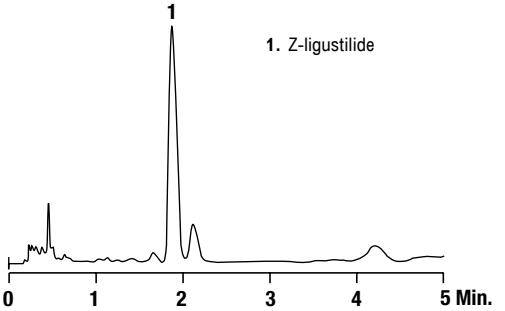
*Sample Application** – Apply 2mL filtrate.

*Wash** – Wash with 2mL water.

Elution – Elute with 3mL methanol.

*Before elution step, repeat load and wash steps until filtrate is consumed.

Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ (Part No. **50605**)
Mobile Phase: Methanol:0.2% Acetic Acid (65:35)
Flow Rate: 4.5mL/min
Detector: UV 270nm
Inj. Vol.: 5µL



1. Z-ligustilide

Additives

Preservatives from Fruit Punch

CHROM 2576

Procedure using GracePure™ Anion-X, 500mg:

Sample Treatment – Use 8mL fruit punch and adjust pH to 10 using potassium hydroxide.

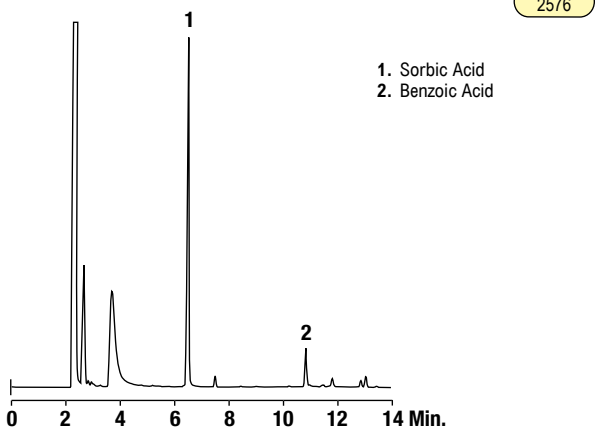
Conditioning – Rinse device 10mL water.

Sample Application – Apply 8mL pH adjusted fruit punch sample.

Wash – Wash with 20mL water.

Elution – Elute with 1mL 1.0N hydrochloric Acid followed by 1mL methanol.

Column: Heliflex® AT™ AquaWax-DA, 30m x 0.25mm x 0.25µm Capillary GC Column (Part No. **14537**)
Temp: 200°C (5 min hold) to 230°C (4 min hold) at 5°C/min
Carrier Gas: Helium at 0.75mL/min (25cm/sec)
Detector: FID at 250°C



Carbohydrates

Fungicides from Red Wine

CHROM 10637 10638

Procedure using GracePure™ C18-Max, 500mg:

Sample Treatment – Add 0.167mg/mL each of carbendazim, thiabendazolem, triadimenol, triadimefon, flusilazole, diniconazole into 1mL water. Combine with 1mL Beaujolais red wine.

Conditioning – Rinse device with 3mL methanol followed by 3mL water.

Sample Application – Apply 2mL red wine mixture.

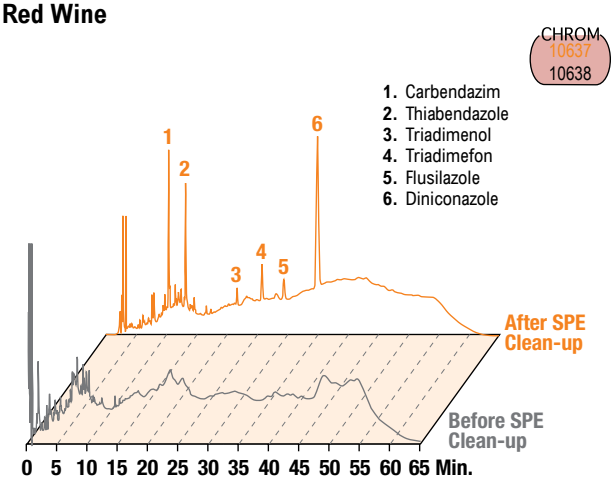
Wash – No wash.

Elution – Elute with 3mL methanol.

Column: Alltima™ HP C18 Amide, 5µm, 250 x 4.6mm (Part No. **87734**)
Mobile Phase: **A:** Water **B:** Acetonitrile
Gradient:

Time:	0	15	50	65
%B:	15	45	45	15

Flow Rate: 1mL/min
Detector: UV at 254nm



Proteins & Peptides/Reversed Phase

Prevail™ SPE Protein Desalting, Cytochrome C and Ribonuclease A Mix in 10mM Sodium Chloride

CHROM 10688

Procedure using Prevail™ C18, 500mg:

Sample Treatment – Cytochrome-C 1mg/mL, Ribonuclease-a 1mg/mL, Blank = water, Salt = 10mM NaCl.

Conditioning – 1 tube volume of methanol (do not dry). 2 tube volumes of 0.025% ammonium hydroxide. Dry tube via vacuum.

Sample Application – Pipette 1mL of sample (either proteins in water blank or proteins in salt solution) onto top of sorbent bed in SPE tube. Run vacuum @ approximately -10 kPa, loading sample in a tight band at top of sorbent column, continue to apply vacuum (@ -10 kPa) until tube is dry. Larger (2mL +) volumes can be de-salted and concentrated. Liquid collected will contain the NaCl

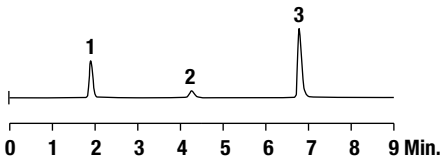
Elution – Pipette 500µL 0.4% TFA and 500µL Acetonitrile containing 0.4% TFA onto top of sorbent bed in spe tube. Run vacuum @ approximately -10 kPa, eluting sample from sorbent column, continue to apply vacuum until tube is dry.

Column: ProSphere™ 300 C4-300, 5µm, 150 x 4.6mm (Part No. **33989**)
Mobile Phase: **A:** 0.2% Trifluoroacetic Acid **B:** Acetonitrile
Gradient:

Time:	0	15
%B:	25	70

Flow Rate: 1mL/min
Detector: ELSD

1. Chloride
2. Ribonuclease A
3. Cytochrome C



Protein Extraction of Ribonuclease and Myoglobin

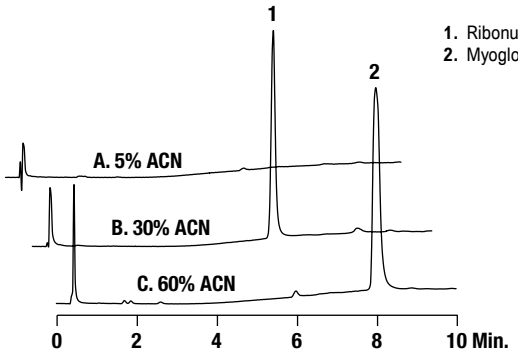
Procedure using Vydac® SPE:

A 3mL SPE cartridge was conditioned with 1mL of Acetonitrile followed by 1mL of 5% Acetonitrile/0.1% Trifluoroacetic Acid. Ribonuclease and myglobin (100mg each) were then loaded in 30% Acetonitrile/0.1% Trifluoroacetic Acid . The cartridge was washed with 1mL of 5% Acetonitrile/0.1% Trifluoroacetic Acid to remove weakly bound compounds, then 1mL of 30% Acetonitrile/0.1% Trifluoroacetic Acid followed by 1mL of 60% Acetonitrile/0.1% Trifluoroacetic Acid. HPLC analysis of the 5% Acetonitrile wash (A) revealed only a small amount of ribonuclease. Most of the ribonuclease eluted in the 30% Acetonitrile wash (B). The myoglobin eluted almost entirely in the 60% Acetonitrile wash (C).

Column: Vydac® C4, 5µm, 50 x 4.6mm (Part No. **214TP5405**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid **B:** Acetonitrile
Gradient:

Time:	0	10
%B:	15	70

1. Ribonuclease
2. Myoglobin



Pesticides

Chlorinated Pesticides in Vegetables

CHROM 2780 2789

Procedure using GracePure™ C18-Max, 500mg:

Sample Treatment – Homogenize 5 grams green bell pepper with 25mL methanol, spike with 0.5µg each of 16 chlorinated pesticides. Filter with 75mL filter column (Part No. **210775**). Dilute to 200mL with DI water and shake.

Conditioning – 2 x 5mL methanol, then 5mL DI water.

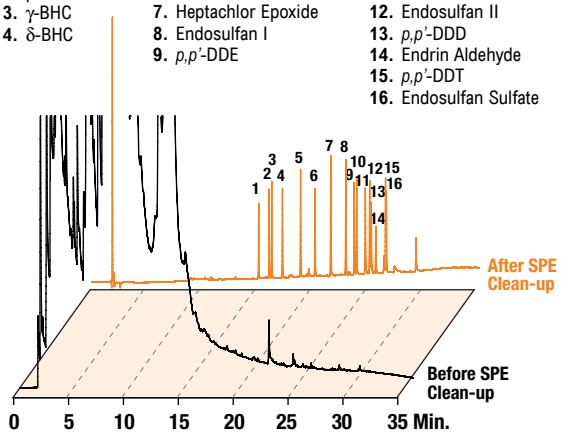
Sample Application – Attach 75mL reservoir (Part No. **210575**) with a syringe adapter (Part No. **210705**) and apply entire sample.

Wash – No wash, apply vacuum for 5 min.

Elution – Elute chlorinated pesticides with 2 x 1mL n-hexane/CH2Cl2 (70:30), evaporated this to dryness and reconstituted with 1mL n-hexane.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 160°C (0 min hold) at 10°C/min, then to 275°C at 5°C/min
Carrier Gas: Helium at 0.8mL/min (26cm/sec)
Detector: ECD at 310°C

1. α-BHC
2. β-BHC
3. γ-BHC
4. δ-BHC
5. Heptachlor
6. Aldrin
7. Heptachlor Epoxide
8. Endosulfan I
9. *p,p'*-DDE
10. Dieldrin
11. Endrin
12. Endosulfan II
13. *p,p'*-DDD
14. Endrin Aldehyde
15. *p,p'*-DDT
16. Endosulfan Sulfate



Chlorinated Pesticides in Soil

CHROM 2779 2788

Procedure using GracePure™ C18-Max, 500mg:

Sample Treatment – Weighed out 5 grams dry soil, spike with 1µg each of 16 chlorinated pesticides, add added 25mL methanol and homogenize. Filter with 75mL filter column (Part No. **210775**). Dilute to 200mL with DI water and shake.

Conditioning – 2 x 5mL methanol, then 5mL DI water.

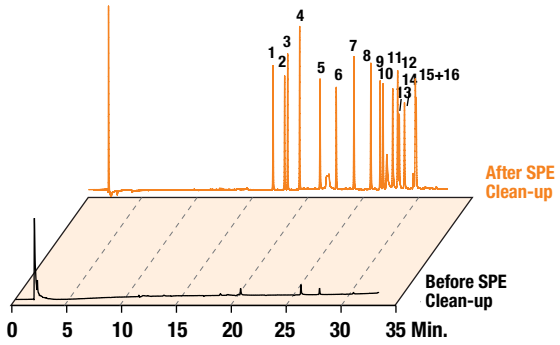
Sample Application – Attach 75mL reservoir (Part No. **210575**) with a syringe adapter (Part No. **210705**) and apply entire sample.

Wash – No wash.

Elution – Elute chlorinated pesticides with 2 x 1mL n-hexane/CH2Cl2 (70:30), evaporate to dryness and reconstitute with 1mL iso-octane.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 160°C (0 min hold) at 10°C/min, then to 275°C at 5°C/min
Carrier Gas: Helium at 0.8mL/min (26cm/sec)
Detector: ECD at 310°C

1. α-BHC
2. β-BHC
3. γ-BHC
4. δ-BHC
5. Heptachlor
6. Aldrin
7. Heptachlor Epoxide
8. Endosulfan I
9. *p,p'*-DDE
10. Dieldrin
11. Endrin
12. Endosulfan II
13. *p,p'*-DDD
14. Endrin Aldehyde
15. *p,p'*-DDT
16. Endosulfan Sulfate



PCBs in Transformer Oil

CHROM 2784

Procedure using GracePure™ Florisil®, 1000mg:

Sample Treatment – Dissolve 0.25grams of transformer oil spiked with Aroclor 1254 (at a concentration of 50mg/kg) in 20mL n-hexane.

Conditioning – 2 x 5mL n-hexane, making sure the cartridge does not dry.

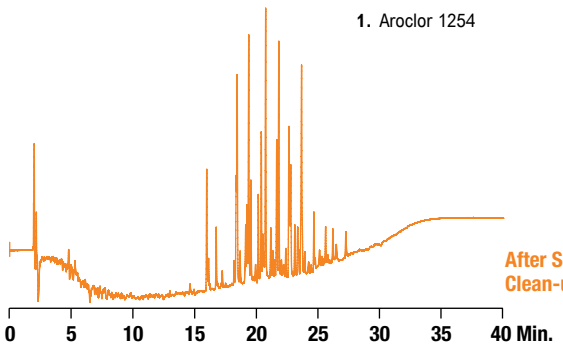
Sample Application – Attach 25mL reservoir (Part No. **210425**) with a syringe adapter (Part No. **210705**) and run sample through conditioned Florisil® tube, aspirating all the solution from the tube. Evaporate the volume down to 4mL and analyzed by GC/ECD.

Wash – No wash.

Elution – No elution, collect sample load.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 150°C (2 min hold) to 300°C (8 min hold) at 5°C/min
Carrier Gas: Helium at 0.75mL/min (25cm/sec)
Detector: ECD at 310°C

1. Aroclor 1254



Pesticides

PCBs in Salmon

CHROM 2781 2787

Procedure using GracePure™ C18-Fast, 1000mg:

Sample Treatment – Weigh out 2.5 grams salmon, spike with 2.5µg Aroclor 1242, add 25mL acetonitrile, homogenize for 30 seconds. Filter with 75mL filter tube (Part No. **210775**). Rinse salmon again with 5mL acetonitrile. Bring up to 100mL with deionized water.

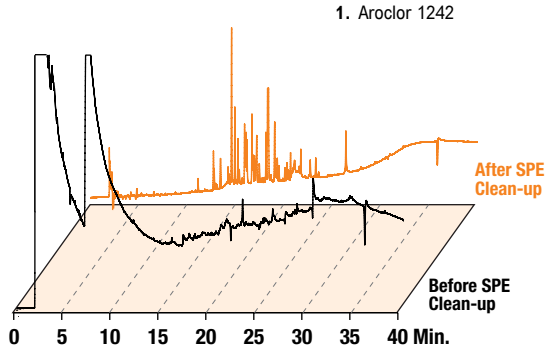
Conditioning – Rinse with 6mL hexanes, 6mL acetone, 2 x 6mL methanol, 2 x 6mL water.

Sample Application – Attach 75mL reservoir, apply entire sample.

Wash – 2 x 5mL water. Dry columns for 10 minutes at full vacuum.

Elution – Elute PCBs with 2 x 3mL 3% toluene/97% n-hexane. Evaporate solvent and reconstitute in 1mL n-hexane.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 150°C (2 min hold) to 300°C (2 min hold) at 5°C/min
Carrier Gas: Helium at 0.75mL/min (25cm/sec)
Detector: ECD at 310°C



Chlorinated Pesticides from Water

CHROM 1668

Procedure using GracePure™ C18-Fast, 1000mg:

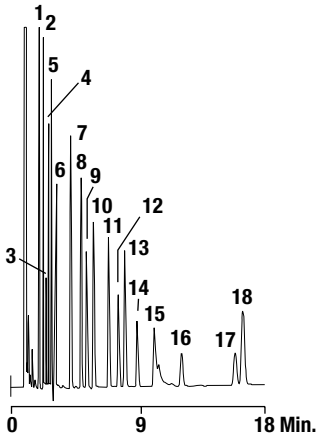
Conditioning – Rinse device with 5mL of methanol followed by 5mL deionized water.

Sample Application – Pass 100mL–500mL (containing 1% methanol) of water sample through the device at 20mL/minute.

Wash – Wash device with 10mL of deionized water then 10mL of methanol:deionized water (20:80). Remove excess by passing air through the device for two minutes.

Elution – Elute with 2mL of hexane:ethyl acetate (70:30). Pass extract through 2g–3g sodium sulfate to remove residual water.

Column: AT™ Pesticide 20m x 0.53mm x 0.60µm Capillary GC Column (Part No. **16846**)
Temp: 210°C
Carrier Gas: Helium, 35cm/sec
Detector: ECD



Recovery (as % Standard)
1. α-BHC (104%)
2. Lindane (109%)
3. β-BHC (106%)
4. Heptachlor (92%)
5. δ-BHC (110%)
6. Aldrin (98%)
7. Heptachlor Epoxide (107%)
8. α-Endosulfan (105%)
9. *p,p'*-DDE (79%)
10. Dieldrin (102%)
11. Endrin (104%)
12. *p,p'*-DDD (92%)
13. β-Endosulfan (103%)
14. *p,p'*-DDT
15. Endrin Aldehyde (102%)
16. Endosulfan Sulfate (103%)
17. Methoxychlor (99%)
18. Endrin Ketone (104%)

Carbamate Pesticides from Water

CHROM 10599 10600

Procedure using GracePure™ C18-Fast, 500mg:

Sample Treatment – Spike 500mL tap water with 125µL carbamate solution for final concentration of 25ppb.

Conditioning – Rinse with 3mL acetonitrile:water (80:20) followed by 3mL water. Dry with vacuum.

Sample Application – Apply 500µL sample.

Wash – 2 x 3mL water.

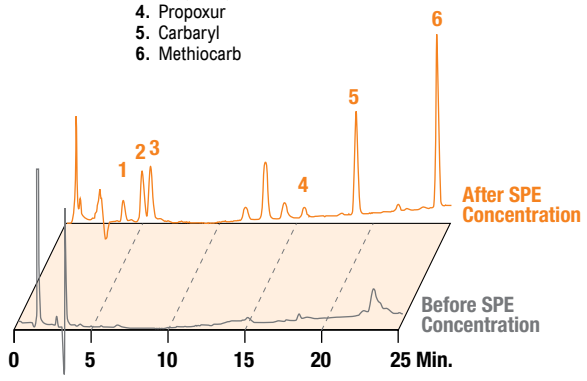
Elution – Elute with 4 x 1mL acetonitrile:water (80:20)

Column: Platinum™ EPS C18, 5µm, 250 x 4.6mm (Part No. **32246**)
Mobile Phase: **A:** DI water **B:** Acetonitrile
Gradient:

Time:	0	5	20	25	30
%B:	25	25	50	50	25

Flow Rate: 1mL/min
Detector: UV at 210nm

1. Aldicarb sulfoxide
2. Methomyl
3. 3-Hydroxycarbofuran
4. Propoxur
5. Carbaryl
6. Methiocarb



environmental

PAHs

PAHs in Water

CHROM 2771 2772

Procedure using GracePure™ C18-Max:

Sample Treatment – 500mL of water and add 75mL of 2-propanol, spiked with 20uL of PAH mixture at 2000ug/mL of each PAH, which corresponds to 40ug/500mL water, or 80ppb of each PAH.

Conditioning – 2 x 5mL of methanol, then 5mL of 85% water/15% 2-propanol.

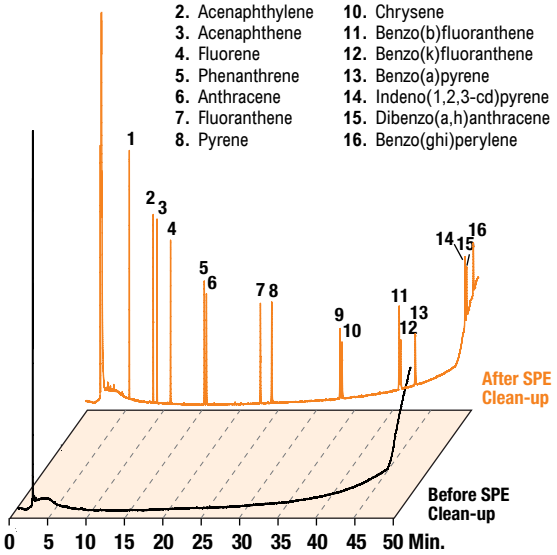
Sample Application – Attach 75mL reservoir (Part No. **210575**) with a syringe adapter (Part No. **210705**) and apply entire sample. Dry columns for 30 minutes at full vacuum.

Wash – No wash.

Elution – Elute PAHs with 3 x 1mL CH2Cl2 and adjusted the volume to 1mL by evaporating under a gentle nitrogen stream.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 80°C (1 min hold) to 160°C (0 min hold) at 25°C/min, then to 300°C (0 min hold) at 3°C/min, then to 325°C (3 min hold) at 25°C/min
Carrier Gas: Helium at 1mL/min (28cm/sec)
Detector: FID at 340°C

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo(a)anthracene
10. Chrysene
11. Benzo(b)fluoranthene
12. Benzo(k)fluoranthene
13. Benzo(a)pyrene
14. Indeno(1,2,3-cd)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(ghi)perylene



PAHs in Toasted Oat Cereal

CHROM 2775 2791

Procedure using GracePure™ C18-Max:

Sample Treatment – 500mL of water and add 75mL of 2-propanol, spiked with 20uL of PAH mixture at 2000ug/mL of each PAH, which corresponds to 40ug/500mL water, or 80ppb of each PAH.

Conditioning – 2 x 5mL of methanol, then 5mL of 85% water/15% 2-propanol.

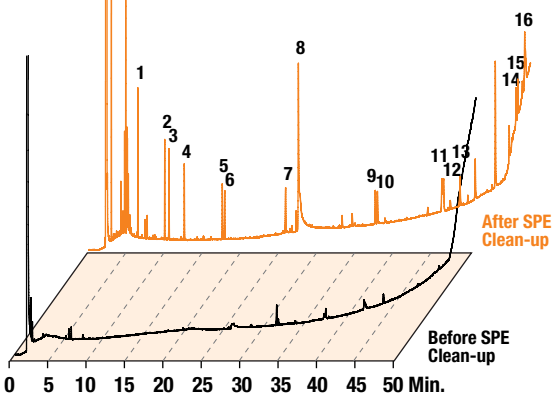
Sample Application – Attach 75mL reservoir (Part No. **210575**) with a syringe adapter (Part No. **210705**) and apply entire sample. Dry columns for 30 minutes at full vacuum.

Wash – 2 x 500µL of 85% water/15% isopropyl alcohol. Vacuum 10 min.

Elution – Elute PAHs with 3 x 1mL CH2Cl2 and adjusted the volume to 1mL by evaporating under a gentle nitrogen stream.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 80°C (1 min hold) to 160°C (0 min hold) at 25°C/min, then to 300°C (0 min hold) at 3°C/min, then to 325°C (3 min hold) at 25°C/min
Carrier Gas: Helium at 1mL/min (28cm/sec)
Detector: FID at 340°C

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo(a)anthracene
10. Chrysene
11. Benzo(b)fluoranthene
12. Benzo(k)fluoranthene
13. Benzo(a)pyrene
14. Indeno(1,2,3-cd)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(ghi)perylene



PAHs

PAHs in Cooking Oil

CHROM 2776 2792

Procedure using DVB Extract-Clean™, 500mg/4mL:

Sample Treatment – Weigh out 1 grams canola oil, spike with 20µg of each PAH, add 10mL n-hexane.

Conditioning – 10mL toluene, applied vacuum until toluene removed, then 5mL n-hexane.

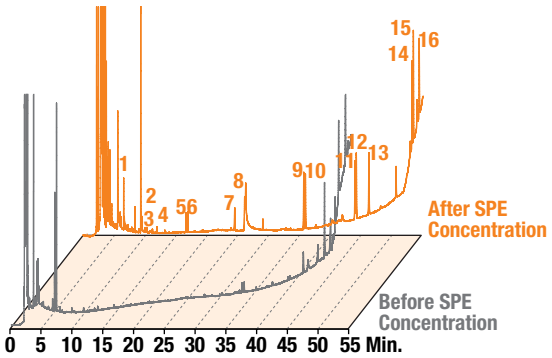
Sample Application – Aspirate the entire canola oil/hexane/PAH solution through the tube.

Wash – 5mL n-hexane.

Elution – Elute PAHs with 4 X 2.5mL toluene and adjusted the sample volume to 0.5mL (500µL) by evaporating under dry nitrogen stream and gentle heat.

Column: AT™ 5ms, 0.25µm, 30m x 0.25mm (Part No. **15807**)
Temp: 80°C (1 min hold) to 160°C (0 min hold) at 25°C/min, then to 300°C (0 min hold) at 3°C/min, then to 325°C (3 min hold) at 25°C/min
Carrier Gas: Helium at 1mL/min (28cm/sec)
Detector: FID at 340°C

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo(a)anthracene
10. Chrysene
11. Benzo(b)fluoranthene
12. Benzo(k)fluoranthene
13. Benzo(a)pyrene
14. Indeno(1,2,3-cd)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(ghi)perylene



Nitroaromatics

Nitroaromatics and Naphthols from Soil

CHROM 10597 10598

Procedure using GracePure™ C18-Max, 500mg:

Sample Treatment – Spike 100g soil with 7.5µg/g of each analyte. Combine soil with 1000mL deionized water, shake for 10 minutes, and filter.

Conditioning – Rinse device with 5mL methanol followed by 5mL water.

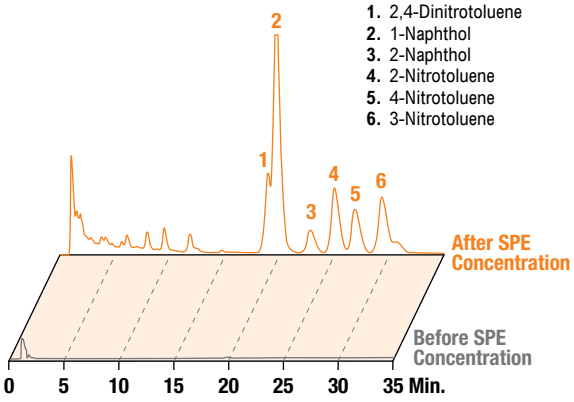
Sample Application – Aspirate 1000mL water sample through SPE at flow rate 1-5mL/min.

Wash – No wash. Air dry for 15 seconds.

Elution – Elute with three 1mL aliquots of methanol:water (50:50). Air dry for 15 seconds between each elution.

Column: Adsorbosphere™ UHS C18, 5µm, 150m x 4.6mm (Part No. **288118**)
Mobile Phase: Methanol:Water (50:50)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

1. 2,4-Dinitrotoluene
2. 1-Naphthol
3. 2-Naphthol
4. 2-Nitrotoluene
5. 4-Nitrotoluene
6. 3-Nitrotoluene



environmental

Hypericins from St. John's Wort

CHROM 9107

Procedure Using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg St. John's Wort powder into five, 3mL aliquots of methanol:water (80:20). Combine and filter extracts to produce 15mL filtrate. Dilute to 30mL with water.

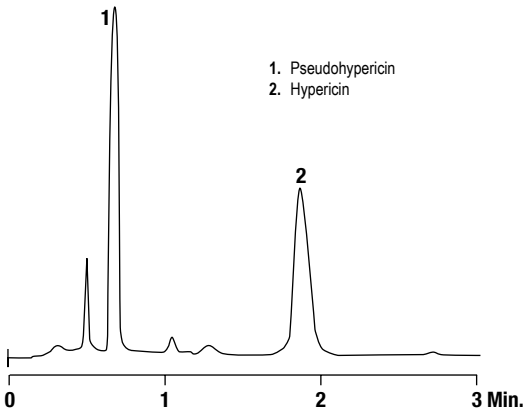
Conditioning – Rinse device with 5mL methanol followed by 5mL deionized water.

Sample Application – Apply 2mL filtrate to the top of the SPE device and draw through the C18 packed bed.

*Wash** – Wash device with 3mL of deionized water.

Elution – Elute with 2mL methanol.

*Repeat load and wash steps consecutively until filtrate is consumed.



Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ HPLC Column, (Part No. **50605**)
Mobile Phase: MeOH:0.2% H_3PO_4 (95:5)
Flow Rate: 4.0mL/min
Detector: VIS at 585nm

Phenolic Acids from Echinacea

CHROM 9110

Procedure Using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg echinacea powder into five, 3mL aliquots of methanol. Combine and filter extracts to produce 15mL filtrate. Dilute to 30mL with water.

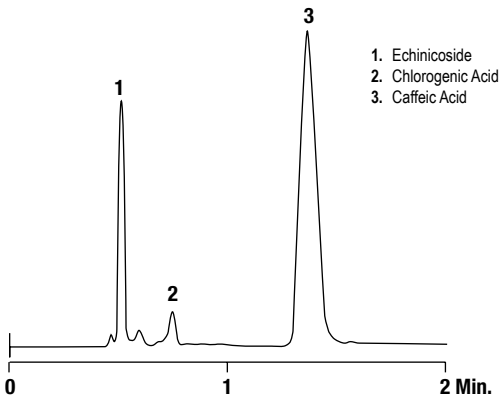
Conditioning – Rinse device with 5mL methanol followed by 5mL deionized water.

Sample Application – Apply 2mL filtrate to the top of the SPE device and draw through the C18 packed bed.

*Wash** – Wash device with 2mL of deionized water.

Elution – Elute with 5mL of methanol:water (50:50).

*Repeat load and wash steps consecutively until filtrate is consumed.



Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ HPLC Column, (Part No. **50605**)
Mobile Phase: ACN:10mM K_2HPO_4, pH 2.6 (20:80)
Flow Rate: 3.5mL/min
Detector: UV at 330nm

Chlorinated Pesticides from Water

CHROM 1668

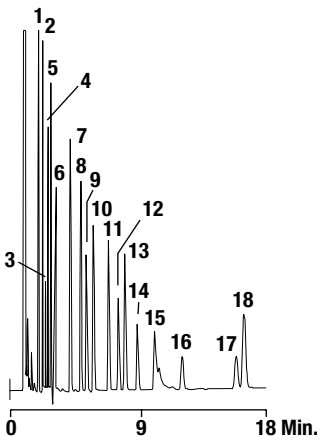
Procedure Using GracePure™ C18-Fast, 1000mg:

Conditioning – Rinse device with 5mL of methanol followed by 5mL deionized water.

Sample Application – Pass 100mL–500mL (containing 1% methanol) of water sample through the device at 20mL/minute.

Wash – Wash device with 10mL of deionized water then 10mL of methanol:deionized water (20:80). Remove excess by passing air through the device for two minutes.

Elution – Elute with 2mL of hexane:ethyl acetate (70:30). Pass extract through 2g–3g sodium sulfate to remove residual water.



Recovery (as % Standard)
1. α-BHC (104%)
2. Lindane (109%)
3. β-BHC (106%)
4. Heptachlor (92%)
5. δ-BHC (110%)
6. Aldrin (98%)
7. Heptachlor Epoxide (107%)
8. α-Endosulfan (105%)
9. p,p'-DDE (79%)
10. Dieldrin (102%)
11. Endrin (104%)
12. p,p'-DDD (92%)
13. β-Endosulfan (103%)
14. p,p'-DDT
15. Endrin Aldehyde (102%)
16. Endosulfan Sulfate (103%)
17. Methoxychlor (99%)
18. Endrin Ketone (104%)

Column: AT™-Pesticide 20m x 0.53mm x 0.60µm Capillary GC Column, (Part No. **16846**)
Temperature: 210°C
Carrier Gas: Helium, 35cm/sec
Detector: ECD

Antioxidants from Chamomile

CHROM 9133

Procedure Using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 1g commercial chamomile tea grounds into 6mL dioxane:methanol (50:50). Filter extract and dilute to 20mL with water.

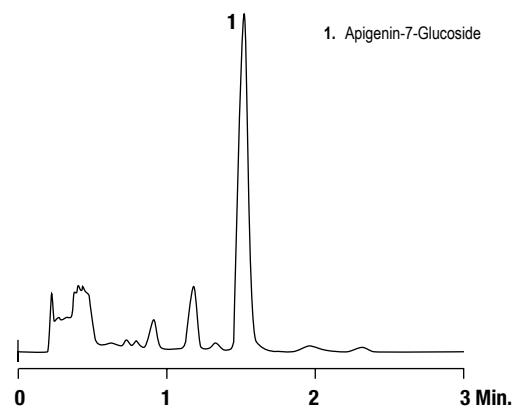
Conditioning – Rinse device with 5mL methanol followed by 5mL water.

*Sample Application** – Apply 1mL filtrate.

*Wash** – Wash with 1mL water.

Elution – Elute first with 2mL methanol:water (50:50). Elute second, fraction and use for this analysis, with 2mL methanol.

*Before elution step, repeat load and wash steps until filtrate is consumed.



Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ HPLC Column, (Part No. **50605**)
Mobile Phase: ACN:20mM K_2HPO_4, pH 7.3 (65:35)
Flow Rate: 3mL/min
Detector: UV 340nm
Inj. Vol.: 5µL

Vasodilators from Dong Quai

CHROM 9132

Procedure using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 1500mg commercial *dong quai* root powder into three, 5mL aliquots of methanol. Combine and filter extracts to produce 15mL filtrate. Dilute to 30mL with water.

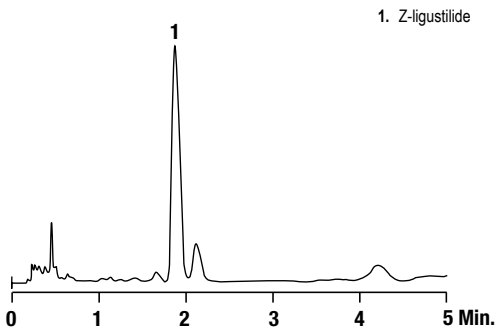
Conditioning – Rinse device with 5mL methanol followed by 5mL water.

*Sample Application** – Apply 1mL filtrate.

*Wash** – Wash with 2mL water.

Elution – Elute with 2mL methanol.

*Before elution step, repeat load and wash steps until filtrate is consumed.



Column: Alltima™ C18, 3µm 53 x 7mm Rocket™ HPLC Column (Part No. **50605**)
Mobile Phase: Methanol:0.2% Acetic Acid (65:35)
Flow Rate: 4.5mL/min
Detector: UV 270nm
Inj. Vol.: 5µL

Sedatives from Kava Kava

CHROM 9125

Procedure using GracePure™ C18-Max, 1000mg:

Sample Treatment – Pulverize 300mg commercial *kava kava* root powder into four, 2mL aliquots of methanol:water (80:20). Combine and filter extracts to produce 8mL filtrate. Dilute to 10mL with water.

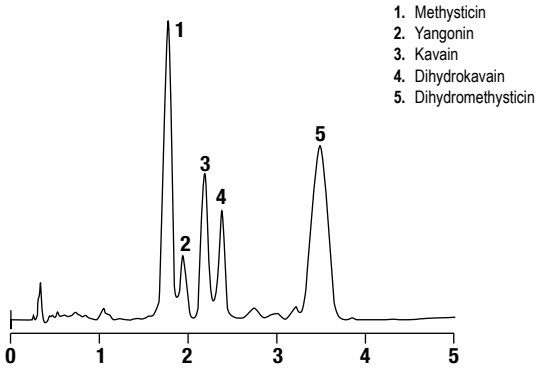
Conditioning – Rinse device with 5mL methanol followed by 5mL water.

*Sample Application** – Apply 2mL filtrate.

*Wash** – Wash with 2mL water.

Elution – Elute with 3mL methanol.

*Before elution step, repeat load and wash steps until filtrate is consumed.



Column: Alltima™ C18, 3µm, 53 x 7mm Rocket™ HPLC Column (Part No. **50605**)
Mobile Phase: ACN:IPA:0.2% Acetic Acid (17:23:60)
Flow Rate: 3.5mL/min
Detector: UV 220nm
Inj. Vol.: 5µL

Fungicides from Red Wine

CHROM 10637 10638

Procedure using GracePure™ C18-Max, 500mg:

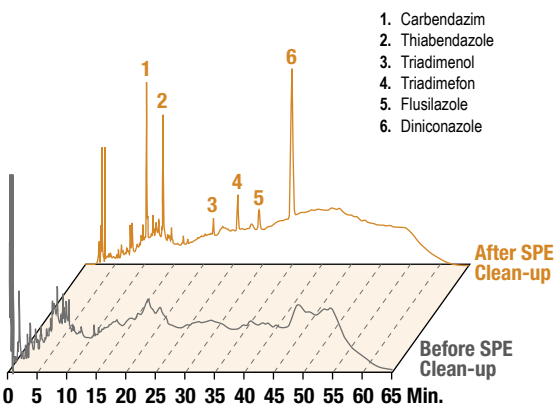
Sample Treatment – Add 0.167mg/mL each of carbendazim, thiabendazolem, triadimenol, triadimefon, flusilazole, diniconazole into 1mL water. Combine with 1mL Beaujolais red wine.

Conditioning – Rinse device with 3mL methanol followed by 3mL water.

Sample Application – Apply 2mL red wine mixture.

Wash – No wash.

Elution – Elute with 3mL methanol.



Column: Alltima™ HP C18 Amide, 5µm, 250 x 4.6mm HPLC Column (Part No. **87734**)
Mobile Phase: **A:** Water **B:** Acetonitrile
Gradient: **(Time, %B):** (0,15%), (15,45%), (50,45%), (65,15%)
Flow Rate: 1mL/min
Detector: UV at 254nm

Nitroaromatics and Naphthols from Soil

CHROM 10597 10598

Procedure using GracePure™ C18-Max, 500mg:

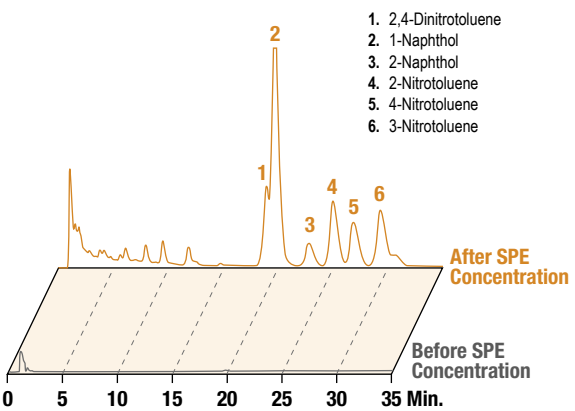
Sample Treatment – Spike 100g soil with 7.5µg/g of each analyte. Combine soil with 1000mL deionized water, shake for 10 minutes, and filter.

Conditioning – Rinse device with 5mL methanol followed by 5mL water.

Sample Application – Aspirate 1000mL water sample through SPE at flow rate 1-5mL/min.

Wash – No wash. Air dry for 15 seconds.

Elution – Elute with three 1mL aliquots of methanol:water (50:50). Air dry for 15 seconds between each elution.



Column: Adsorbosphere™ UHS C18, 5µm, 150 x 4.6mm HPLC Column (Part No. **288118**)
Mobile Phase: Methanol:Water (50:50)
Flow Rate: 1.0mL/min
Detector: UV at 254nm
Temperature: Ambient

Benzodiazepines from Human Plasma

CHROM 10606 10608

Procedure using GracePure™ C18-Aq, 500mg:

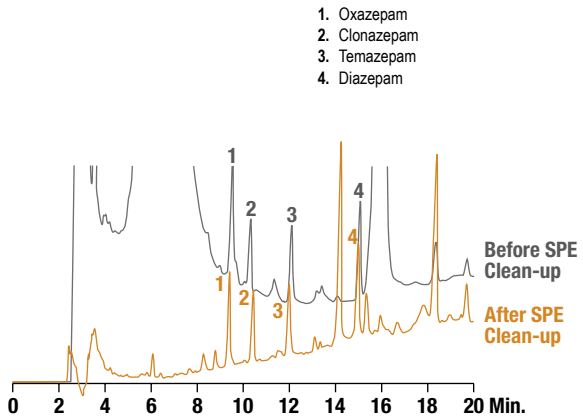
Sample Treatment – Spike 500µL plasma with 10µL of a 0.1mg/mL standard solution. Combine with 100µL of 0.1M sodium carbonate buffer. Vortex and centrifuge for 10 minutes.

Conditioning – Rinse device 2mL water.

Sample Application – Apply entire sample.

Wash – Wash with 2mL water and then 50µL methanol.

Elution – Elute with 600µL methanol.



Column:	Alltima™ HP C18, 5µm, 250 x 4.6mm HPLC Column (Part No. **87680**)
Mobile Phase:	**A:** Water **B:** Acetonitrile
Gradient:	**(Time, %B):** (0,30), (30,80)
Flow Rate:	1mL/min
Detector:	UV at 254nm
Temperature:	Ambient

Carbamate Pesticides from Water

CHROM 10599 10600

Procedure using GracePure™ C18-Fast, 500mg:

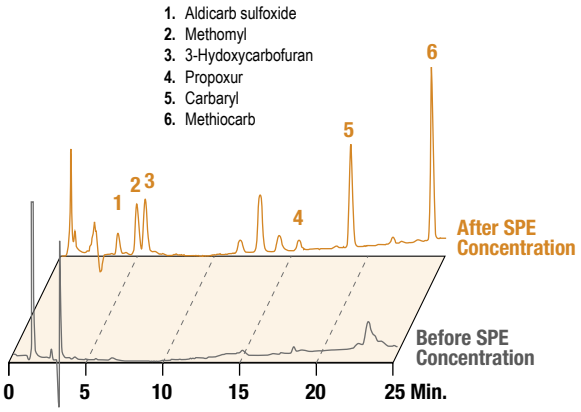
Sample Treatment – Spike 500mL tap water with 125µL carbamate solution for final concentration of 25ppb.

Conditioning – Rinse with 3mL acetonitrile:water (80:20) followed by 3mL water. Dry with vacuum.

Sample Application – Apply 500µL sample.

Wash – 2 x 3mL water.

Elution – Elute with 4 x 1mL acetonitrile:water (80:20)



Column:	Platinum™ EPS C18, 5µm, 250 x 4.6mm HPLC Column (Part No. **32246**)
Mobile Phase:	**A:** DI water **B:** Acetonitrile
Gradient:	**(Time, %B):** (0,25,), (5,25), (20,50), (25,50), (30,25)
Flow Rate:	1mL/min
Detector:	UV at 210nm
Temperature:	Ambient

Preservatives from Fruit Punch

CHROM 2576

Procedure using GracePure™ Anion-X, 500mg:

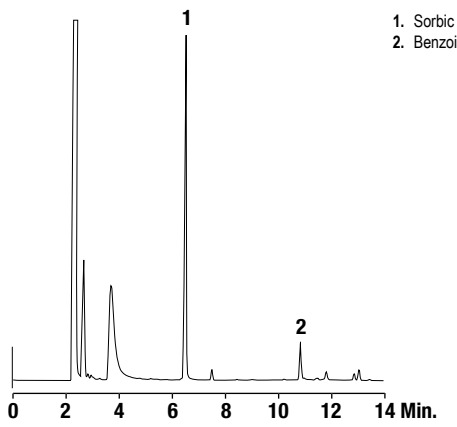
Sample Treatment – Use 8mL fruit punch and adjust pH to 10 using potassium hydroxide.

Conditioning – Rinse device 10mL water.

Sample Application – Apply 8mL pH adjusted fruit punch sample.

Wash – Wash with 20mL water.

Elution – Elute with 1mL 1.0N hydrochloric acid followed by 1mL methanol.



Column:	Heliflex® AT™ AquaWax-DA, 30m x 0.25mm x 0.25µm Capillary GC Column (Part No. **14537**)
Temp:	200°C (5 min hold) to 230°C (4 min hold) at 5°C/min
Carrier:	Helium at 0.75mL/min (25cm/sec)
Detector:	FID at 250°C

Diuretics from Urine

CHROM 10289 10290

Procedure using GracePure™ C18-Max, 1000mg:

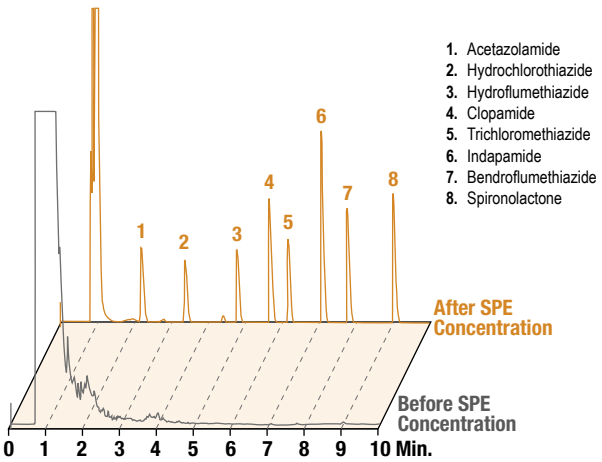
Sample Treatment – Spike synthetic urine with 8 diuretics to a concentration of 1.25µg/mL each.

Conditioning – Rinse device 5mL methanol, followed by 5mL water.

Sample Application – Apply 15mL spiked urine sample at 1mL/min.

Wash – Wash with 5mL water.

Elution – Elute with 2mL methanol. Evaporate solvent and reconstitute in 250µL HPLC mobile phase.



Column:	Alltima™ C18, 3µm, 100 x 4.6mm HPLC Column (Part No. **81382**)
Mobile Phase:	**A:** 25mM Ammonium Acetate, 0.1%TFA **B:** Acetonitrile, 0.1%TFA
Gradient:	**(Time, %B):** (0,20,), (10,90)
Flow Rate:	1.0mL/min
Detector:	ELSD

Applications Chart

Analyte Class	Matrix	Analytes per Application	GracePure™ Product	Pretreatment
Amphetamines	Urine	Amphetamine and Methamphetamine	C18-Aq, 500mg	Spike urine with 1µg/mL target analytes. Dilute with equal volume of 2% ammonium hydroxide in DI water.
Anticonvulsants	Serum	Phenobarbital, Primidone, Carbamazepine, 5,5-Diphenylhydantoin, MPPH (5-Methylphenyl-5-phenylhydantoin)	C18-Low, 500mg	Add 100µL of 0.1M KH_2PO_4 buffer, pH 3.5 to 500µL of serum in a test tube. Add 200µg/mL MPPH, 5-methylphenyl-5-phenyl-hydantoin as internal standard. Vortex 1 minute.
Benzodiazepines	Serum	Norchlordiazepoxide, Demoxepam, Chlordiaz-epoxide, Nitrazepam, Nordiazepam (Metabolite of diazepam), Diazepam	C18-Low, 500mg	Use 500µL serum. Add 500µL internal standard solution: 50µg/mL benzodiazipine. Vortex 1 minute.
BHA	Soy Oil	BHA (3-*tert*-Butyl-4-hydroxyanisole)	Amino, 200mg	Add 10mg BHA into 1mL soy oil and dilute to 10mL with *n*-Pentane.
Caffeine	Coffee	Caffeine	C18-Aq, 500mg	None, will work equally well for any beverage containing caffeine.
Carbohydrates	Molasses	Fructose, Glucose, Sucrose	C18-Low, 500mg	Dilute 20g molasses to 250mL with DI water.
Carbohydrates	Wine	Ethanol, Glucose, Sucrose	C18-Max, 100mg	None.
Chlorinated Pesticides	Water	α-BHC, Lindane, β-BHC, Heptachlor, Aldrin, Heptachlor Epoxide, p,p'-DDE, Dieldrin, o,p'-DDD, Endrin, o,p'-DDT, p,p'-DDD, p,p'-DDT	C18-Fast, 500mg	Due to the large sample volume, attach large volume reservoir to SPE device.
Chlorotetracycline	Ointment	Chlorotetracycline	Diol, 500mg	Add 2mL of hexane to 50mg of ointment. Vortex 1 minute.
Chlorphenoxy Acid Herbicides	Water	2,4-D; 2,4,5-T; Silvex	C18-Fast, 500mg	Acidify 100mL water sample to pH 2.2.
Desalting	Protein Solution	Cytochrom C, Ribonuclease-A	C18-Aq, 500mg	None.
Lactic Acid	Water	Lactic Acid	Anion-X, 500mg	None.
Lidocaine, Metabolites	Serum	GX (Glycinexylidide), MEGX (Monoethylgly-cinexylidide), Lidocaine, Mepivacaine (internal standard),	C18-Low, 500mg	Use 500µL serum. Add 500µL internal standard solution: 50µg/mL Mepivacaine HCl in 0.1M NaH_2PO_4. Vortex 1 minute.
Nitroaromatics and Naphthols	Water	2,4-DNT, 2-NT, 4-NT, 3-NT, 1- Naphthol, 2-Naphthol	C18-Fast, 500mg	Spike 1000mL tap water with 0.75µg/mL of analytes.
Off Flavors	Wine	4-Ethyl Phenol, 4-Ethyl Gualacol	C18-Low, 500mg	None.
Paraben Preservatives	Cosmetics	Methyl Paraben, Propyl Paraben	C18-Low, 500mg	Weigh one gram of cosmetic (hand cream, toothpaste, liquid soap) into a test tube. Add 10mL methanol and vortex one minute. Centrifuge resulting mixture to remove insoluble materials. Remove a 100µL aliquot to a 2mL volumetric flask and dilute to volume with methanol.
Perchlorate	Biological Matrix	Perchlorate	Anion-X, 500mg	None.
Phenylpropanolamine	Urine	Phenylpropanolamine	C18-Low, 100mg	1mL urine sample is placed in a small test tube. Add 250mL of carbonate buffer ($NaHCO_3/Na_2CO_3$, 5:1 w/w) Vortex 1 minute.
Phthalate Esters	Drinking Water	Dimethyl Phthalate, Diethyl Phthalate, Diallyl Phthalate, Dibutyl Phthalate, Diamyl Phthalate	C18-Low, 500mg	None.
Polyaromatic Hydrocarbons	River Water	Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenz[ah]anthracene, Benzo[ghi]perylene, Indeno[1,2,3-cd]pyrene	C18-Aq, 500mg	None.
Polyaromatic Hydrocarbons	Tap Water	Acenaphthalene, Fluorene, Phenanthrene, Anthracene, Fluoranthrene, Pyrene, Benz[a]anthracene, Benzo[b]fluoranthrene, Benzo[k]fluoranthrene, Benzo[a]pyrene, Dibenz[a,h]anthracene, Indeno[1,2,3-cd]pyrene, Benzo[ghi]perylene	C18-Low, 500mg	None.
Preservatives	Beverages	Propionic Acid, Butyric Acid, Valeric Acid, Caproic Acid, Heptanoic Acid, Caprylic Acid	Anion-X, 500mg	Adjust beverage pH to 10 using KOH.
Quinidine	Urine	Quinidine	Diol, 500mg	Add 1mL HCl and 1mL urine sample to a 5mL volumetric flask. Heat to 65ºC in a water bath for 10 minutes. Cool and add 1mL ammonium hydroxide. Dilute to volume with distilled water.
Salicylic Acid	Urine	Salicylic Acid, Acetylsalicylic Acid	C18-Max, 100mg	Spike 2mL synthetic urine with 100ppm salicylic acid and 100ppm acetylsalicylic acid.
Sedatives/Hypnotics	Serum	Barbital, Methyprylon, Amobarbital, Phenacetin, Secobarbital, Meprobamate, Glutethimide, Caf-feine, Phenobarbital, Methaqualone, Oxazepam, 4-Methyl Primidone, Diazepam, Nodiazepam	C18-Low, 500mg	Use 500µL serum. Add 200µL internal standard solution: 10µg/mL 4-methyl primidone in 0.1M KH_2PO_4, pH 4. Vortex 1 minute.
Steroids	Hydrocortisone Cream	Hydrocortisone	Silica, 500mg	Weigh one gram of cream into a 20mL vial. Add 10mL hexane: ethyl acetate (50:50). Vortex 3 minutes. Decant supernatant into a 50mL volumetric flask. Repeat extraction and combine super-natants. Dilute to volume with hexane:ethyl acetate (50:50).
THC	Urine	Δ9-Tetrahydrocannabinol	C18-Low, 500mg	Place 10mL urine sample in a centrifuge tube. Add 0.9mL of 10N NaOH. Cap tube and place in boiling water bath for 15 minutes. Cool to room temperature. Adjust pH to 2. Vortex 1 minute.
THC, Metabolites	Urine	Δ9-Tetrahydrocannabinol Methyl Ester, 9-Carboxy-11-nor-Δ9-THC Methyl Ester (Metabolite of #1)	C18-Low, 500mg	Add 1mL methanolic KOH (10% w/v) to 10mL of urine in a test tube. Cap and heat tube to 100ºC for 15–20 minutes. Cool to room temperature and adjust pH to 3.
Theophylline	Serum	β-Hydroxyethyl Theophylline (internal standard), Theophylline	C18-Low, 100mg	Add 2mL of 0.1M KH_2PO_4 (pH 4) buffer to 1mL serum. Vortex for one minute.
Topical Anesthetics	Serum	Benzocaine, Procaine, Mepivacaine	C18-Low, 500mg	Use 500µL serum. Add 500µL internal standard solution: 50µg/mL Mepivacaine HCl in 0.1M NaH_2PO_4. Vortex 1 minute.

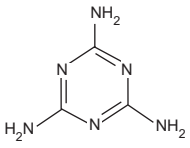
Contact your office or distributor for pricing.

GracePure™

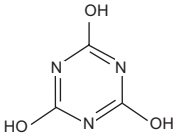
Precondition	Load	Wash	Elute
5mL methanol followed by 5mL DI water.	Apply 10mL sample.	2mL DI water, followed by 1mL IPA:DI water (25:75). Vacuum 2 minutes. Next wash 1mL hexane, vacuum 2 minutes. Final wash with 1mL IPA.	3 x 1mL IPA containing 2% ammonium hydroxide.
5mL methanol followed by 5mL DI water.	Add the prepared sample.	9mL of DI water, vacuum 2 minutes.	500µL of methanol.
5mL methanol followed by 5mL DI water.	Add the serum sample.	6mL DI water, vacuum 2 minutes.	1mL of methanol.
3mL pentane.	Add 1mL sample.	1.5mL *n*-pentane.	2mL ethanol.
5mL methanol followed by 5mL DI water.	Add 1mL prepared sample.	6mL DI water, vacuum 10 minutes.	3mL of chloroform.
5mL methanol followed by 5mL DI water.	Add 2mL prepared sample.	No wash, apply vacuum for 5 minutes.	Collect eluate. Filter through a 0.45µm syringe filter.
2mL methanol followed by 2mL DI water.	Add 2mL of wine with the vacuum turned off.	No wash, allow wine to remain in contact with cartridge for 2 minutes.	Turn on vacuum and collect eluant. The organic acids and anthocyanins will retain while the carbohydrates pass through.
5mL methanol followed by 5mL DI water.	Add 100mL of water sample.	No wash, apply vacuum for 5 minutes.	2mL of ethyl acetate.
3mL of hexane.	Add 500µL prepared sample.	2mL of hexane, continue vacuum for 3 minutes.	2mL of a methanol:0.1N HCl solution (50:50).
5mL methanol followed by 5mL DI water.	Add acidified sample.	Wash with 6mL of DI water.	3mL of chloroform.
3mL methanol followed by 0.025% ammonium hydroxide.	Apply 1mL protein salt solution.	No wash.	500µL 0.4% TFA followed by 500µL acetonitrile containing 0.4% TFA. Apply vacuum until dry.
2mL 1M NaCl followed by 10mL DI water.	1mL, 1mL/min. (pH 7).	DI water, 2mL.	0.1M HCl, 500µL.
5mL methanol followed by 5mL DI water.	Add sample.	8mL DI water:methanol (75:25), vacuum 2 minutes.	500µL methanol.
5mL methanol followed by 5mL DI water.	Add 1000mL sample at flow rate of 5mL/min.	No wash.	Elute with 3 x 1mL methanol:water (50:50). Air dry after each elution.
5mL methanol followed by 5mL DI water.	Apply 10mL wine sample.	5mL water.	1mL isopropyl alcohol.
5mL methanol followed by 5mL DI water.	Add 2mL prepared sample.	3mL DI water, vacuum 2 minutes.	1mL methanol.
3mL 0.5M NaCl followed by 3mL DI water.	Apply 1mL sample.	No wash.	3 x 0.75mL of 0.1M NaCl.
2mL methanol followed by 2mL DI water.	Add the buffered urine.	2mL DI water, vacuum 2 minutes.	6mL of chloroform:isopropanol (90:10) through the cartridge. Repeat with an additional 0.2mL.
5mL methanol followed by 5mL DI water.	Add 200mL water sample.	3mL DI water.	Pass two 500µL aliquots of ethyl acetate.
5mL methanol followed by 5mL DI water.	Apply 200mL water containing PAH's.	2mL DI water followed by 2mL IPA:Water (20:80).	2 x 2mL methanol.
6mL 2-propanol:DI water (15:85).	Add 100mL water sample.	2mL 2-propanol:DI water (15:85).	1mL methylene chloride.
10mL DI water.	Apply 8mL beverage sample.	20mL DI water.	1mL 1.0N HCl followed by 1mL methanol.
3mL methanol followed by 3mL DI water adjusted to pH 9.	Add 500µL prepared sample.	1mL of distilled water, continue vacuum for 2 minutes to remove residual wash solution.	Pass two aliquots of 500µL methanol.
3mL methanol followed by 3mL DI water.	Add 2mL spike urine.	2mL 50mM phosphate buffer monobasic, pH 2.	2mL methanol:water (50:50).
5mL methanol followed by 5mL DI water.	Add prepared serum sample.	6mL DI water, vacuum 2 minutes.	500µL acetone.
2mL, hexane:acetone (80:20).	Add 1mL prepared sample.	2mL of hexane:acetone (80:20) vacuum 2 minutes.	Pass two aliquots of 500µL methanol.
5mL methanol followed by 5mL DI water.	Add prepared urine sample.	Wash first: 10mL of 0.1M HCl . Wash second 25mL of 50µM phosphoric acid containing 10% acetonitrile. Vacuum 2 minutes.	3mL of acetone through the cartridge. Collect eluate and add 1.5mL of methylene chloride, centrifuge 5 minutes. Remove upper phase and add 1.5mL of hexane. Centrifuge for 5 minutes. Remove upper phase once again and dry the treated sample. Redissolve in 200µL of chloroform for subsequent GC analysis.
5mL methanol followed by 5mL DI water.	Add prepared urine sample.	5mL DI water followed by 5mL of acetonitrile:water (40:60). Vacuum 2 minutes.	2mL of methanol.
2mL methanol followed by 2mL DI water.	Add buffered serum.	2mL DI water, vacuum 2 minutes.	1mL of methanol.
5mL methanol followed by 5mL DI water.	Add sample.	8mL DI water:methanol (75:25), vacuum 2 minutes.	Pass 500µL of methanol and dry. Redissolve in 200µL of chloroform for subsequent analysis by gas chromatography.

Grace Davison Discovery Sciences

HPLC and UHPLC Methods for Melamine



Melamine



Cyanuric Acid

Milk, infant formula and other dairy products were recently found contaminated with melamine, following an earlier melamine contamination outbreak in pet food in 2007.

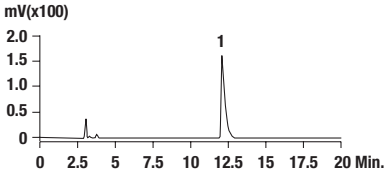
Toxicology studies show ingestion of melamine in large quantities may lead to reproductive damage or bladder cancer due to the formation of bladder/kidney stones. At lower levels, melamine and cyanuric acid are absorbed into the bloodstream. Together, they concentrate and form melamine cyanurate in the urine-filled renal microtubules. Cystallization blocks and damages the renal cells that line the tubes, causing the kidneys to malfunction.

After 2007 and more recent melamine contamination outbreaks, there is an urgent need for analytical methods that can identify and quantify melamine in food. Current melamine detection methods involve LC-MS and GC-MS. GC-MS methods require derivatization, and LC-MS methods generally use gradient conditions that require column clean up and re-equilibration.

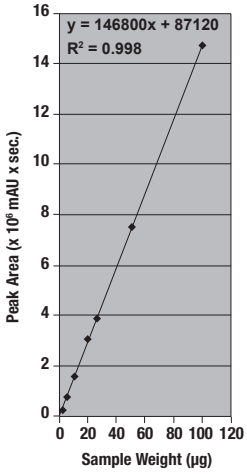
Grace has developed MS-compatible LC methods for Melamine using an HILIC media platform that can be applied to both traditional HPLC as well as UHPLC systems. Melamine was analyzed with a standard HPLC system using a 5µm particle HILIC phase packed into a 250 x 4.6mm column. The 1.5µm version of this phase was then packed into a high throughput format conducive to UHPLC and fast LC systems. Both methods deliver excellent linearity and use isocratic elution for fast analysis without the need for re-equilibration.

HPLC Method for Melamine

This HPLC analytical method for melamine fulfills the FDA requirements using a HILIC column and an ionizable mobile phase compatible with mass spec. Low UV detections offers excellent linearity between 40ng and 100µg.

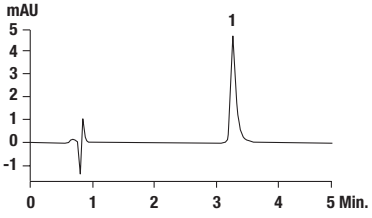


HPLC Column: Alltima™ HP HILIC, 5µm, 4.6 x 250mm (Part No. **86466**)
Mobile Phase: Acetonitrile:10mM Ammonium Acetate in Water (95:5)
Flow Rate: 1mL/min
Detection: UV@240nm
Column Temperature: 30ºC
Injection: 40µg/mL x 20µL



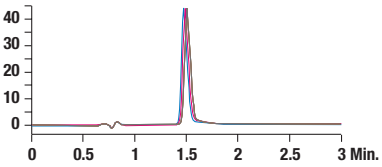
UHPLC Method for Melamine

Compared to the conventional HPLC method, the UHPLC method is 4 times faster. With the use of 1.5µm particles, optimal linear velocities extend over a wider range. Therefore, it is possible to maintain efficiency and resolution while running samples at faster flow rates.

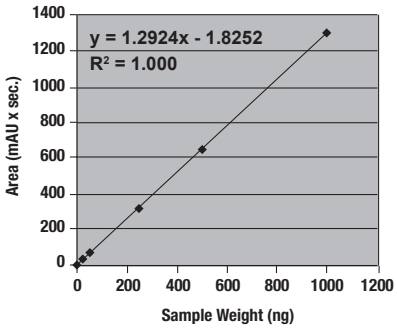


UHPLC Column: VisionHT™ HILIC, 1.5µm, 2 x 50mm (Part No. **5141919**)
Mobile Phase: Acetonitrile:10mM Ammonia Acetate in Water (95:5)
Flow Rate: 0.2mL/min
Detection: UV@240nm
Column Temperature: 30ºC
Injection: 50µg/mL x 0.5µL

9 injections in parallel shows good reproducibility



UHPLC Column: VisionHT™ HILIC, 1.5µm, 2 x 50mm (Part No. **5141919**)
Mobile Phase: Acetonitrile:Water(20mM Ammonium Formate) (90:10)
Flow Rate: 0.2mL/min
Detection: UV@240nm
Column Temperature: 30ºC
Injection: 50µg/mL x 0.1µL

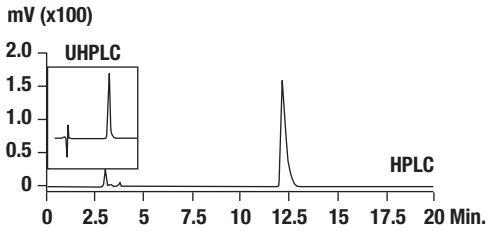


Conc.	Inj. (µL)	Weight (ng)	Peak Area
50µg/mL	0.1	5	7.3
50µg/mL	0.5	25	32
50µg/mL	1	50	63
50µg/mL	5	250	318
50µg/mL	10	500	641
50µg/mL	20	1000	1293

This method exhibits excellent linear response between 5ng and 1000ng for accurate quantitation.

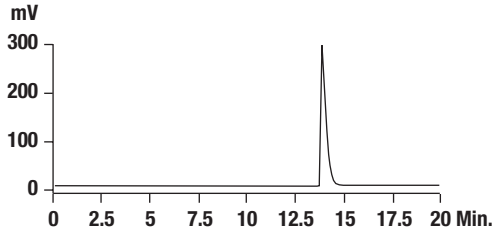
HPLC Method Transfer to UHPLC

The recent adoption of Fast LC systems within the laboratory environment means there is a period of time when most labs have a variety of LC systems types – Traditional, UHPLC, and/or alternate Fast LC systems. Optimizing and transferring methods between systems has not been a simple and intuitive task. However, when the identical bonded phase is available in sub2, 3, 5, and 10µm particle sizes, it can be applied to appropriate formats to suit the system type. A simple calculation to determine equivalent linear velocity is all that's necessary to seamlessly transfer methods between systems, or to optimize a method on one system and apply to an entirely different type of system. Compared to a conventional HPLC method, the UHPLC method is 4 times faster.



	HILIC Column	Flow Rate	Time (min)	Conc.	Inj. Range	Loading Range
HPLC	Alltima™ HP HILIC, 5µm, 4.6 x 250mmL	1.0mL/min	12.090	40µg/mL	1-100µL	40ng-4mg
UHPLC	VisionHT™ HILIC, 1.5µm, 2.0 x 50mmL	0.2mL/min	3.259	50µg/mL	0.1-20µL	5ng-100ng

ELSD Methods for Melamine



HPLC Column: Alltima™ HP HILIC, 5µm, 4.6 x 250mm (Part No. **86466**)
Detector: Alltech® 3300 ELSD (Part No. **5135834**)
Mobile Phase: Acetonitrile:10mM Ammonium Acetate in Water (95:5)
Flow Rate: 1mL/min
Detection: UV@240nm
Column Temperature: 30ºC
ELSD 3300 Settings: drift tube 40ºC, gas 1.8L/min, gain x 4
Injection: 40µg/mL x 20µL

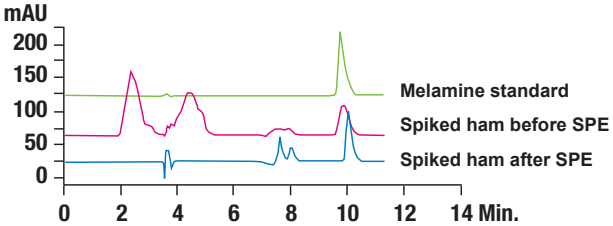
SPE Methods for Melamine

Extracting melamine from various food matrices using solid phase extraction is often required to obtain accurate quantitation. GracePure™ Cation-X, a strong cation exchange resin delivers a cleaner, more concentrated sample.

Melamine from Ham

SPE Column Type: GracePure™ SPE Cation-X, 500mg/3mL (Part No. **5138770**)
Sample Pretreatment: weight ham 2g
mix with 20mL 1% TCA and 5mL Acetonitrile
Sonicate for 30mins
Centrifuge for 15mins
Filter the supernatant with 0.45µm syringe filter, then spike with melamine standard solution

SPE Steps:
Column Conditioning: 2 column volumes methanol, then 2 column volumes water
Sample Application: slowly apply 10mL egg extract through the column
Column Washing: 3 column volumes water, then 2 column volumes methanol
Eluent: 4mL Methanol:0.5M Ammonium Hydroxide (1V:1V)



SPE recovery*=92%

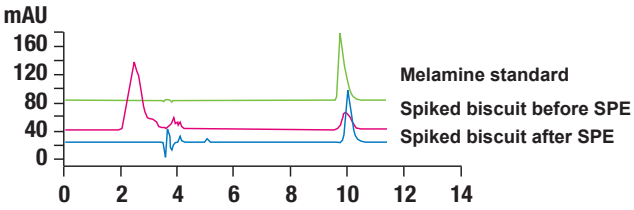
Mobile Phase: 10mM Ammonium Acetate:Acetonitrile (5:95)
HPLC Column: Alltima™ HP HILIC, 5µm, 4.6 x 250mm
Detector: UV @ 240nm
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL

SPE Methods for Melamine continued

Melamine from Biscuit

SPE Column Type: GracePure™ SPE Cation-X, 500mg/3mL, (Part No. **5138770**)
Sample Pretreatment: weight biscuit 2g
mix with 20mL 1% TCA and 5mL Acetonitrile
Sonicate for 30mins
Centrifuge for 15mins
Filter the supernatant with 0.45µm syringe filter, then spike with melamine standard solution

SPE Steps:
Column Conditioning: 2 column volumes methanol, then 2 column volumes water
Sample Application: slowly apply 10mL egg extract through the column
Column Washing: 3 column volumes water, then 2 column volumes methanol
Eluent: 4mL Methanol:0.5M Ammonium Hydroxide (1V:1V)



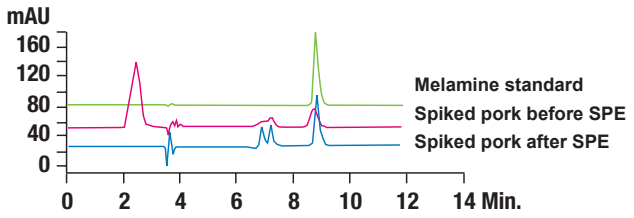
SPE recovery*=80%

Mobile Phase: 10mM Ammonium Acetate:Acetonitrile (5:95)
Column: Alltima™ HP HILIC, 5µm, 4.6 x 250mm
Detector: UV @ 240nm
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL

Melamine from Pork

SPE Column Type: GracePure™ SPE Cation-X, 500mg/3mL (Part No. **5138770**)
Sample Pretreatment: weight pork 2g
mix with 20mL 1% TCA and 5mL Acetonitrile
Sonicate for 30mins
Centrifuge for 15mins
Filter the supernatant with 0.45µm syringe filter, then spike with melamine standard solution

SPE Steps:
Column Conditioning: 2 column volumes methanol, then 2 column volumes water
Sample Application: slowly apply 10mL egg extract through the column
Column Washing: 3 column volumes water, then 2 column volumes methanol
Eluent: 4mL Methanol:0.5M Ammonium Hydroxide (1V:1V)



SPE recovery*=103%

HPLC Mobile Phase: 10mM Ammonium Acetate:Acetonitrile (5:95)
Column: Alltima™ HP HILIC, 5µm, 4.6 x 250mm
Detector: UV @ 240nm
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL

Conclusions

Fast, isocratic UHPC and HPLC methods were developed for the determination of melamine. Linearity range for melamine is 40 - 4000 ng. Since the Grace® VisionHT™ HILIC UHPLC column and the Alltima™ HP HILIC column are based on the same media, methods developed on the HPLC column can be easily transferred to UHPLC and vice versa. Compared to the HPLC method, UHPLC is 4 times faster. Mobile phases used in these methods are MS and ELSD compatible.

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The Use of Solid Phase Extraction for the Isolation and Concentration of Polynuclear Aromatic Hydrocarbons and Pesticides from Food

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Laura Kaepplinger
Scott Anderson
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GRACE

Abstract

The presence of suspected carcinogens such as polynuclear aromatic hydrocarbons (PAHs) and pesticides in foods is a concern for both producers and consumers. Analytical techniques such as gas chromatography (GC) can be used for the analysis of PAHs and pesticides, but they can be difficult because the concentration of these compounds found in food approach the limit of detection and because of interferences from other organic compounds found in food. This poster will demonstrate the use of Solid Phase Extraction (SPE) to concentrate PAHs and pesticides from food, thereby facilitating accurate qualitative and quantitative analysis by GC.

Discussion

Solid Phase Extraction (SPE) is a well-established method for the isolation and concentration of compounds of interest in analytical chemistry, primarily GC and HPLC. By utilizing the proper SPE method, all of the target analytes from a large sample are retained on the SPE device, which allows accurate quantitative and qualitative analysis of compounds that may be present at levels that otherwise would be too low for accurate analysis. Part of the SPE method development includes the judicious choice of elution solvents to reduce or eliminate interfering peaks in the resulting chromatogram. One of the few alternatives to SPE is liquid-liquid extraction, but SPE has several advantages, including the use of far less solvent, faster extraction methods, and the selective elution of analytes reduces interfering peaks.

Polynuclear aromatic hydrocarbons (PAHs) and pesticides are two classes of undesirable substances found in foods at concentrations that approach or exceed the lower limit of detection of chromatographic methods. PAHs can result from exposure to high temperatures in cooking or roasting, or from processing procedures such as smoking. PAHs can also originate from contamination from particles from industrial areas and emissions from diesel exhaust.

Restrictions in the use and production of chlorinated pesticides in the United States since the mid-1970s has reduced the risk of direct exposure to these compounds. General use of DDT was no longer permitted after January 1, 1973 (see http://www.epa.gov/history/topics/ddt/01.htm). However in much of the world, the general population is still exposed to chlorinated pesticides in food because of the persistence of the compounds in the environment.

The following procedures will demonstrate the efficiency and ease of SPE for the isolation and concentration of PAHs and chlorinated pesticides spiked into foods.

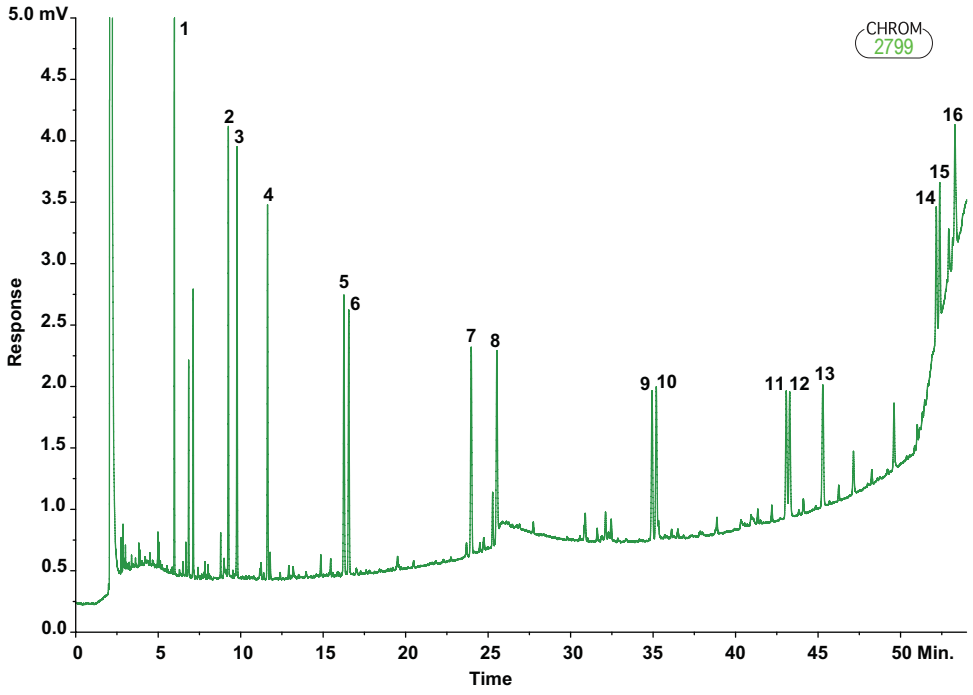
Experimental

PAHs in Roasted Grains- SPE Conditions

Tube: GracePure™ C18-Max SPE tube, 500mg/6mL, part #5138767

Sample: 5 grams crushed roasted grains

SPE Procedure: Weigh out and crush 5 grams of roasted grains, spike with 10µL of PAH mixture, which is 2000µg/mL of each of 16 PAHs. Add 30mL of 2-propanol and homogenize. Filter through a 75mL filter column (part #210775). Add 200mL of distilled water and shake. Condition GracePure™ C18-Max SPE tube, 500mg/6mL, part #5138767 with 2 x 3mL of methanol, then 3mL of 85% water/15% 2-propanol. Apply sample through the SPE tube using a 75mL reservoir (part #210575), connecting it to the top of the SPE tube with a syringe adapter (part #210705). After the sample was pulled completely through the SPE tube wash with 2 x 500µL of 85% water/15% 2-propanol. Continue vacuum through the tube for about 15 minutes to dry the SPE packing. Elute the PAHs with 2 x 1mL CH_2Cl_2 and adjusted the volume to 1.0mL by evaporating under a gentle nitrogen stream. Inject 1.0µL onto an AT™-5ms, 30 meter x 0.25mm I.D., 0.25µm film thickness, part #15807, as follows: oven= 80°C (1minute hold) to 160°C at 25°C/minute, to 300°C at 3°C/minute, to 325°C (3 minute hold) at 25°C/min, injector=270°C, FID=340°C, split 50:1, 14.0psig, helium, linear velocity=28cm/second (at 80°C).



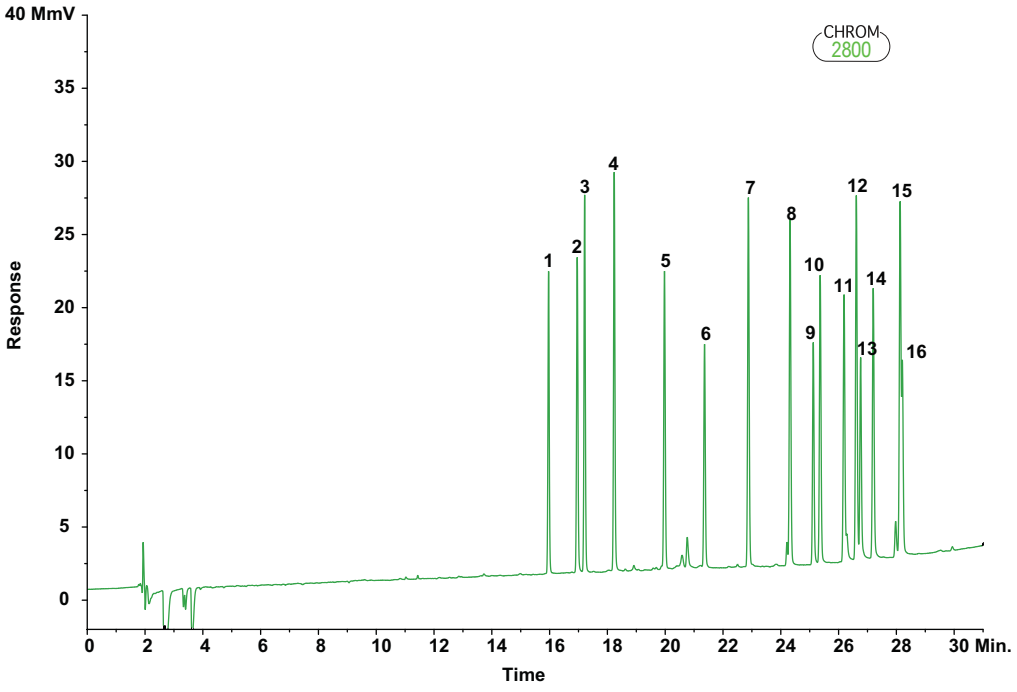
Compound	% Recovery
1. Naphthalene	92%
2. Acenaphthylene	97%
3. Acenaphthene	98%
4. Fluorene	95%
5. Phenanthrene	93%
6. Anthracene	89%
7. Fluoranthene	87%
8. Pyrene	74%
9. Benzo[a]anthracene	64%
10. Chrysene	59%
11. Benzo[b]fluoranthene	59%
12. Benzo[k]fluoranthene	61%
13. Benzo[a]pyrene	61%
14. Indeno[1,2,3-cd]pyrene	53%
15. Dibenzo[a,h]anthracene	48%
16. Benzo[ghi]perylene	42%

Chlorinated Pesticides in Vegetables - SPE Conditions

Tube: GracePure™ C18-Max SPE tube, 500mg/6mL (part #5138767)

Sample: 5 grams green bell pepper

SPE Procedure-Weigh out 5 grams of green bell pepper, spike with 0.5µg each of 16 chlorinated pesticides. Add 25mL methanol and homogenize. Filter through a 75mL filter column (part #210775). Add 200mL of distilled water and shake. Condition GracePure™ C18-Max SPE tube, 500mg/6mL, part #5138767 with 2 x 3mL methanol, then 3mL DI water. Aspirate the filtered green pepper with pesticides through the tube, then wash with 5mL DI water, then pull dry for 15 minutes. Elute the pesticides with 3 x 1mL n-hexane/CH_2Cl_2 (70:30), evaporate this to dryness and reconstitute with 1mL n-hexane. Inject 1.0µL onto an AT™-5ms, 30 meter x 0.25mm I.D., 0.25µm film thickness, part #15807, as follows: oven= 80°C (1minute hold) to 100°C at 15°C/minute, then to 160°C (0 min hold) at 10°C/minute, then to 275°C at 5°C/minute, injector= 250°C, ECD=310°C, split 65:1, 14.0psig, helium, linear velocity=26cm/second (at 80°C).



Compound	% Recovery
1. α-BHC	67%
2. β-BHC	87%
3. γ-BHC	78%
4. δ-BHC	87%
5. Heptachlor	59%
6. Aldrin	54%
7. Heptachlor Epoxide	79%
8. Endosulfan I	79%
9. p,p'-DDE	59%
10. Dieldrin	79%
11. Endrin	86%
12. Endosulfan II	90%
13. p,p'-DDD	74%
14. Endrin Aldehyde	85%
15. p,p'-DDT	98%
16. Endosulfan Sulfate	76%

Conclusion

Recoveries of PAHs spiked into roasted grains are in the range of 98% acenaphthene to 42% benzo[ghi]perylene with SPE and subsequent analysis by capillary GC with flame ionization detection. Similarly, recoveries of chlorinated pesticides spiked into vegetables are in the range of 98% p,p'-DDT to 54% aldrin after SPE with analysis by capillary GC with electron capture detection.