Derivatization

In the context of HPLC

Introduction

- Derivatization is part of
  1)sample preparation or
  2)chromatographic analyses

where analyte modified via chemical reactions so that it can be analysed whith chosen method.

Aims

- Impoving selectivity (peak resolution)
  - e.g. Separation on ionic substances with reversed phase chromatography.
- Improving detection (sensitivity)
  - e.g. Introducing chromophore or fluorophore
  - Nowadays also for improving MS sensitivity
- Resolving chiral compounds
  - See lecture Types of liquid chromatography I

Aims

HPLC → Detector

- Increasing retention
- Making reversed phase analyses possible for some analytes
- Improving analyte resolution

- UV-Vis
- Flourescence
- ESI/MS

Realization

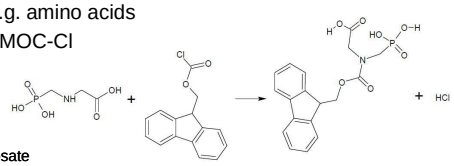
- Pre-column
  - Reagent is added in the vial (either by hand or via autosampler). Easy to control the condition (solvent, temperature, time). Formed derivate has to be stable.
- Post-column
  - Reagent is added after alaytical column with an additional pump. Automated. Reaction needs to be fast.

Reactions

- Oxydation and reduction are usually too not specific.
- Mainly condensation reactions

Changing chromatography

- Ionic/ionizable compounds
  - e.g. amino acids
  - FMOC-Cl



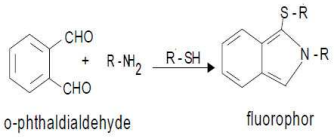
Glyphosate
The most widely used pestiside in EU.
Very polar, elutes from RP at dead time.

FMOC-Cl

The product has large hydrophobic parts and RP can be used for analyses.

Detecting

- UV-Vis
  - A chromophore needs to be inserted
- Fluorescence
  - A fluorophore needs to be inserted
  - Fluorescence reagents are also suitable for UV-Vis

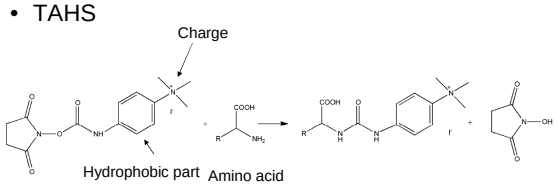


o-phthaldialdehyde

fluorophor

Detecting

- ESI/MS
  - Charge
  - Hydrophobisity
- TAHS



Optimizing derivatization

- Concentration of the reagent
- Amount of reagent solution
- Reaction media
  - Solvent
  - pH
- Reaction time
- Stability of the product