Sample Preparation?
Einige Zahlen

61% of time is spend for sample processing

30% of errors are coming from sample processing
Standard Addition
Serial Dilution
Standard Addition – Serial Dilution

General Steps:
1. Dilute
2. Vortex
3. Add STD
4. Vortex
5. Inject

Stock solution

(1:10)
1:50
1:100
1:200
1:1000

Fully automated standard addition method for the quantification of 29 polar pesticide metabolites in different water bodies using LC-MS/MS.
Dilution Workstation
Dilution Workstation

- Show the reproducibility and accuracy of automated dilutions with a PAL RTC
- Test is done on a 14 compound mixture
- Range of dilution
  - Stock solution at 4 mg/mL
  - Dilutions from 400 to 1 µg/mL (9 vials) in hexane

Many thanks to Philippe Mottay, Brechbühler AG, Schlieren, Switzerland
PAL Setup

• PAL RTC equipped with
  • 2 park stations
  • 2x 1000 µL syringe
  • 2x 100µL syringe
  • 2x 10µL syringe
  • Vortex mixer
  • Solvent module
  • Fast wash station
  • VT54 tray
  • VT15 tray
• Software PAL Sample Control
Verification of method with GC compatible compounds

- The method was tested with GC compatible compounds
- Mixture of 14 compounds at 4 mg/ml
- Range of dilutions
  - 400; 200; 100; 40; 20; 10; 4; 2; 1 µg/ml in Hexane
- Measured by GC/FID (Thermo Trace 1310)
- Method:
  - 40°C, 4 min to 260°C @15°C/min hold 1.5 min.
  - Split injection (20/1) at 260°C, column flow 2 ml/min
  - Detector at 270°C
## Calibration results

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyl acetate</td>
<td>0.9999</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>0.9998</td>
</tr>
<tr>
<td>Ethyl valerate</td>
<td>0.9996</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.9996</td>
</tr>
<tr>
<td>Beta-pinene</td>
<td>0.9995</td>
</tr>
<tr>
<td>C10</td>
<td>0.9995</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.9995</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.9995</td>
</tr>
<tr>
<td>Benzyl acetate</td>
<td>0.9995</td>
</tr>
<tr>
<td>Menthol</td>
<td>0.9995</td>
</tr>
<tr>
<td>Citronellol</td>
<td>0.9995</td>
</tr>
<tr>
<td>Geraniol</td>
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</tr>
<tr>
<td>Coumarin</td>
<td>0.9997</td>
</tr>
<tr>
<td>Alpha Ionone</td>
<td>0.9995</td>
</tr>
</tbody>
</table>
Determination Fatty Acids as FAME by GC/MS

- Determination of fatty acid composition and content of foods
- Determination of Biodiesel composition
- Trans-esterification of fatty acids to FAME is a very common and at the same time tedious procedure.
- Automation increases productivity and prevents exposure of humans to hazardous chemicals.
Derivatisation Workflow FAME

Generation of Fatty Acid Methylesters (FAME) with 1 min. Transesterification for GC/MS analysis

According to Eidg. Untersuchungsmethode 269.1
5 Port Dilutor Module

- Addition of Methyl ester / Heptane / Citrate
- Wash steps through the dilutor
Conclusions:

- Fast and reliable derivatization
- Very good accuracy and precision
- Excellent separation of different FAMEs
- High productivity
- Traceability
Application Note(s) auf www.palsystem.com
Integrated Platform Including Bligh and Dyer Extraction and Dual-Column UHPLC-MS/MS Separations for Metabolomics Studies

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CTC Sunday Workshop @ IMSC 2014
Sunday, August 24th 2014, Geneva, Switzerland
Identification of Endogenous Metabolites from *Chlamydomonas reinhardtii* Algae

b) Automated on-line sample preparation with RTC platform

Add 225 µL H₂O + 225 µL CHCl₃
Vortex for 10 s
Centrifuge for 5 min. (4000 rpm, ≈ 900 x g)

1) Aspirate 500 µL of upper fraction
   (H₂O-MeOH fraction)
   Dilution 5-fold by adding 60 µL to 240 µL of dilution solvent
   (= 10% MeOH in samples)
   Add chromatographic standards
   Alternately inject 25 µL on C₁₈ column
   (UHPLC system 1)

2) Aspirate 250 µL of lower fraction
   (CHCl₃ fraction)
   Evaporate to dryness with N₂ gas
   (0.35 bar, 35°C, 10 min.)
   Reconstitute in 150 µL MeOH
   Add chromatographic standards
   Inject 5 µL on C₈ column
   (UHPLC system 2)
UHPLC Conditions and Timings

**UHPLC system 1**  
Aqueous fraction (AQ)

- Flow = 400 µl/min
- A) 5 mM NH₄Formate (pH 3.0)
- B) ACN + 0.1% FA
- C) 0.025% NH₄OH (pH 8.3)
- D) ACN + 0.0125% NH₄OH

**UHPLC system 2**  
Organic fraction (ORG)

- Flow = 300 µl/min
- A) 5 mM NH₄Acetate (pH 4.2)
- B) ACN + 0.1% AA

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**UHPLC 1**  
(20 min runs)

- 100% AQ-pH8 (1)  
- 0% AQ fractn dilution + STD addition

- Blank (1)

**UHPLC 2**  
(30 min runs)

- 100% ORG fractn evap. & recon.

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**Sample 1 Bligh & Dyer fractions analysis (2 hours)**

- AQ-pH8 (1) AQ fractn dilution + STD addition
- AQ-pH3 (1) AQ fractn dilution + STD addition
- Blank (1)
- ORG (1) ORG fractn evap. & recon.

**Sample 2 Bligh & Dyer fractions analysis**

- AQ-pH8 (2) AQ fractn dilution + STD addition
- AQ-pH3 (2) AQ fractn dilution + STD addition
- Blank (2)

• Robotic Tool Change (RTC) PAL system with several modules (CTC Analytics)
• Two quaternary LPG Nexera LC30AD UHPLC pumps (Shimadzu)
• TripleTOF 5600 mass spectrometer with CDS device (AB SCIEX)
Thak you very much!