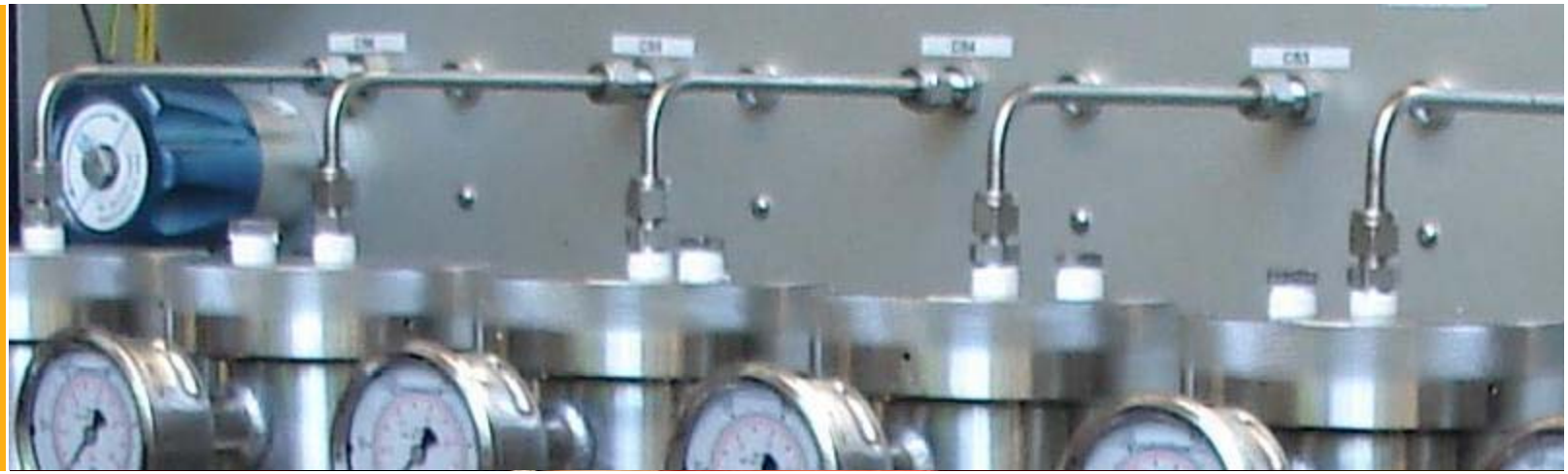


SFC – A Versatile Tool in the Analyis and Purification of Pharmaceutical Compounds

Georg Diehl, Aldo Meishammer, Eric Francotte

Novartis Pharma AG, Basel/Switzerland

3rd International Conference Packed-Column SFC, 22.-23.07.09

Philadelphia, USA

NOVARTIS

INSTITUTES FOR BIOMEDICAL RESEARCH

Table of Content

INTRODUCTION

The Separations Group @ NIBR, Basel

Applied Separation Technologies in the Separations Group (SEP) within Discovery Chemistry

- Analytical
  - HPLC (MS/MS)
  - SFC (MS)
  - GC (MS/MS)
  - CE (MS)
- Preparative
  - HPLC (MS)
  - SFC
  - SMB

Typical Fields of Application in the Separations Group within Discovery Chemistry

- Chiral Separations
- Achiral Purification
  - Metabolite Purification
- Analysis of Complex Mixtures
- Purity Determinations
- Chemical Stability (Stress Test)

SFC - TECHNICAL ASPECTS

SFC Systems Used in This Work

Berger Analytical SFC

- Max flow rate CO_2: 10 ml/min
- Max flow rate modifier: 10 ml/min
- Max operating pressure: 400 bar
- No fraction collection

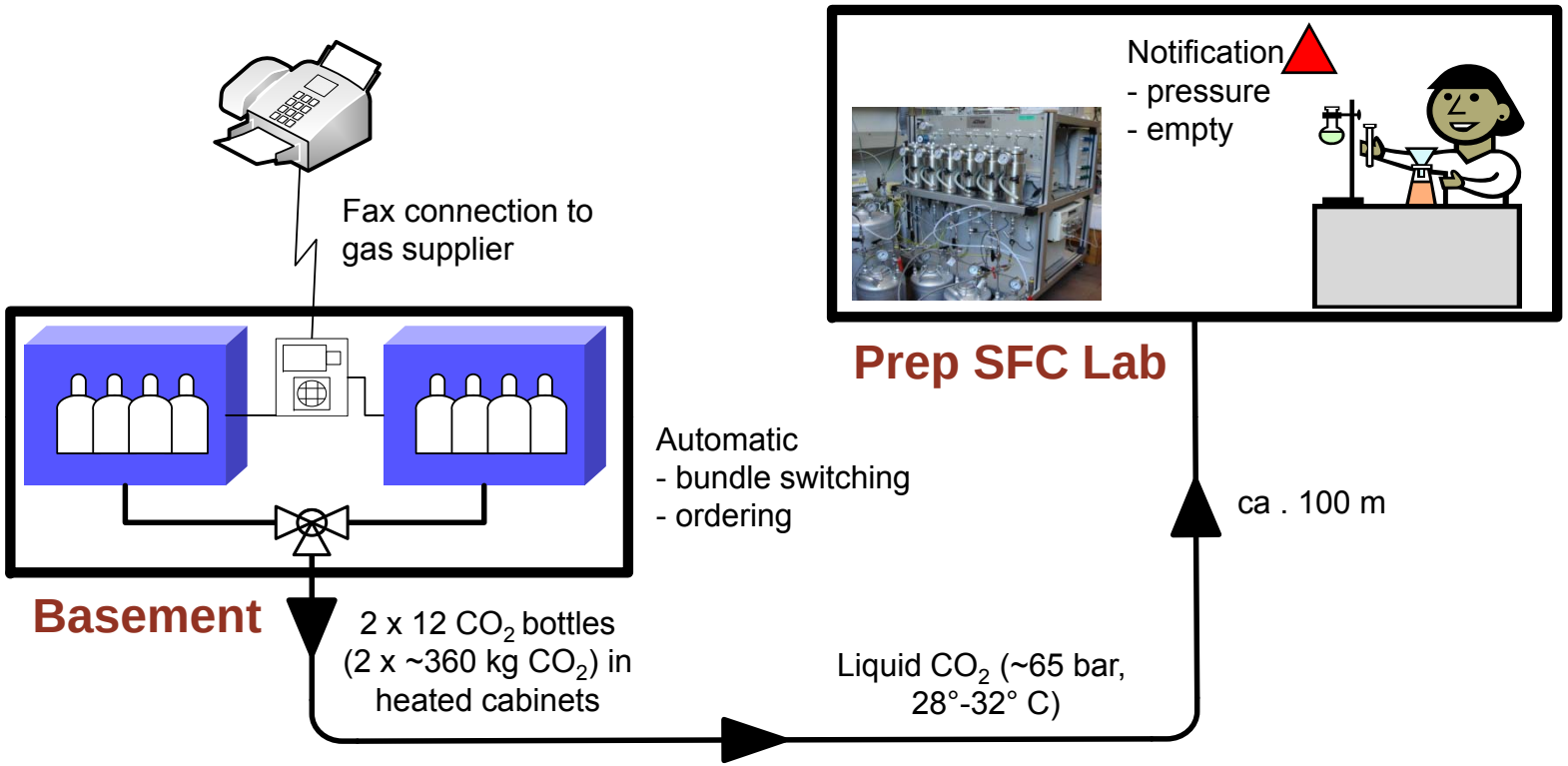
Thar Investigator SFC

- Max flow rate CO_2: 10 ml/min
- Max flow rate modifier: 10 ml/min
- Max operating pressure: 400 bar
- Semi-Prep fraction collection possible
- MS coupling with Micromass Platform II

Thar Prep SFC-200

- Max flow rate CO_2: 200 g/min
- Max flow rate modifier: 50 ml/min
- Max operating pressure: 380 bar
- Fraction collection: 6 cyclones à 1 l
  $\Rightarrow$ 1 for waste, 5 for fractions

CO_2 Delivery System for Prep SFC

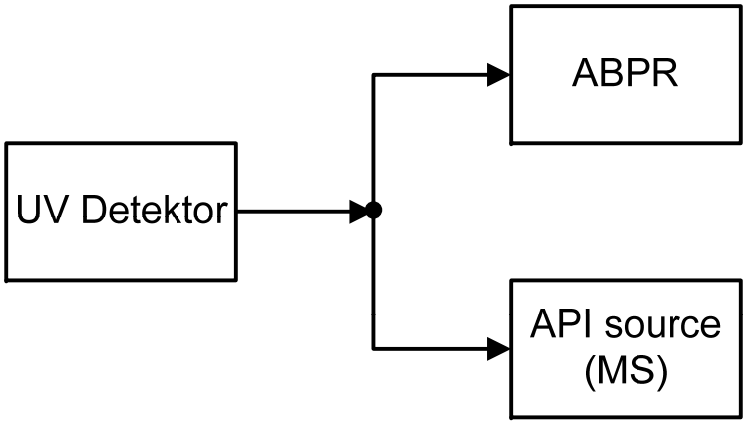


Contract with Gas Supplier:

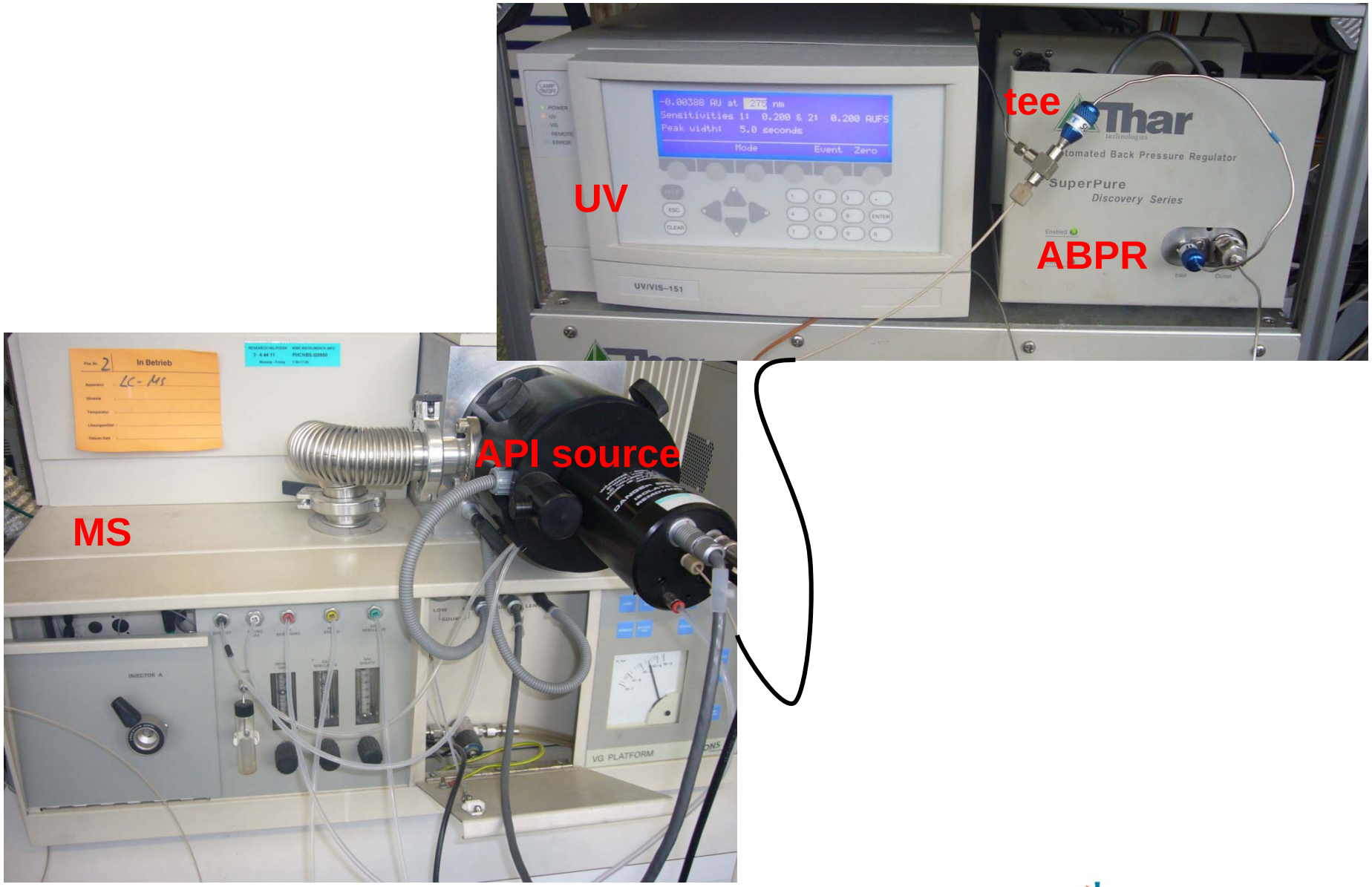
- Guaranteed disruption-free supply of at least 170 kg/day (118 g/min) of liquid CO_2, exception: long weekends
- Automatic ordering and installation of new bundles (no user action required)
- Always one full bundle as reserve for weekends and in winter
- Internet portal for CO_2 consumption and status
- CO_2 und O_2 sensors and alarms

SFC-MS Coupling

- Thar Investigator SFC coupled to Micromass Platform II mass spectrometer
- Two instrument control softwares (SuperChrome + MassLynx) running on two different PCs with contact closure
- Mainly APCI but also ESI mode employed
- First used with additional make-up flow for better ionization (2-propanol/H_2O/HCOOH 1000:10:2) but tests in APCI mode showed only slightly lower ionization efficiency without make-up flow
- MS only used in analytical mode (flow rate approximately 4 ml/min)
- Used in achiral work with mass scans as well as in chiral gradient screening with selected ion traces



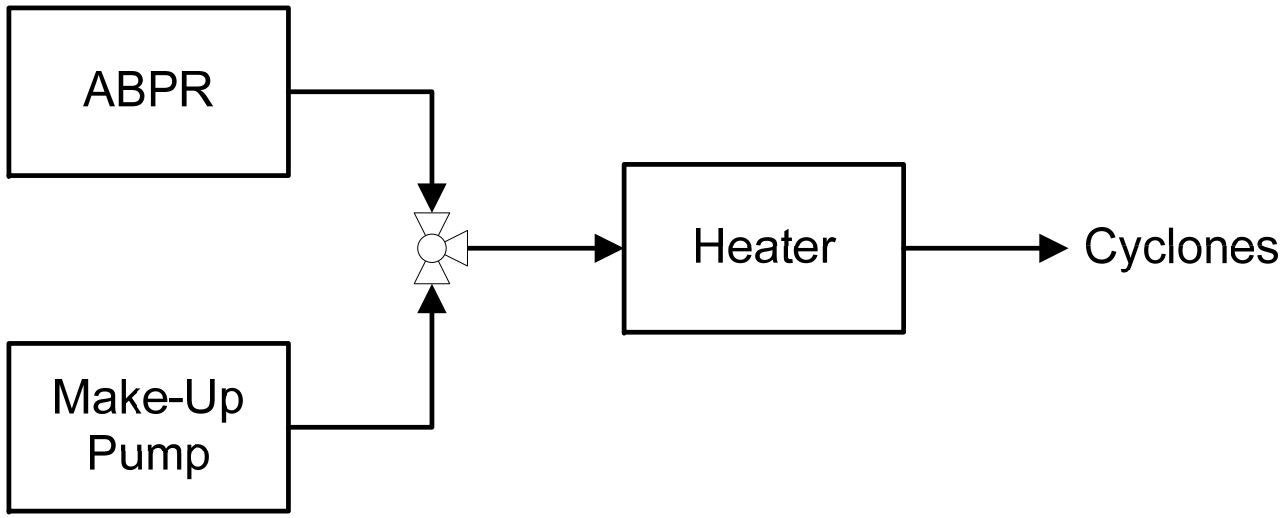
SFC-MS Coupling



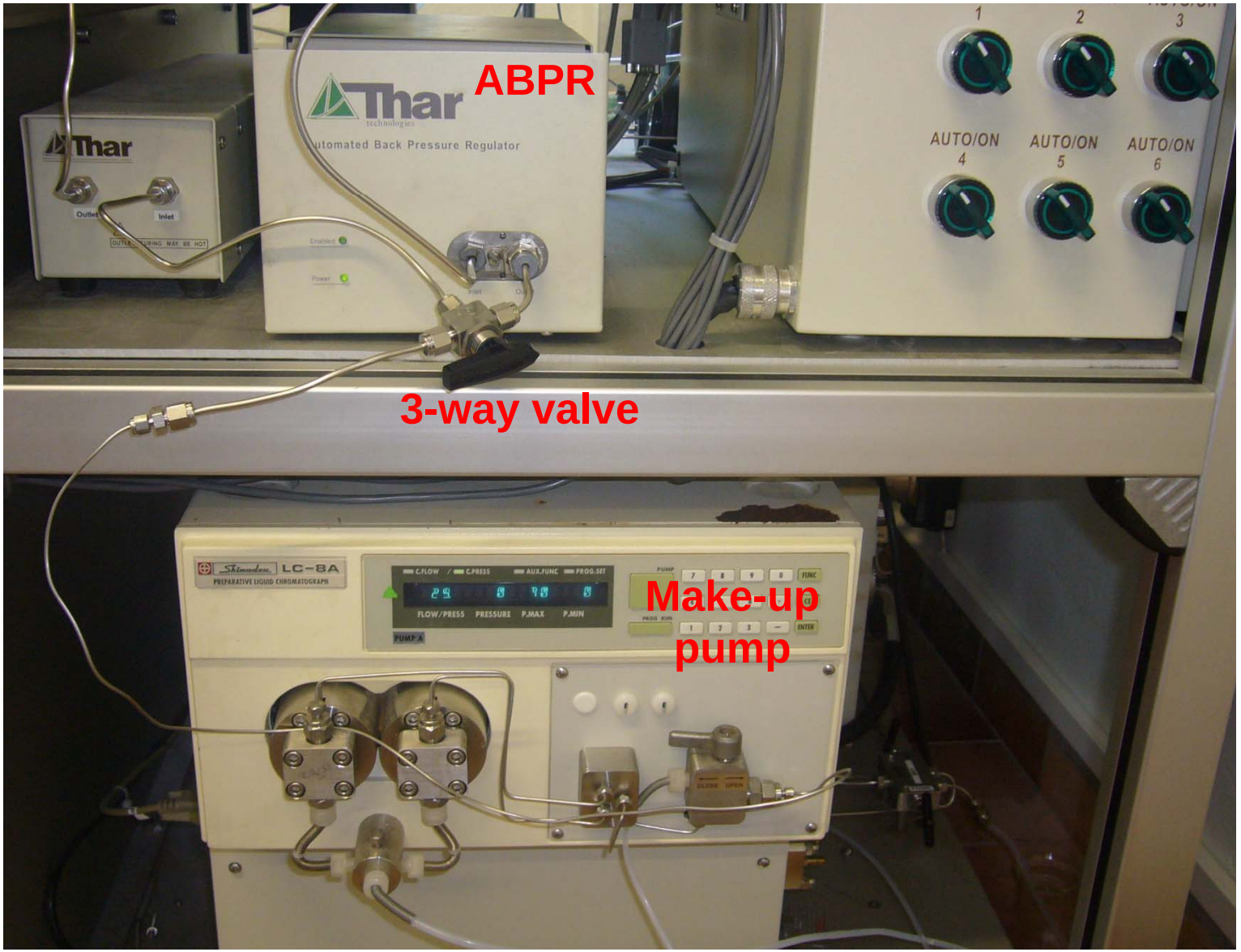
NOVARTIS

Make-Up Pump for Low Modifier Percentage

- Shimadzu Prep HPLC pump connected with a three way valve between ABPR and heater of Thar SFC-200
- Only used if modifier percentage <10% and solubility in mobile phase low
- Typical additional flow rate of 10-15 ml/min
- No restrictions on solvent used
- Tests have demonstrated improved purity of fractions for closely eluting peaks when using this setup
- Make-up pump on/off controlled by SFC-200 (overnight runs!)



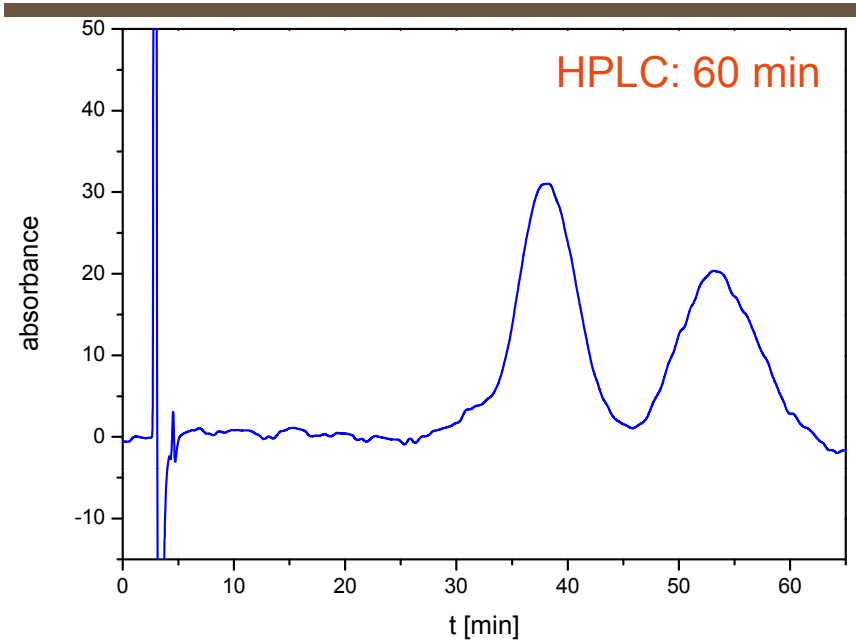
Make-Up Pump for Low Modifier Percentage

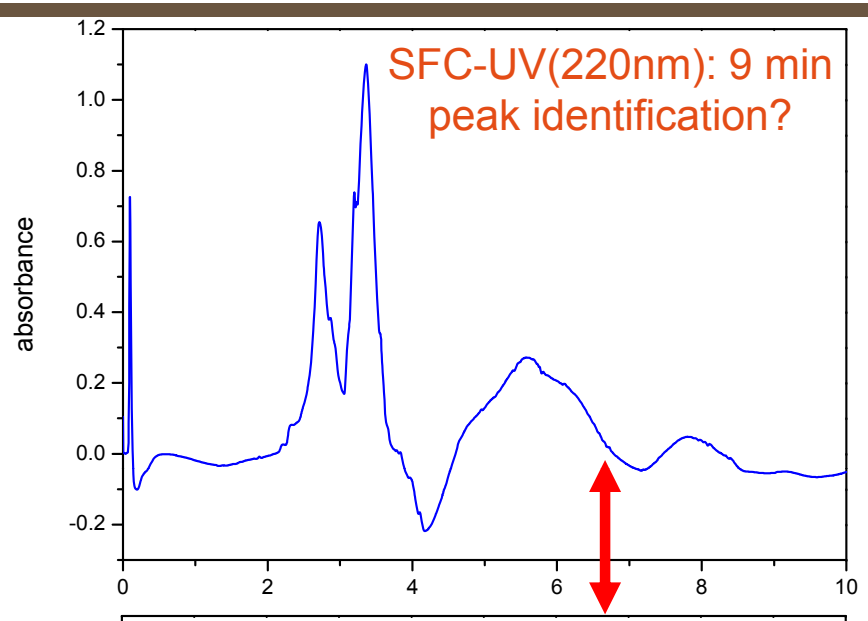


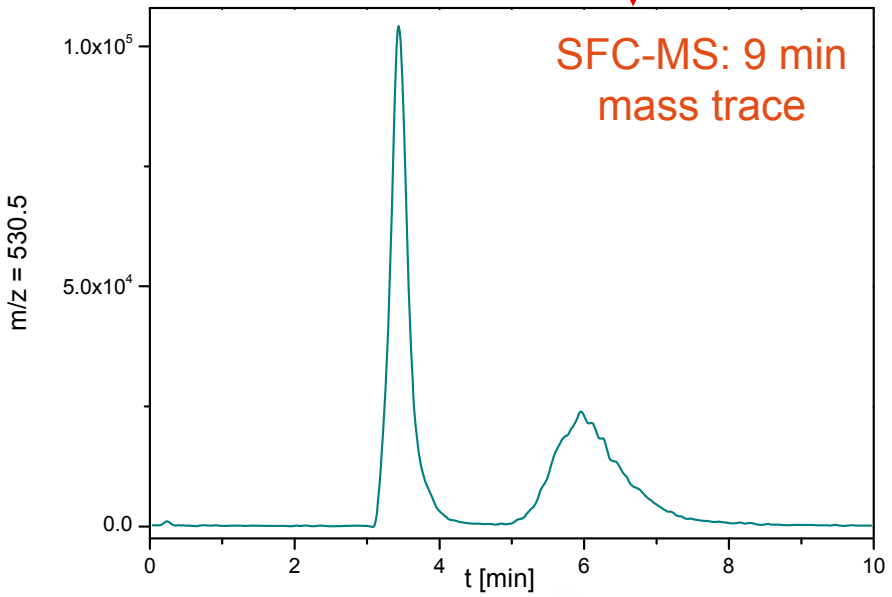
NOVARTIS

CASE STUDIES

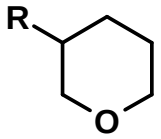
Chiral Resolution of Renin Inhibitor





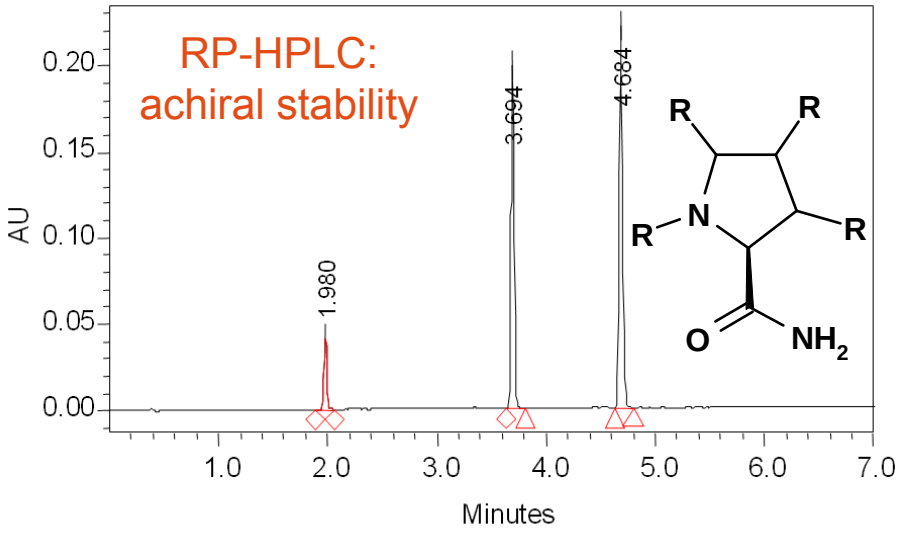


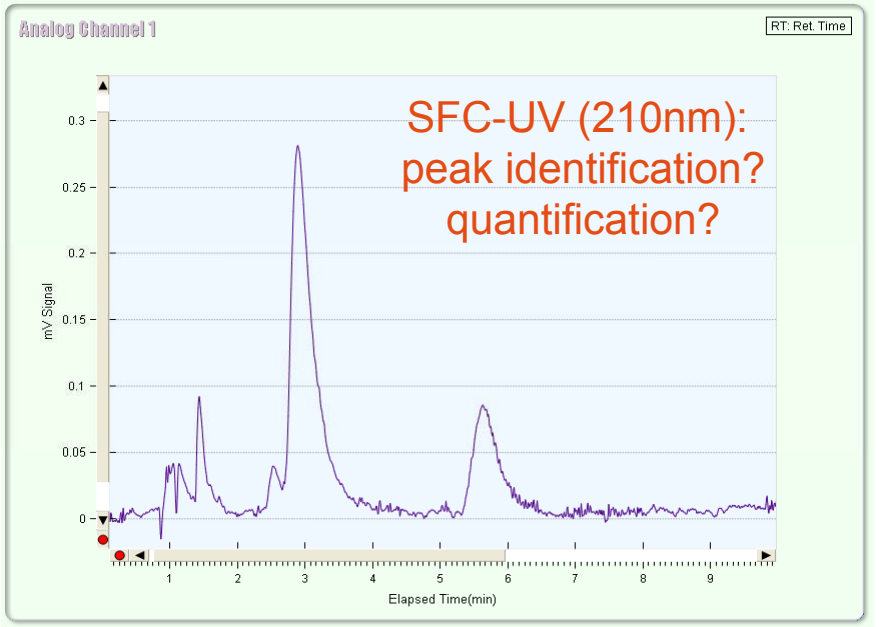
- Request for chiral resolution of 280 mg of renin inhibitor
- Method development in HPLC resulted in 60 min run time
  ⇒ try SFC
- Run time on SFC: 8 min
- Conditions: 3 ml/min CO_2, 20% EtOH, AD-H column, 4.6 x 150 mm
- **Improved peak identification by MS if sample concentration is low or no chromophore in molecule**



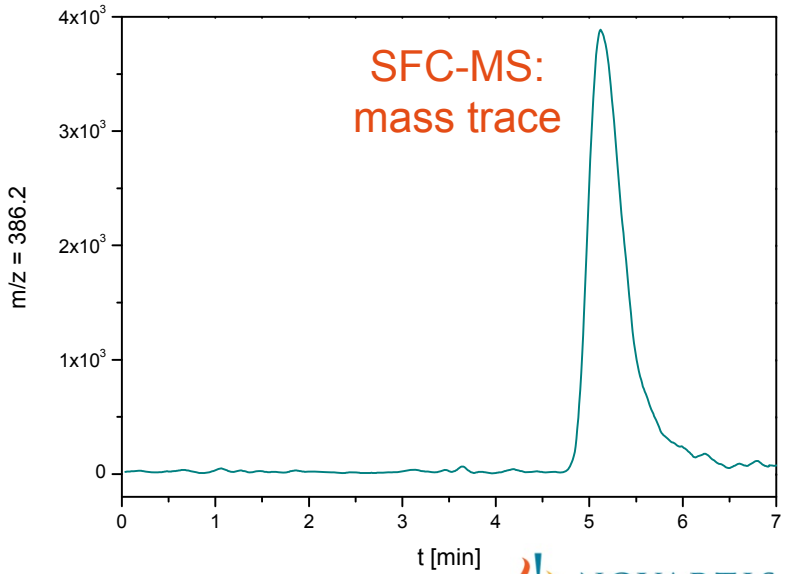
NOVARTIS

Stability Study Using SFC-MS





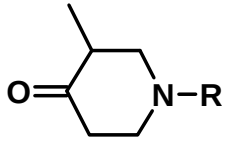
- Request for achiral and chiral stability of research compound
- Stability studies done in batch and solutions of different pH
- Achiral stability routinely done with UPLC(UV,MS)
- Chiral stability problematic on NP-HPLC(UV) because of coeluting peaks of degradants and limit of quantification
- No NP-HPLC-MS in our lab
- Best solution: SFC-MS (APCI+)
- This sample was stressed for one week at pH 7.4, 80°C
- Conditions: 4 ml/min CO_2, 40% EtOH, AD-H column, 4.6 x 250 mm
- **Improved quantification by MS also with chiral samples if sample concentration is low and sample is complex (degradants etc.)**



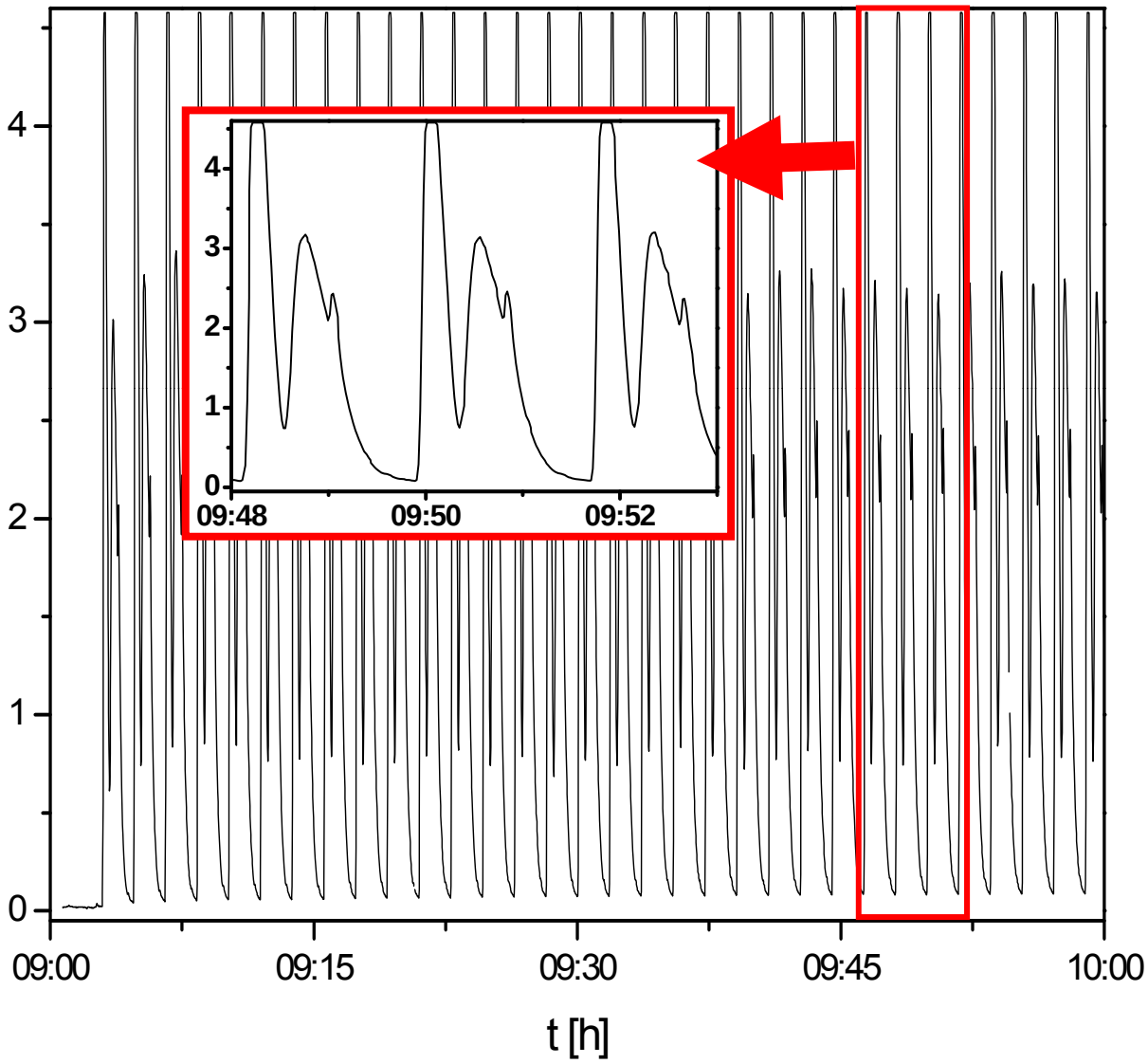
NOVARTIS

Chiral Resolution of Intermediate

Request for chiral resolution of **50 g** of building block



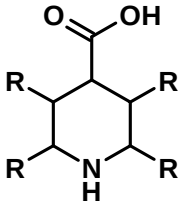
- Column: Chiralpak AD-H, 5 µm, 30 x 250 mm
- Mobile phase: CO_2/EtOH 95:5, 120 g/min
- **Cycle time: 1.8 min**
- 240 **stacked injections** (à 208 mg) in 7 h 22 min
- **Productivity: 6.8 g/h ≈ 2.33 kg/24h/kg CSP**
- Organic solvent consumption: 23 l EtOH in total
- 32 stacked injections shown
- P1: 23.1 g, ee = 99.0%; P2: 22.0 g, ee = 98.8%



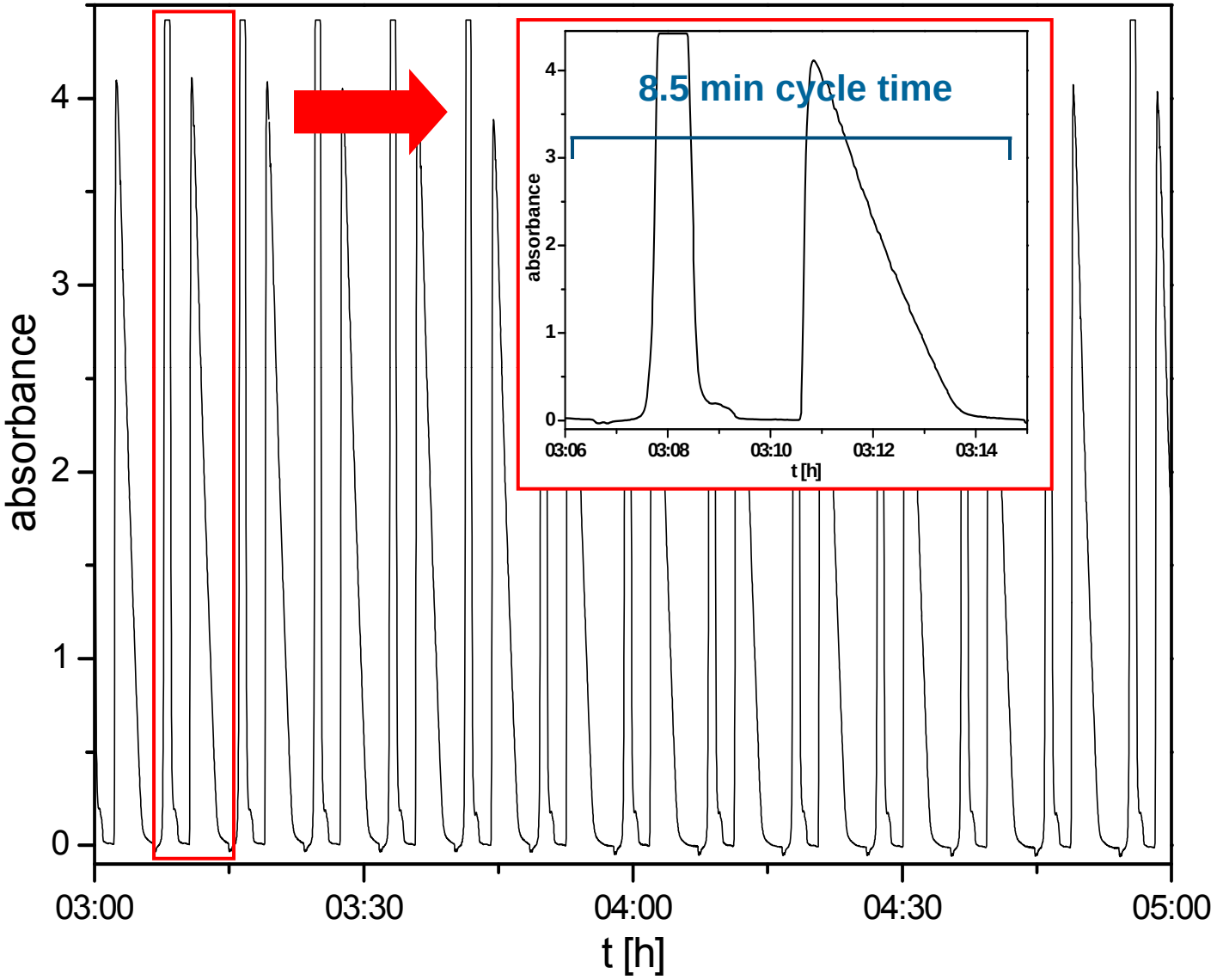
NOVARTIS

Chiral Separation of Instable Compound

- Request for chiral separation of 2.6 g rac. carboxylic acid



- Significant esterification with TFA/ethanol
- Acidity of supercrit. CO_2 enables prep. separation without acidic additives
- Column: Chiralpak AD-H, 5 µm, 30 x 250 mm
- Mobile phase: CO_2/methanol 70:30
- Cycle time of 8.5 min
- 25 stacked injections (à 104 mg racemate) in 3.5 hours
- P1: 1.18 g, ee > 99.9%
  P2: 1.21 g, ee > 99.9%



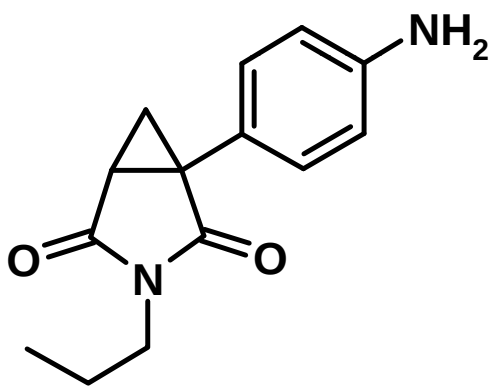
NOVARTIS

Preparative Separation of Aromatase Inhibitor

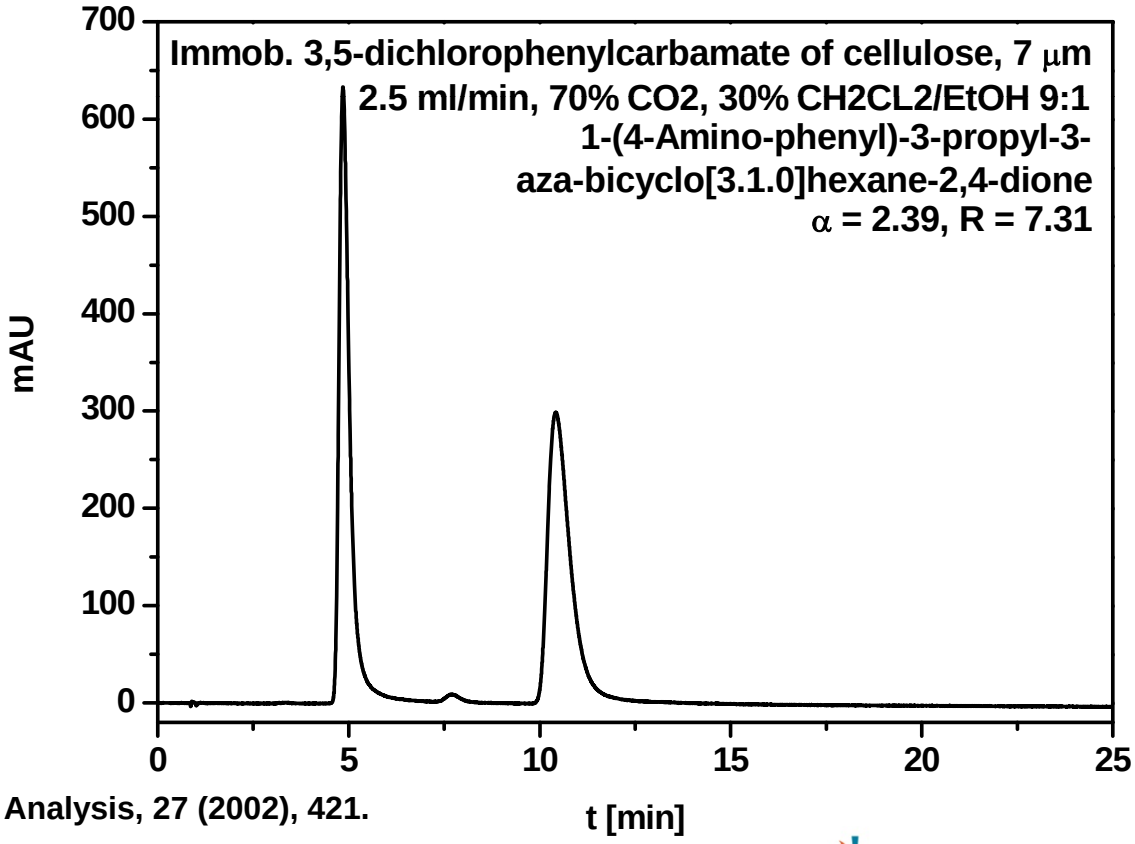
- Low solubility in classical mobile phase, high solubility in chlorinated solvents:

n-heptane/IPA 90:10	⇒	3 mg/ml	CH_2Cl_2	⇒	>40 mg/ml
TBME	⇒	17 mg/ml	$CHCl_3$/MeOH 9:1	⇒	>40 mg/ml
THF	⇒	>40 mg/ml			

- SFC separation on immobilized halogenophenylcarbamates of cellulose



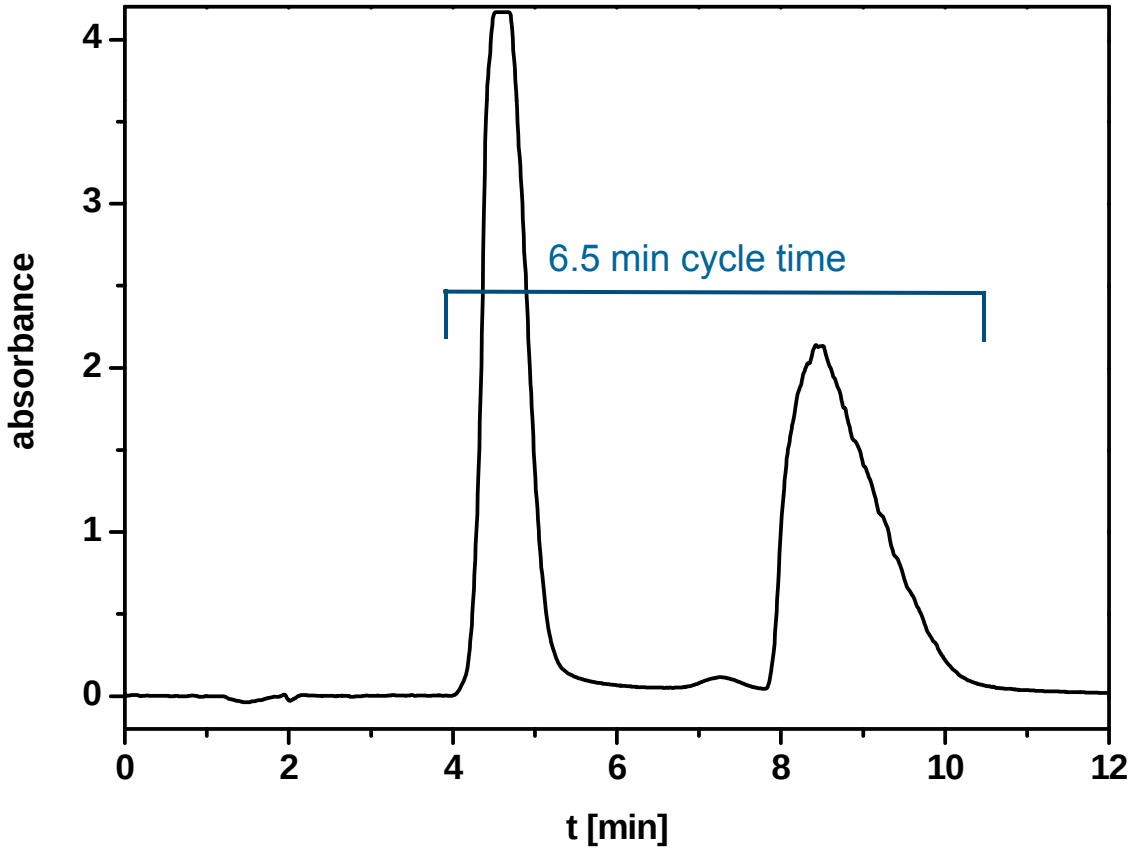
1-(4-Amino-phenyl)-3-propyl-
3-aza-bicyclo[3.1.0]hexane-2,4-dione



E. Francotte, D. Huynh; J. Pharm. & Biomed. Analysis, 27 (2002), 421.

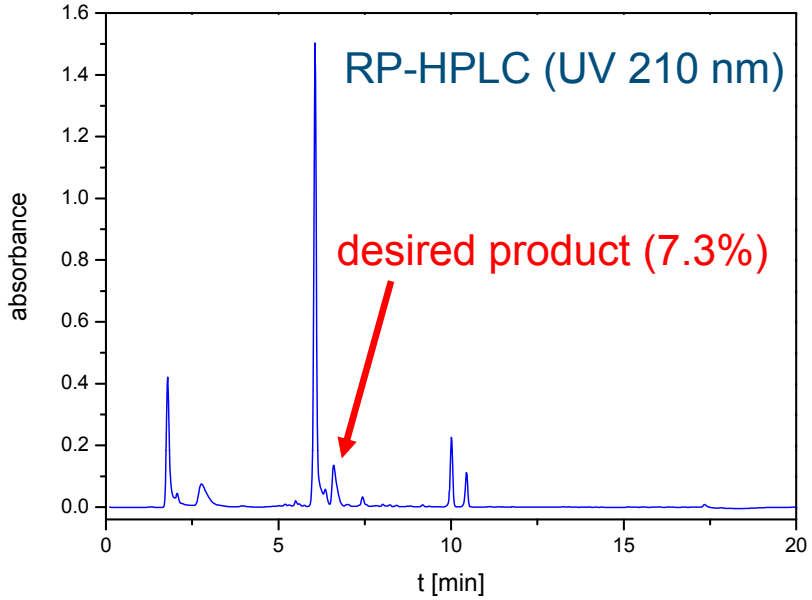
Preparative Separation of Aromatase Inhibitor

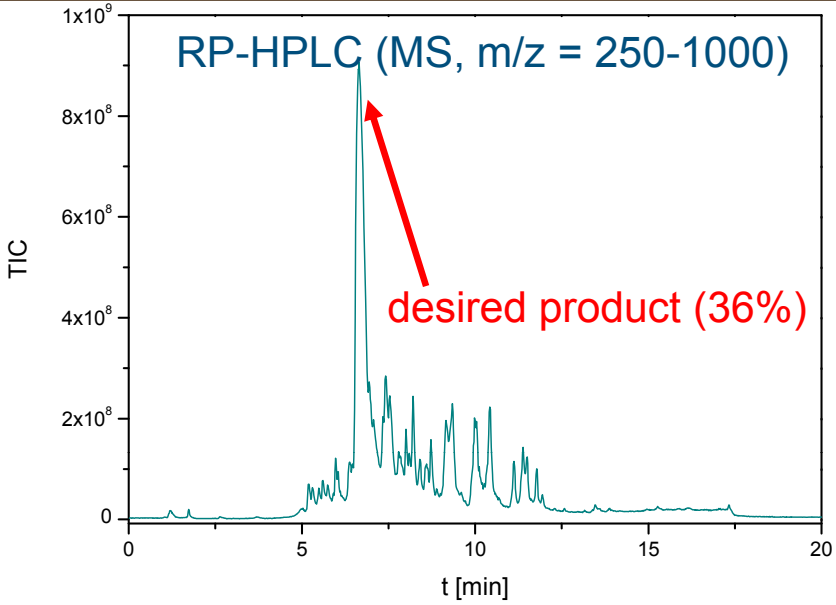
- Prep. SFC on immob. CSP, 21.2 x 250 mm (7 µm)
- High feed concentration in modifier solvents (CH_2Cl_2/EtOH 8:2) ⇒ 70 mg/ml
- Column load: 140 mg, 590 mg of compound resolved by SFC
- Flow: 50 g/min, 70% CO_2, 30% modifier; 150 bar, 40°C
- Productivity: 0.477 kg/kg CSP/24 h



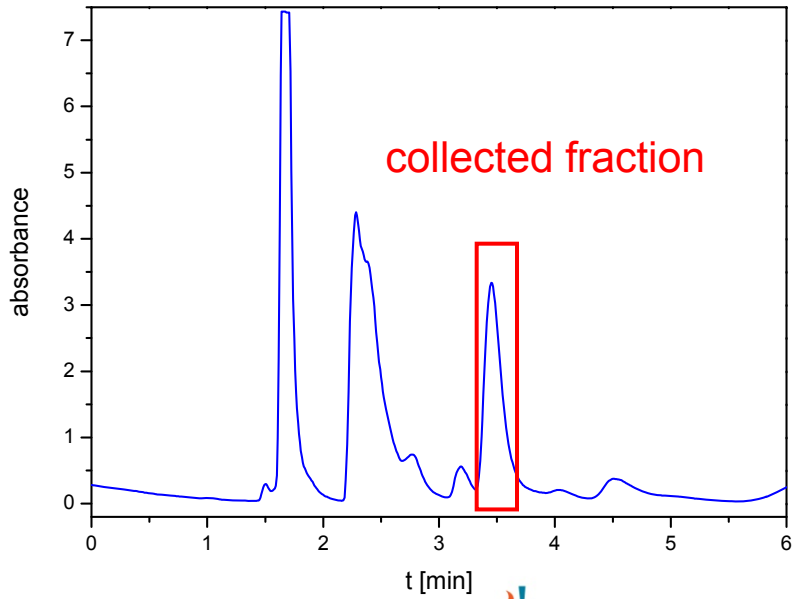
NOVARTIS

Achiral purification of reaction mixture





- Request for purification of 9.35 g of reaction mixture **containing only 7.4% (UV) of desired product**
- Column: Princeton 2-Ethylpyridine, 5 µm, 30 x 250 mm
- Mobile phase: CO_2/MeOH 85:15, 120 g/min
- Cycle time: 6 min
- 200 stacked injections
- Purity (UV, 210 nm): 98%
- Recovery: 0.68 g (7.2%)

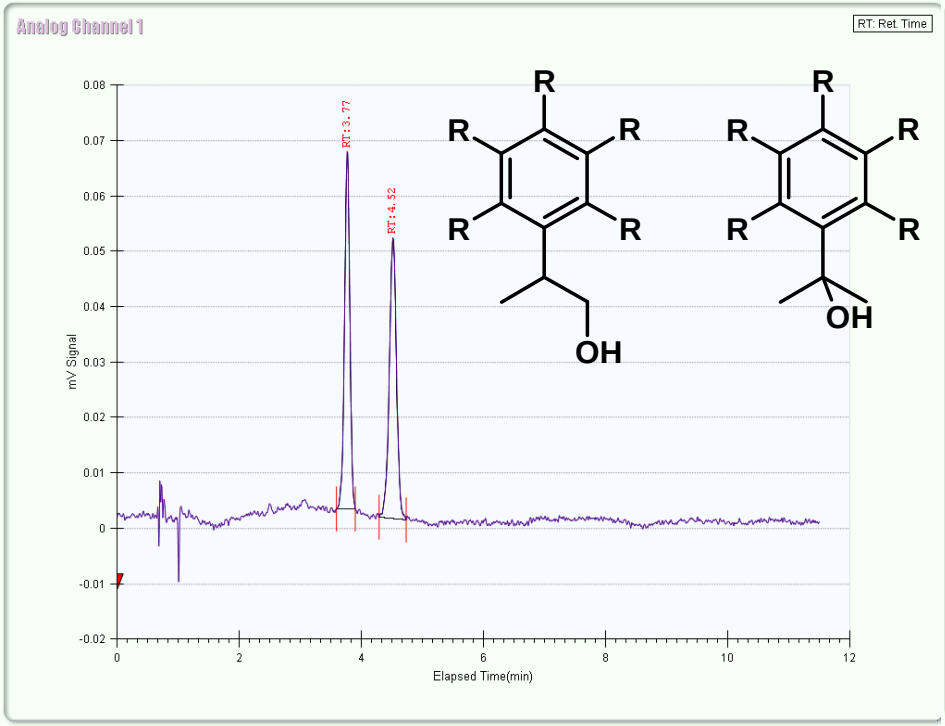


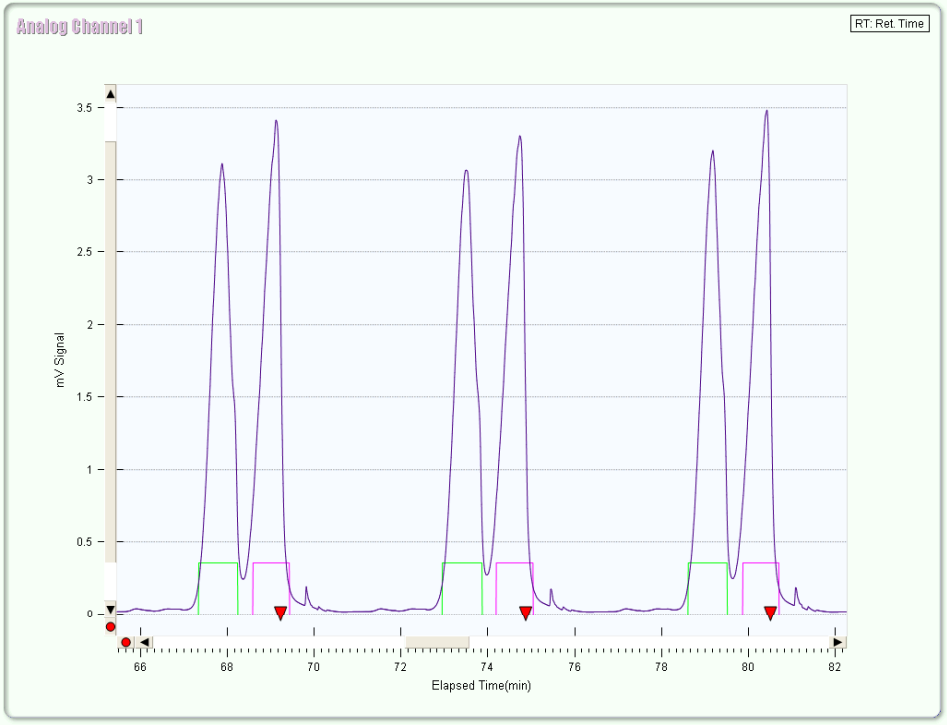
NOVARTIS

Purification of Metabolites

Purification of hydroxylated metabolites produced by microbial biotransformation

- First step: prep. RP-HPLC/MS but further purification necessary, very good selectivity on SFC
- Only small amounts, therefore preparative separation on 4.6 mm column
- Column: Princeton DEAP, 5 µm, 4.6 x 250 mm
- Mobile phase: CO_2/MeOH 90:10, 6 ml/min, cycle time: 5.7 min
- P1: 4 mg, P2: 5 mg, both 98.0% (UV), sent to NMR for structure elucidation
- NMR analyst enthusiastic about purity of NMR samples (removal of fatty acids and other impurities not easily detectable by HPLC-UV and HPLC-MS)

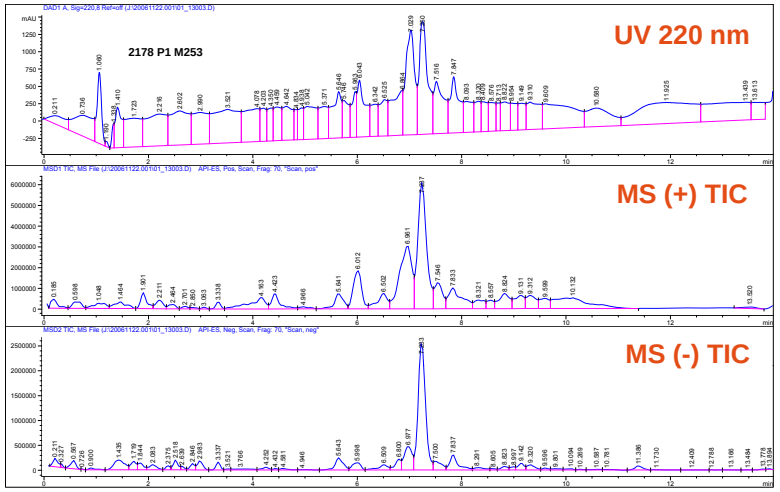




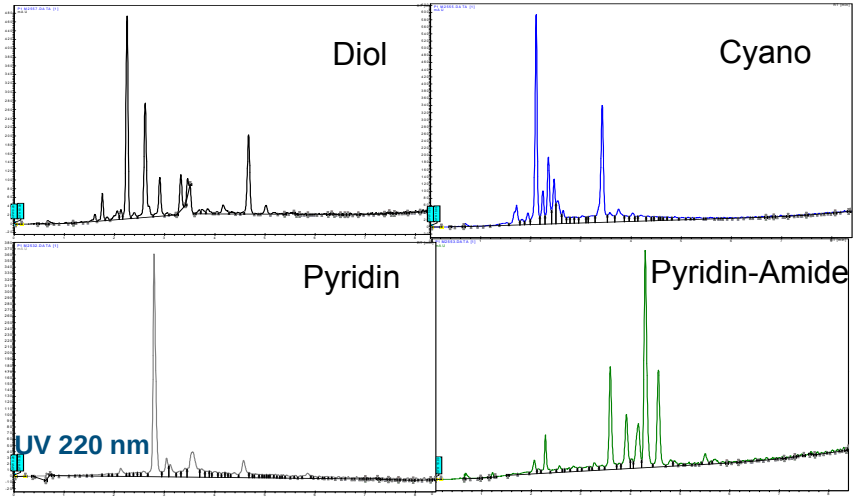
NOVARTIS

SFC Separation of Novel Ascosalitoxins

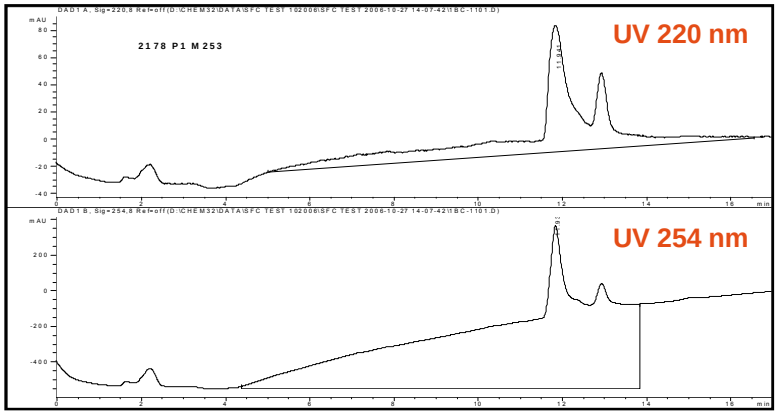
HPLC separation on reversed phase



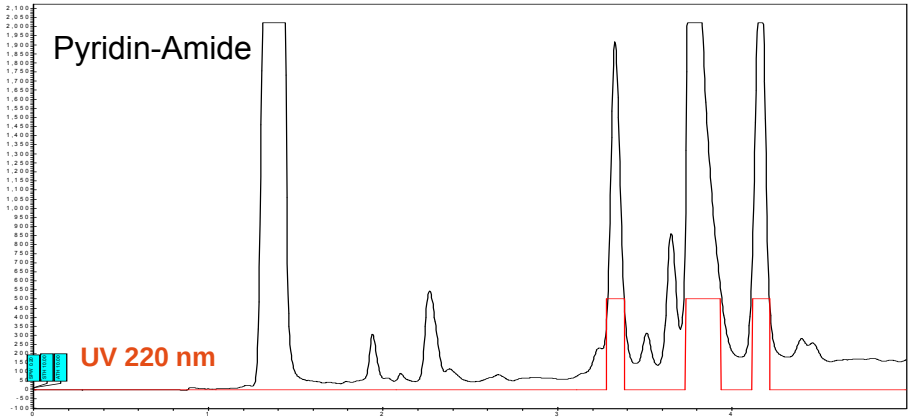
Analytical SFC separation



HPLC separation on normal phase



Preparative SFC separation



NOVARTIS

Summary

By showing

- how SFC is done at NIBR Basel
- how we have technically improved the commercially available instrumentation
- several examples encompassing chiral, achiral, analytical, and preparative SFC with different detection techniques and different types of compounds

it was demonstrated that SFC is indeed a versatile tool in the analysis and purification of pharmaceutical compounds.

Acknowledgement

Dan Huynh

Martin Grosup