GPC

Gel Permeation Chromatography

The proved clean-up method
for food, feed, and environmental laboratories

www.LCTech.de
Preparative Gel Permeation Chromatography

- **GPC** = *Gel Permeation Chromatography*
  - Separation of analytes from matrix by size exclusion via a soft gel
  - Separation according to molecular size (hydrodynamic volume)
Preparative Gel Permeation Chromatography

- GPC = Gel Permeation Chromatography
- Or SEC = Size Exclusion Chromatography
- Applications
  - Molecular weight determination (e.g., polymer industry/sciences)
  - Sample cleanup
- A well known and accepted method all over the world which is actually a standard in sample clean up for feed and food analyses – an partly for environmental samples as well.
- GPC = Essential part of many international methods
Preparative Gel Permeation Chromatography

- Pesticides (X-, P-, N-containing)
  Pyrethroids
- Polyhalogenated Biphenyls
- Polycyclic Aromatic Hydrocarbons
- PCDD / PCDF – Dioxin-like PCB
- Plastisizer
- Mycotoxins
- And many more ….
Preparative Gel Permeation Chromatography

- Food / Feed
  - Fatty (Fish, meat, poultry, milk products, seeds)
  - Non-fatty (vegetables, fruit, cereals)
- Environmental Samples
  - Soil
  - Sediments
  - Sludge / sewage sludge
- Herbal drugs
- Suitable for non-polar and semi-polar compounds
- Routine labs analyze up to 550 pesticides
Preparative Gel Permeation Chromatography

Sampling/Homogenization → Extraction → Concentration → Clean-up → Concentration → Analysis

Where to use preparative GPC?
Preparative Gel Permeation Chromatography

Extracted Matrices

GPC Column

GPC

GC

Analytes for Analysis

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Preparative Gel Permeation Chromatography

- Flow
- High Molecular Compounds
- Forerun
- Main run
  - Collect
  - Analytes
  - Waste
- Bio-Beads

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The Solution - GPC

The diagram shows the elution profiles of different food sources. The x-axis represents the volume in milliliters (ml) and the y-axis represents the concentration in milligrams (mg).

- **Beef tallow**
- **Butter**
- **Bovine liver**
- **Herring**
- **Egg**
- **Mussel (Mytilus edulis)**
- **Human milk**

The peaks indicate the elution points for each food source.
Preparative Gel Permeation Chromatography

<table>
<thead>
<tr>
<th></th>
<th>Compound</th>
<th>Retention Time</th>
<th>% Yield</th>
<th>Molecular Weight</th>
<th>Molecular Weight</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>corn oil</td>
<td>11.867</td>
<td>8.888%</td>
<td>39544328</td>
<td>375572</td>
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<tr>
<td>2</td>
<td>ethylhexyl phthalat</td>
<td>17.835</td>
<td>8.888%</td>
<td>51542352</td>
<td>468454</td>
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<td>3</td>
<td>methoxychlor</td>
<td>26.357</td>
<td>8.888%</td>
<td>95820968</td>
<td>592365</td>
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<tr>
<td>4</td>
<td>perylene</td>
<td>42.100</td>
<td>8.888%</td>
<td>82256376</td>
<td>344264</td>
</tr>
<tr>
<td></td>
<td><strong>Totals</strong></td>
<td></td>
<td>8.888%</td>
<td>269164032</td>
<td>1728355</td>
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</tbody>
</table>
### GPC (DFG S19)
- **Injection Volume:** 5.0 mL
- **Eluent:** Ethyl acetate / cyclohexane (1/1, v/v)
- **Flow:** 5.0 mL/min

#### Table 2: Gel permeation chromatography

<table>
<thead>
<tr>
<th>Module</th>
<th>Description</th>
<th>Use / Application</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
<td>Extract from E 1 to E 9</td>
<td>All samples</td>
</tr>
</tbody>
</table>

#### Table 4 (continued)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>GPC elution volume range</th>
<th>Silica gel eluates (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cinofenate</td>
<td>100-140</td>
<td>0</td>
</tr>
<tr>
<td>Cyanazine</td>
<td>110-135</td>
<td>0</td>
</tr>
<tr>
<td>Cyanoethylphos</td>
<td>115-145</td>
<td>0</td>
</tr>
<tr>
<td>Cyanothos</td>
<td>115-150</td>
<td>0</td>
</tr>
<tr>
<td>Cytin®</td>
<td>090-120</td>
<td>0</td>
</tr>
<tr>
<td>1-Methylpyrrol®</td>
<td>090-110</td>
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<td>Cypermic®</td>
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<tr>
<td>Cypermethrin</td>
<td>100-135</td>
<td>0</td>
</tr>
<tr>
<td>n-p-DDE</td>
<td>110-140</td>
<td>5</td>
</tr>
<tr>
<td>n-p-DDD</td>
<td>115-140</td>
<td>5</td>
</tr>
<tr>
<td>n-p-DCE</td>
<td>120-150</td>
<td>5</td>
</tr>
<tr>
<td>p-p-DDE</td>
<td>120-150</td>
<td>5</td>
</tr>
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<td>p-p-DCT</td>
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<td>DEF®</td>
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<td>Deltamethrin</td>
<td>100-135</td>
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<tr>
<td>Deltamethrin-s-methyl</td>
<td>125-155</td>
<td>0</td>
</tr>
<tr>
<td>Deltamethrin-s-methyl sulfone</td>
<td>120-160</td>
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<tr>
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<td>N-Dimethyl-primaphos-methyl</td>
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<td>Delfox®</td>
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<td>Deltal®</td>
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<td>106-135</td>
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<tr>
<td>Dichlorobenzene®</td>
<td>125-155</td>
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<tr>
<td>Dichlorodichlorobenzene®</td>
<td>110-140</td>
<td>3</td>
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<tr>
<td>Dichlorfluorid®</td>
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<tr>
<td>p,p'-Dichlorobenzene®</td>
<td>125-155</td>
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<tr>
<td>Dichloromethane®</td>
<td>115-140</td>
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<tr>
<td>Dichloromethane-methyl</td>
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<tr>
<td>Dichloro®</td>
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<tr>
<td>Dichloro® N</td>
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<td>2</td>
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<td>Dichlorophos®</td>
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<tr>
<td>Dieldrin®</td>
<td>120-150</td>
<td>0</td>
</tr>
<tr>
<td>Deltamethrin-s-methyl</td>
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<td>0</td>
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<tr>
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<tr>
<td>Dichlorfluorid®</td>
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</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
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</tr>
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<td>Dichlorobenzene®</td>
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<tr>
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<td>100-140</td>
<td>0</td>
</tr>
<tr>
<td>p,p'-Dichlorobenzene®</td>
<td>125-155</td>
<td>0</td>
</tr>
</tbody>
</table>
| Dichloromethane®     | 115-140                  | 0 | 1 | 3 | 0 | 0 | *to be continued*
Preparative Gel Permeation Chromatography

- Most common resin used: Bio-Rad Bio-Beads S-X3
  - Styrene-divinylbenzene-copolymer
  - Exclusion limit 2,000 Dalton
  - 200 – 400 mesh, 30 – 90 µm particles
  - Typical filling weights 10, 50 or 70 g
Typical solvents used
- Ethyl acetate / Cyclohexane (1/1 // v/v)
- Dichloromethane
- About 320 mm bed length (50 g column)
• Load Utilization Period: 1 to 2 Years
• Washing with
  • Acetone
  • Methanol
Preparative Gel Permeation Chromatography

- GPC is comparatively simple
- Easiest sample preparation for vegetable oils
- Suitable for non-fatty and fatty matrices
- Suitable for very difficult matrices (e.g. herbal drugs, tea)
- Reduces matrix interferences
- Reduces instrument down time
- New low limits for infant food set in EU (3 – 8 ppb)
Some established methods
- Extendend DFG S19 (Pesticides/PCB)
  - EN 12393 (non-fatty food)
  - EN 1528 (fatty food)
- US EPA SW-846 3640 A (POPs)
- AOAC 924.81 (OCP in animal fat)
- US EPA 1614 (PBDE)
- FSIS CLG-CHC3 (OCP in animal fat)
- USP Monographs: Lanolin (OCP/OPP in lanolin)
Preparative Gel Permeation Chromatography

- GC-PND Chromatograms

Without GPC

With GPC
Preparative Gel Permeation Chromatography

With GPC

Without GPC
Matrix burden corrected for 1 g/mL
Dried orange powder

Steinbach P, Sinderhauf K, Vögler P, Schwack W

a) CLF – Central Laboratories Friedrichsdorf, Bahnstraße 14-30, 61348 Friedrichsdorf, GERMANY
b) Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, 70599 Stuttgart, GERMANY
### GPC Clean-Up Efficiency

<table>
<thead>
<tr>
<th>Clean-up efficiency rates for</th>
<th>DFG-S19 - GPC [%]</th>
<th>QuEChERS - PSA [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>organic acids</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>sugars</td>
<td>95</td>
<td>62</td>
</tr>
<tr>
<td>free fatty acids</td>
<td>84</td>
<td>14</td>
</tr>
<tr>
<td>carotenoids</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>flavour compounds</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>mean (of all determined compounds)</td>
<td>89</td>
<td>37</td>
</tr>
</tbody>
</table>

Steinbach P\(^a\), Sinderhauf K\(^a\), Vögler P\(^a\), Schwack W\(^b\)

\(^a\) CLF – Central Laboratories Friedrichsdorf, Bahnstraße 14-30, 61348 Friedrichsdorf, GERMANY

\(^b\) Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, 70599 Stuttgart, GERMANY
Results

- No Cross Contamination
  - 1 ppm (black) – blank (blue)
Results

- OC-Pesticide Standard 500 ppb – GPC Online
  - Recoveries and Standard Deviations (n=3)
Results

- PAH in Vegetable Oil - GPC Online Modus
  
  Recoveries and Standard Deviations (n=3)
Results

- Organochlorine–Pesticide Standard 250 ppb Concentrator Modus
  - Recoveries and Standard Deviations (n=3)
Application Overview

• PAH in Edible Oil and Fatty Food
• Pesticides in Herbal Drugs and Tea
  • QuEChERS
• Plastizisers in Fat
• Zearalenone in Edible Oil
• Environmental Samples
  • US EPA SW-846 Method 3640A
• Azo dyes in Spice
• Dioxins/PCDF/Coplanar PCB
Applications and Positioning

Pesticides/PCB in Fat:

- DFG S19
- USP Monographs Lanolin – Lanolin DFG S19
- AOAC 981.24
- FSIS CLG CH3
Pesticides/PCB in Fat:

**DFG S19 (§ 64 LFGB L 00.00-34)**

- Analytes: Organochlorine pesticides, PCB
- Suitable for meat, poultry, fish, cheese
- Cold extraction of samples with acetone/hexane (E 8)
- GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
- (Mini silica column cleanup)
- Measurement GC-ECD or GC-MS

→ Routine food laboratories – Monitoring agencies
Pesticides/PCB in Fat:

FSIS CLG-CHC3

- Analytes: Organochlorine pesticides (quantification) 
  OCP, OPP, PBB (identification)
- Suitable for animal fat, eggs
- Fat rendered at 100 ± 5 °C from adipose tissue
- GPC Bio-Beads S-X3, 24 g, ethyl acetate/cyclopentane (7/3)
- Measurement GC-ECD

→ Routine food laboratories – Monitoring agencies
Pesticides/PCB in Fat:

**USP Monographs Lanolin – Lanolin DFG S19**

- Analytes: Organochlorine pesticides, pyrethroids, organophosphorous pesticides
- Lanolin = Wool wax from sheep
- Melt lanolin, filter and dilute in GPC eluant
- DFG: GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
  USP: GPC Bio-Beads S-X3, 35 g, dichloromethane/hexane (1/1)
- (Mini silica column cleanup – DFG S19)
- Measurement GC-ECD, GC-FPD

→ **Routine laboratories pharmaceutical supply**
Pesticides/PCB in Fat:

AOAC 984.21

- Analytes: 18 (+) Organochlorine pesticides
- Suitable for beef and poultry fat
- GPC Bio-Beads S-X3, 60 g, dichloromethane/cyclohexane (1/1)
- Measurement GC-ECD

→ Routine food laboratories – Monitoring agencies
Applications and Positioning

Overlay of 5 Chromatograms with 0.5 g Beef Fat

Flow: 4.8 mL/min
Dump: 10 min
Collect 10 min
Applications and Positioning

Chromatograms US EPA Test Mix

OI Column

LCTech Column
Applications and Positioning

Optimum Parameters for Process 5 (Solvent Exchange):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent: Ethylacetate/cyclopentane (7/3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature Vacuum Chamber: 60 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum Phase 1:</td>
<td>300 mbar</td>
<td></td>
</tr>
<tr>
<td>Vacuum Phase 2:</td>
<td>240 mbar</td>
<td></td>
</tr>
<tr>
<td>Solvent Exchange:</td>
<td>180 mbar</td>
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</tr>
<tr>
<td>α-HCH</td>
<td>89%</td>
<td></td>
</tr>
<tr>
<td>HCB</td>
<td>82%</td>
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</tr>
<tr>
<td>β-HCH</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td>γ-HCH</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td>Heptachlor</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td>Aldrin</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td>t-Heptachlor</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td>α-Endosulfan</td>
<td>90%</td>
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</tr>
<tr>
<td>4,4'-DDE/Die</td>
<td>91%</td>
<td></td>
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<tr>
<td>Endrin</td>
<td>91%</td>
<td></td>
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<tr>
<td>β-Endosulfan</td>
<td>92%</td>
<td></td>
</tr>
<tr>
<td>2,4'-DDT</td>
<td>95%</td>
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<tr>
<td>4,4'-DDD</td>
<td>74%</td>
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<tr>
<td>4,4'-DDT</td>
<td>104%</td>
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<tr>
<td>Methoxychlor</td>
<td>85%</td>
<td></td>
</tr>
</tbody>
</table>
Applications and Positioning

PAH in Edible Oil and Fatty Food:

- Analytes: Polycyclic aromatic hydrocarbons
- Suitable for edible oils (direct cleanup)
  Fatty food (saponification)
- Fortify edible oil with IS and dilute with GPC eluant
- GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
- Measurement GC-MS
- SOP available

→ Routine food laboratories – Oil producers
Applications and Positioning

Pesticides in Herbal Drugs and Tea

- Analytes: OCP, OPP, ONP, Pyrethroids, PCB
- Dried and fresh plant material (analogous to DFG S19)
- Extraction E1/E3 or E4/E5
- GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
- (Mini silica cleanup)
- Measurement GC-MS, GC-FPD, GC-NPD, GC-ECD

→ Routine laboratories pharmaceutical supply or import control
Applications and Positioning

Plastizisers in Fat:

- Analytes: Plastizisers (phthalates) from food packing material
- Suitable for fatty food
- Water/acetone extraction → dichlormethane partitioning; deuterated IS
- GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
  Dump: 18 min Collect: 10 min
- Measurement GC-MS or GC-FID (ppb to ppm range)

→ Routine food laboratories – Monitoring agencies
Zearalenone in Edible Oil:

- Analytes: Zearalenone
- Suitable for edible oils (corn oil)
- Fortify edible oil with IS and dilute in GPC eluant
- GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
- Immunoaffinity column cleanup
- Measurement LC-MS/MS or HPLC-FLD

→ Routine food laboratories
Examples

Zearalenone in Oil

Fractionation range of zearalenone

Elution volume [mL/min]

Zearalenone [µg/kg]
Environmental Samples:

- **US EPA SW-846 Method 3640A**
  - Analytes: POP
  - Environmental samples
  - GPC Bio-Beads S-X3, 70 g, dichloromethane
  - Immunoaffinity column cleanup
  - Measurement LC-MS/MS or HPLC-FLD
  → Routine environmental laboratories
Azo Dyes in Spice:

- Analytes: Azo dyes (Sudan Red I – IV, para red, butter yellow)
- Spice (mixtures), instant sauces
- Extraction water/acetone → ethyl acetate/cyclohexane partitioning
- GPC Bio-Beads S-X3, 50 g, ethyl acetate/cyclohexane (1/1)
- Measurement GC-MS

→ Routine food laboratories
Examples

GPC of Sudan Red I

Extraction According to DFG S19, c = 1 mg/kg
Applications and Positioning

**Dioxins/PCDF/Coplanar PCB**

- Analytes: PCDD/PCDF/dlPCB
- Feed, food, environment, waste
- All kind of extraction types
- GPC Bio-Beads S-X3 column types
- Cleanup Al$_2$O$_3$, Silica (acidic/alkaline), charcoal
- Measurement HRGC-MS

→ Extremely time consuming → GPC quattro

→ Dioxin laboratories
Thanks for your attention!

LC Tech

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