Implementing SFC for the analysis of veterinary antibiotics in manure

SFC User Meeting, 08.10.2025

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UNIVERSITÄT DUISBURG ESSEN

Offen im Denken

Introduction & Context

ReFarM

wetsus

Agricycling

DLV ADVIES &RESULTAAT

BioEnergieSieringhoek GmbH & Co KG

Kompetenzzentrum Niedersachsen • Netzwerk Nachwachsende Rohstoffe und Bioökonomie e.V. 3N

HOCHSCHULE OSNABRÜCK UNIVERSITY OF APPLIED SCIENCES

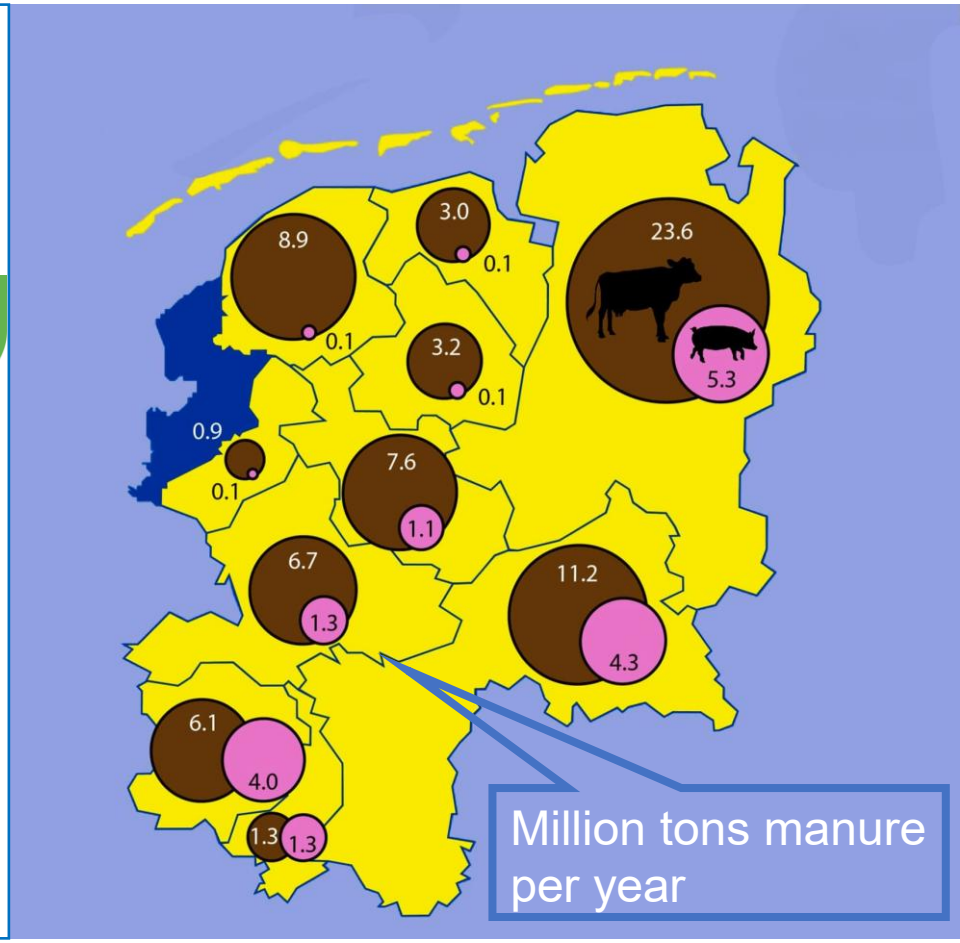
Koninklijke oosterhofholman

UNIVERSITÄT DUISBURG ESSEN Offen im Denken

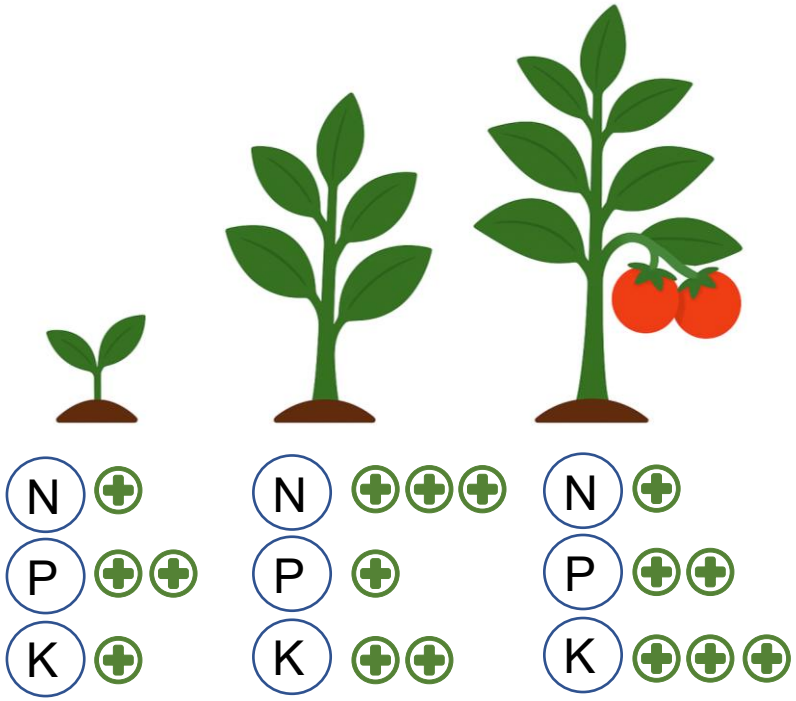
Humus Guru.nl MAAKT DE BODEM WEER GEZOND

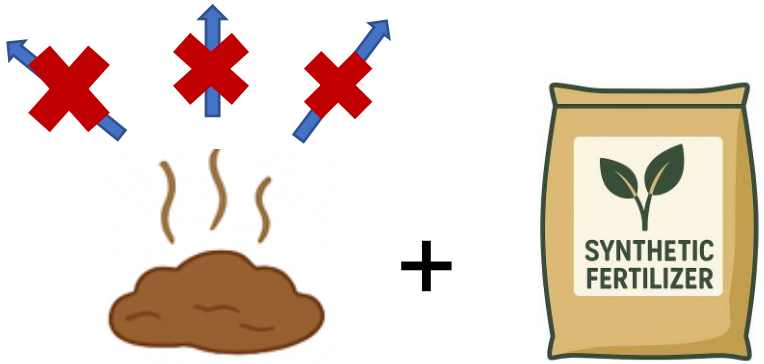
ReFarM: Rethinking Farming and Manure Management

Motivation: Agricultural transformation through sustainable manure management



Problem conventional manure management





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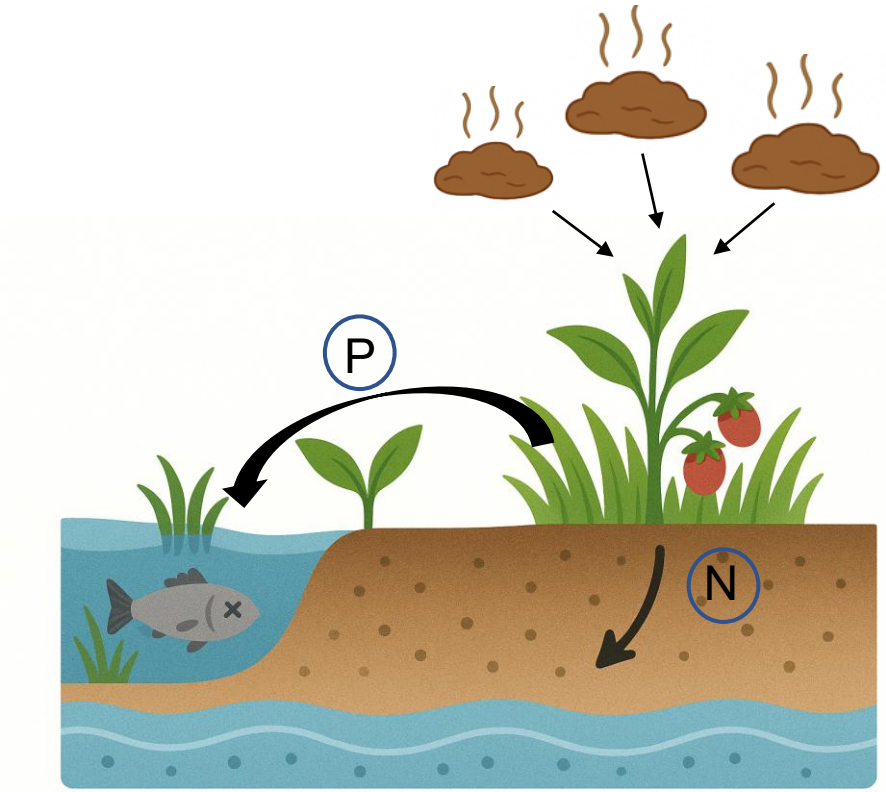
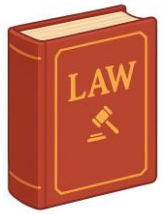
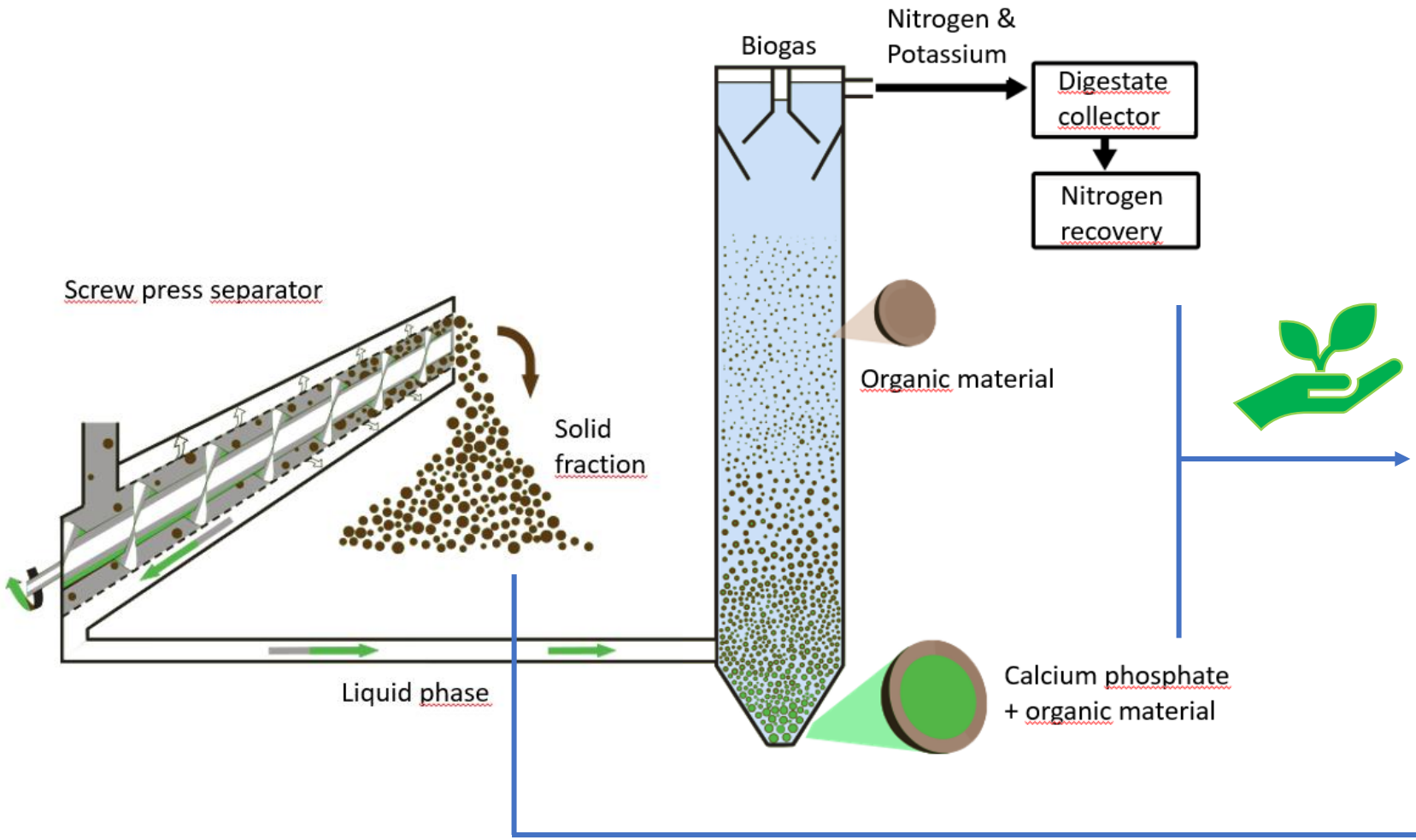




Project goal

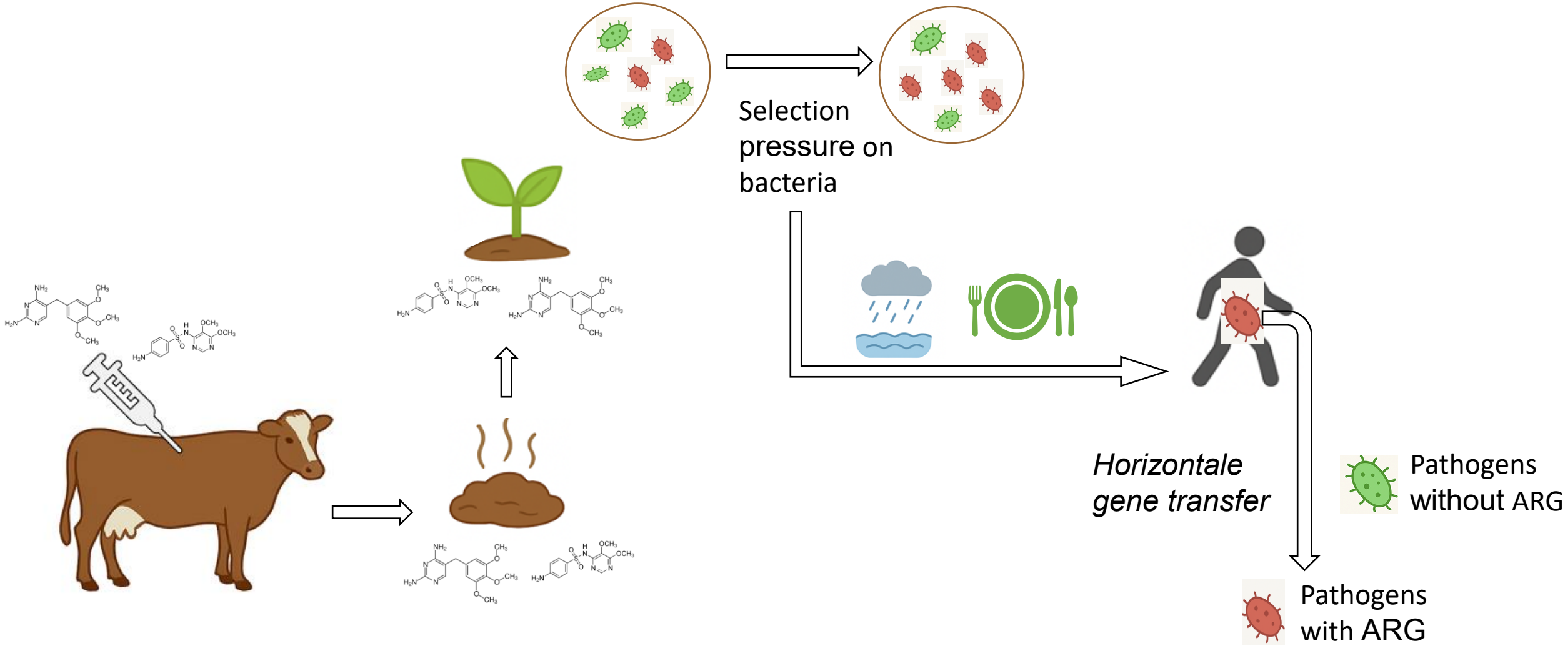
Recover valuable nutrients via an innovative **Upflow anaerobic sludge blancet reactor (UASB)**



Application of **sustainable** & **efficient** manure (products)

Contaminants in Manure - Antibiotics

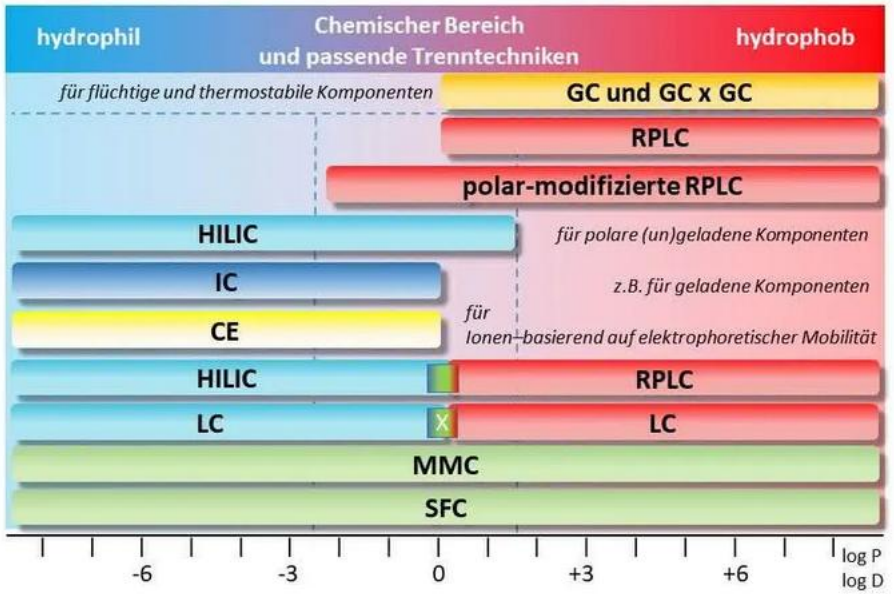
Our part: Analytical Challenges, i.e. Pollutant analysis



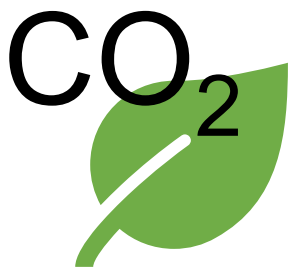
Analysis of antibiotics in manure

Analytical Challenge: low concentrations (ng/L), complex matrix, diverse physico-chemical properties

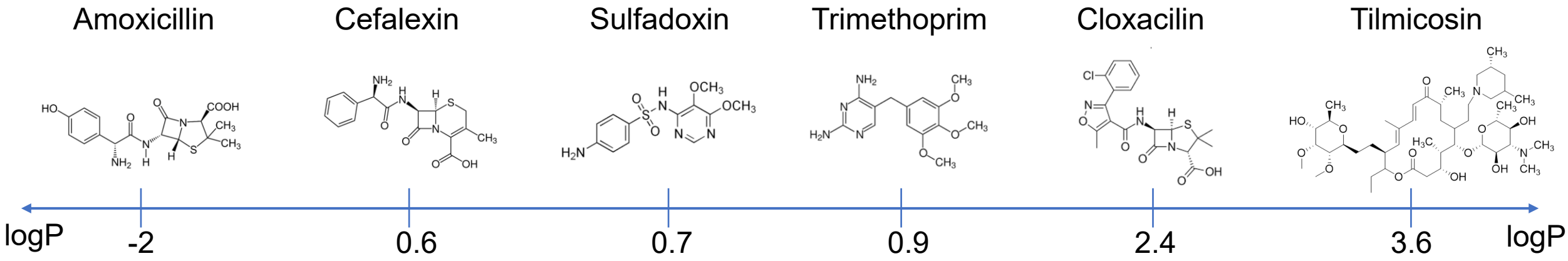
Analysis: LC–MS/MS as main analytical method → Why not implementing SFC?

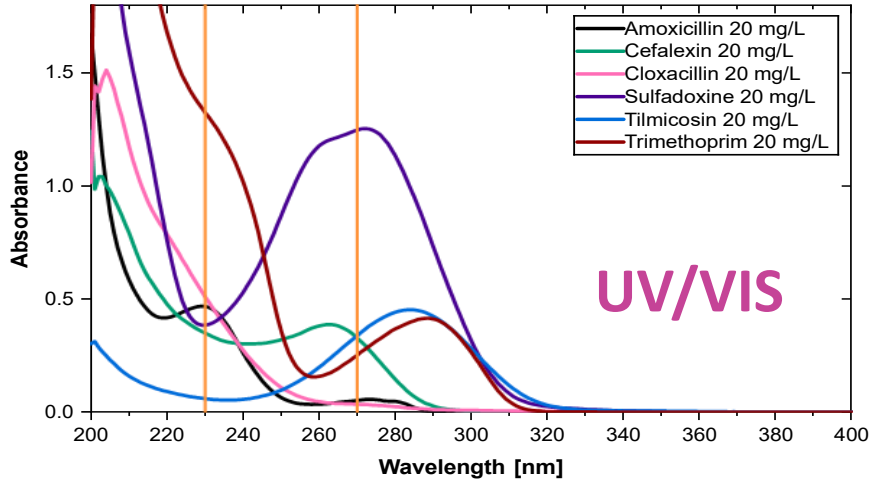


S. Bieber, T. Letzel (2024). SFC-HRMS – eine grüne, polaritätserweiternde Methode für die Spurenstoffanalytik. *LABO* [Online] Verfügbar unter https://www.labo.de/chromatographie/sfc-hrms---eine-gruene--polaritaetserweiternde-methode-fuer-die-spurenstoffanalytik.htm



Analysis of antibiotics in manure: Analyte Overview



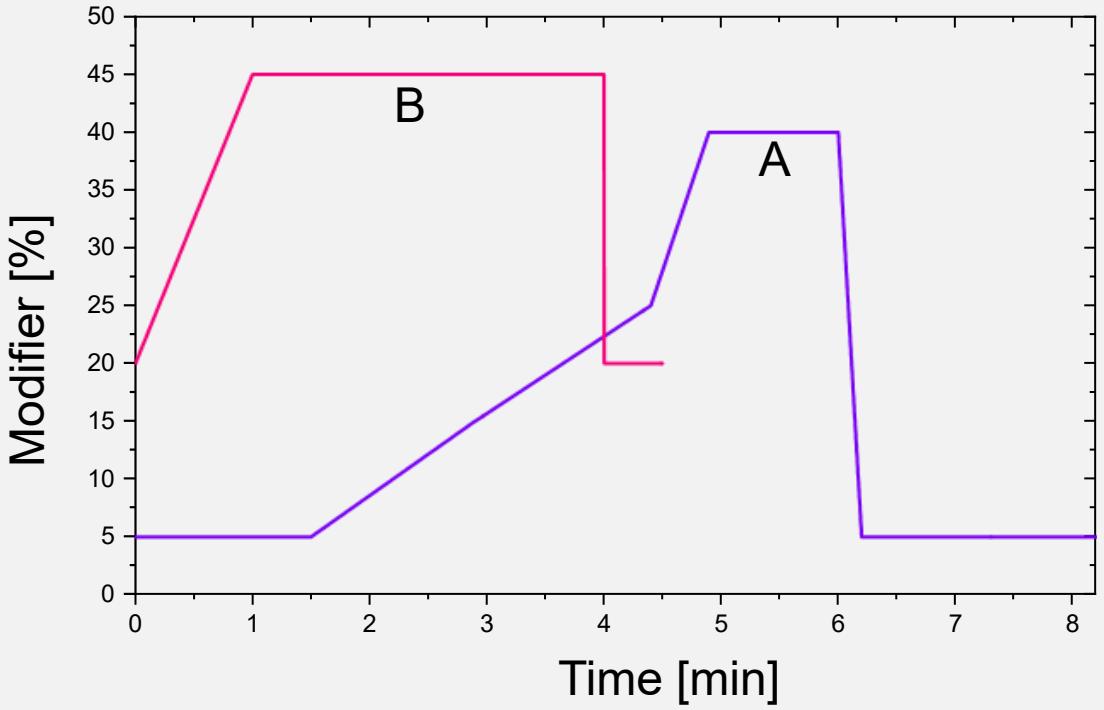


→ 230 nm and 270 nm chosen

Method settings

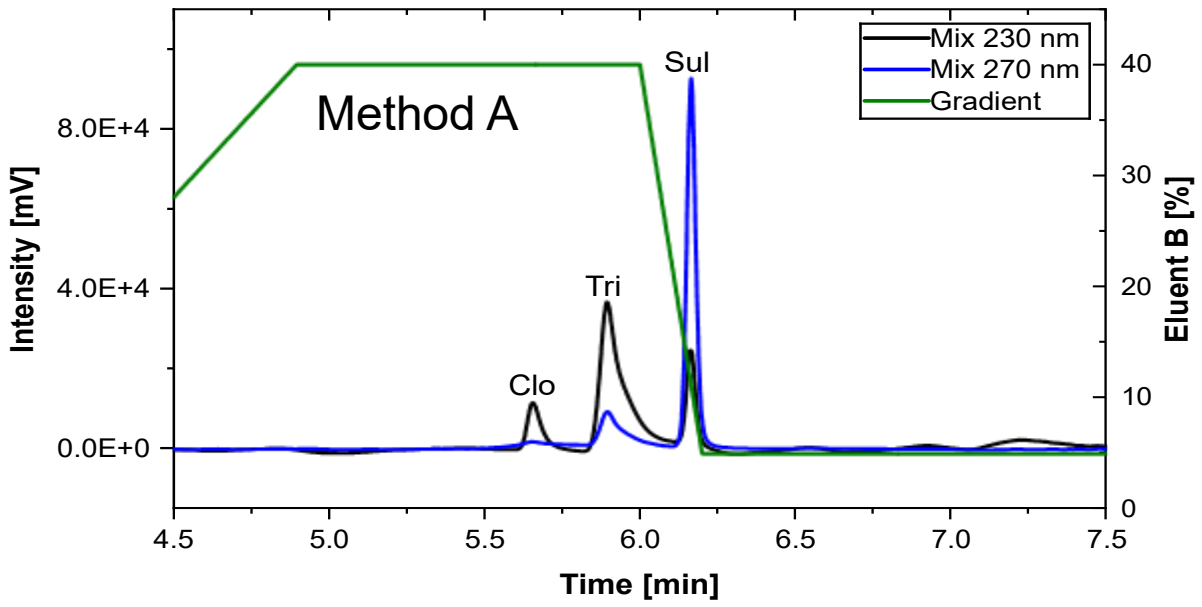
Parameter	Method A[1]	Method B[2]
Column	Torus Diol II	Torus Diol II
Autosampler [°C]	5	5
BPR [Bar]	150	140
Column oven [°C]	55	55
Flow rate [mL]	1.00	0.75
Modifier	MeOH + $0.1\%_{vol}$ FA	MeOH + $5\%_{vol}$ AF 10mM

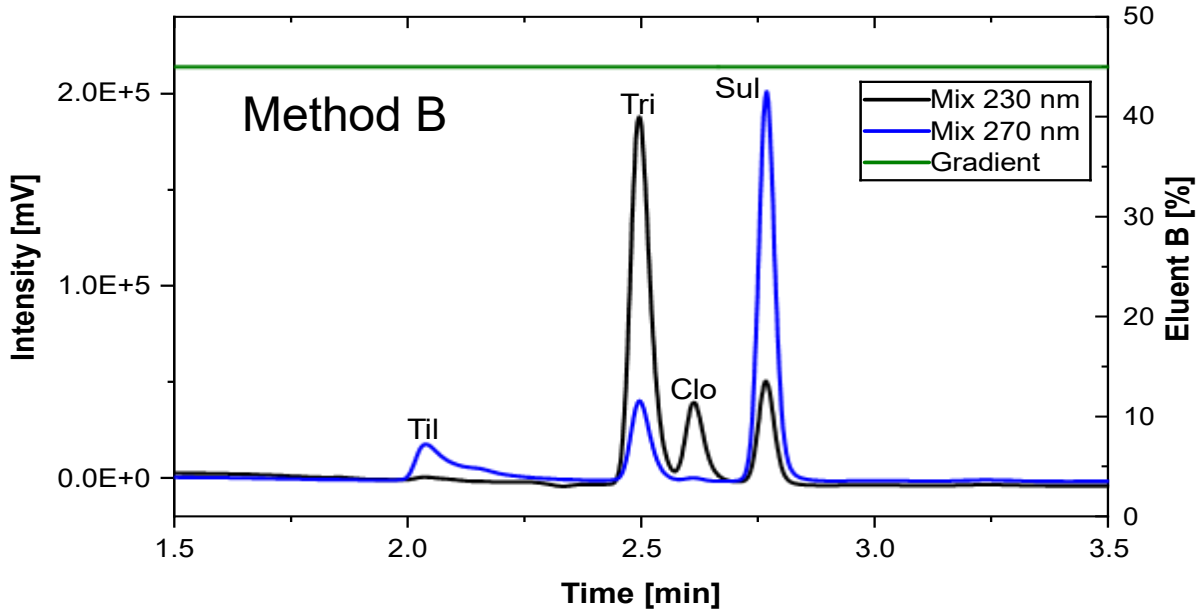
Gradient



[1] Lu, X. f.; Zhou, Y.; Zhang, J.; Ren, Y. *J. Sep. Sci.* **2019**, *42* (6), 1281-1288
[2] Demir, Z.; Bahmany, S.; Bethlehem, C.; van Oldenrijk, J.; Bos, P. K.; Koch, B. C. P. J. Chromatogr. **2021,** *1173*, 122696-122696.

Results SFC-UV/VIS

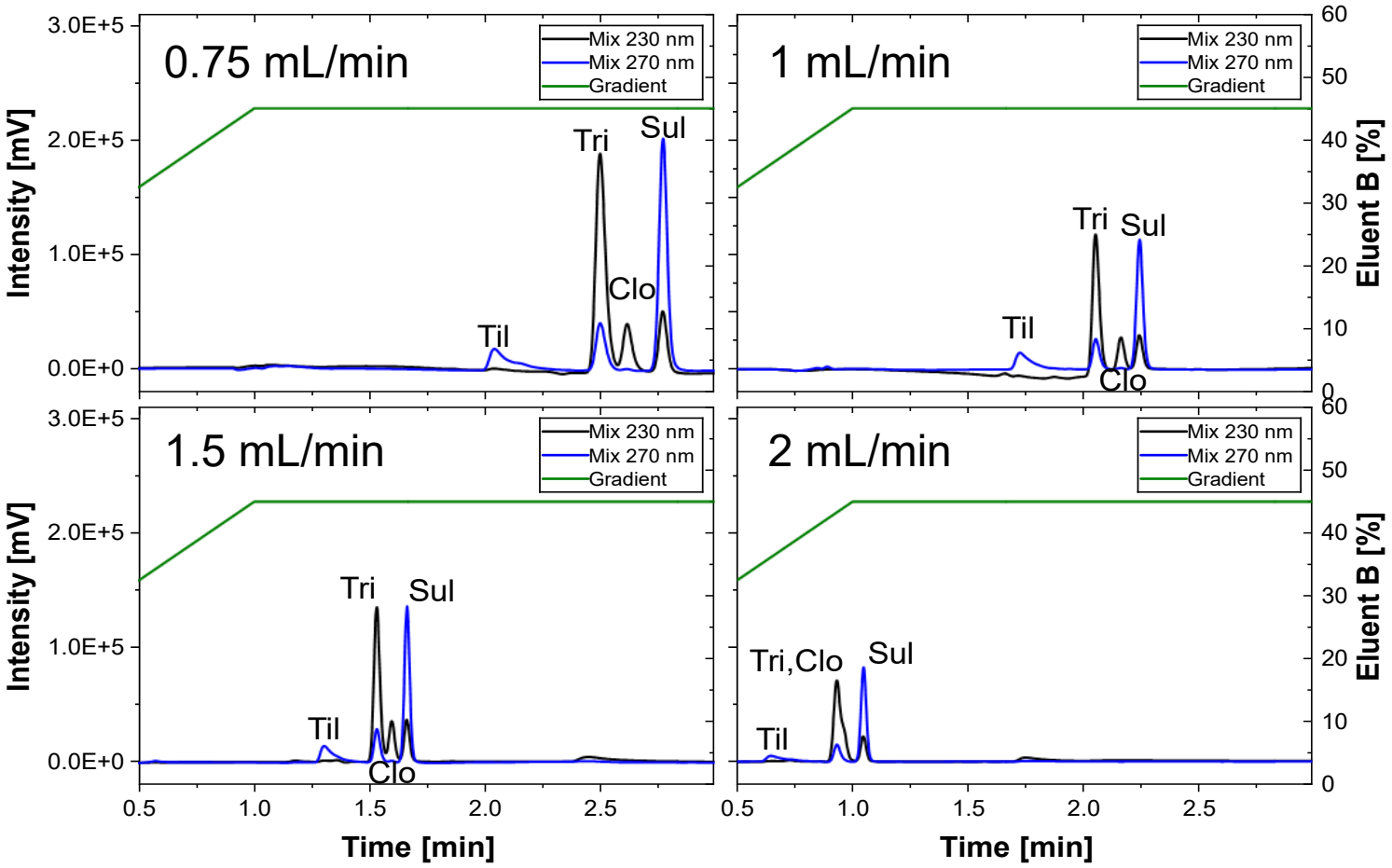




Method B	
Baseline separation (r > 1.5)	✓ ; ✗ ; ✓
Skewness (compared to method A)	Til : - Clo : + Tri : + Sul : =

- Rapid separation of four antibiotics
- Amoxicillin and Cefalexin could not be eluted within the scope of the method

Influence of flow rate; LOD/LOQ determination



Analyte	R^2	LOD [mg/L]	LOQ [mg/L]
Tri	0.999	0.92	2.75
Clo	0.996	2.06	6.17
Til	0.999	1.01	3.03
Sul	0.999	0.97	2.90

- Low flow rate required under these method conditions to maintain baseline separation
- UV/Vis LOD/LOQ exceeds expected analyte concentrations ⟶ MS/MS detection required

Sample preparation

Extracting fractions of **manure**[3]**:**
Solid vs **Liquid**

1. Acetonitrile–methanol–0.1 M EDTA–McIlvaine buffer (pH = 4)
2. Ultrasonication
3. Centrifugation
4. mQ water dilution

1. Glacial acetic acid EDTA– McIlvaine buffer (pH = 4.0)
2. Methanol
3. Centrifugation
4. mQ water dilution

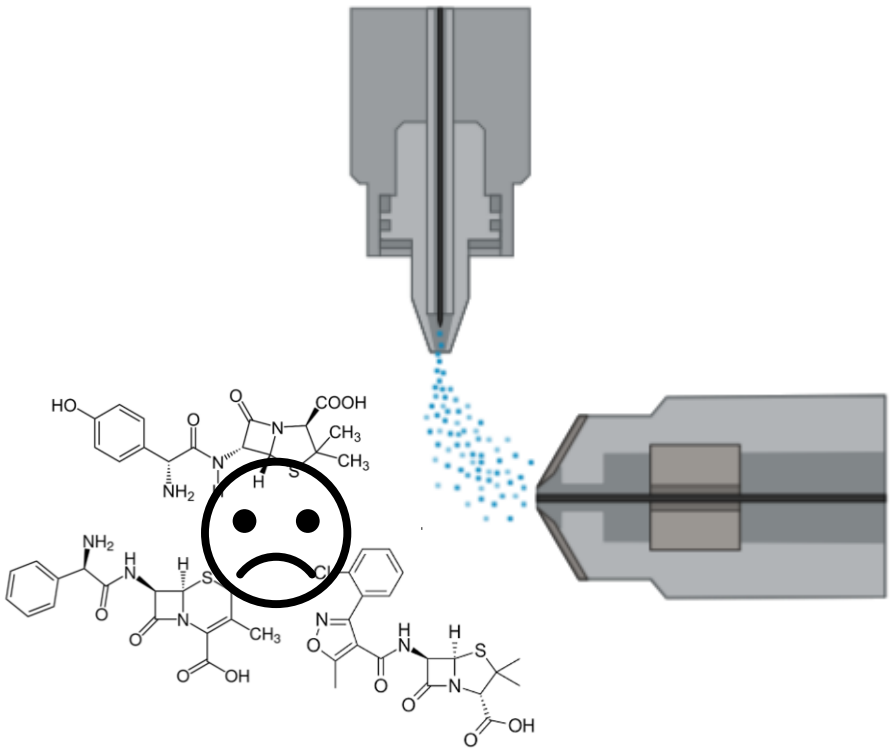
→ Precipitation and separation of substantial quantity of proteins and NOMs

SPE

1. Conditioning with MeOH and EDTA[3]
2. Rinsing with 5% MeOH[3]
3. **Elution 1** with 10 mL of 9:1 ethyl acetate /methanol[3]
4. **Elution 2** with 0.5 mL of methanol, water and formic acid (90/9/1, v/v/v)[4]

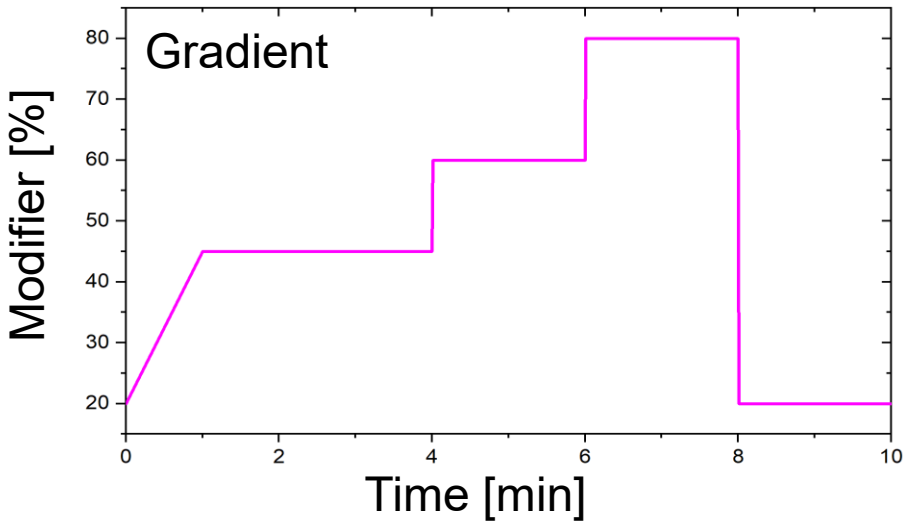
[3] S. Wallace, D. S. Aga *J. of Env. Quality.*, **2016**, *45*, 471-479. [4] J. Rossmann, S. Schubert, R. Gurke, R. Oertel, W. Kirch *J Chromatogr. B*,**2014**, *969*, 162-170.

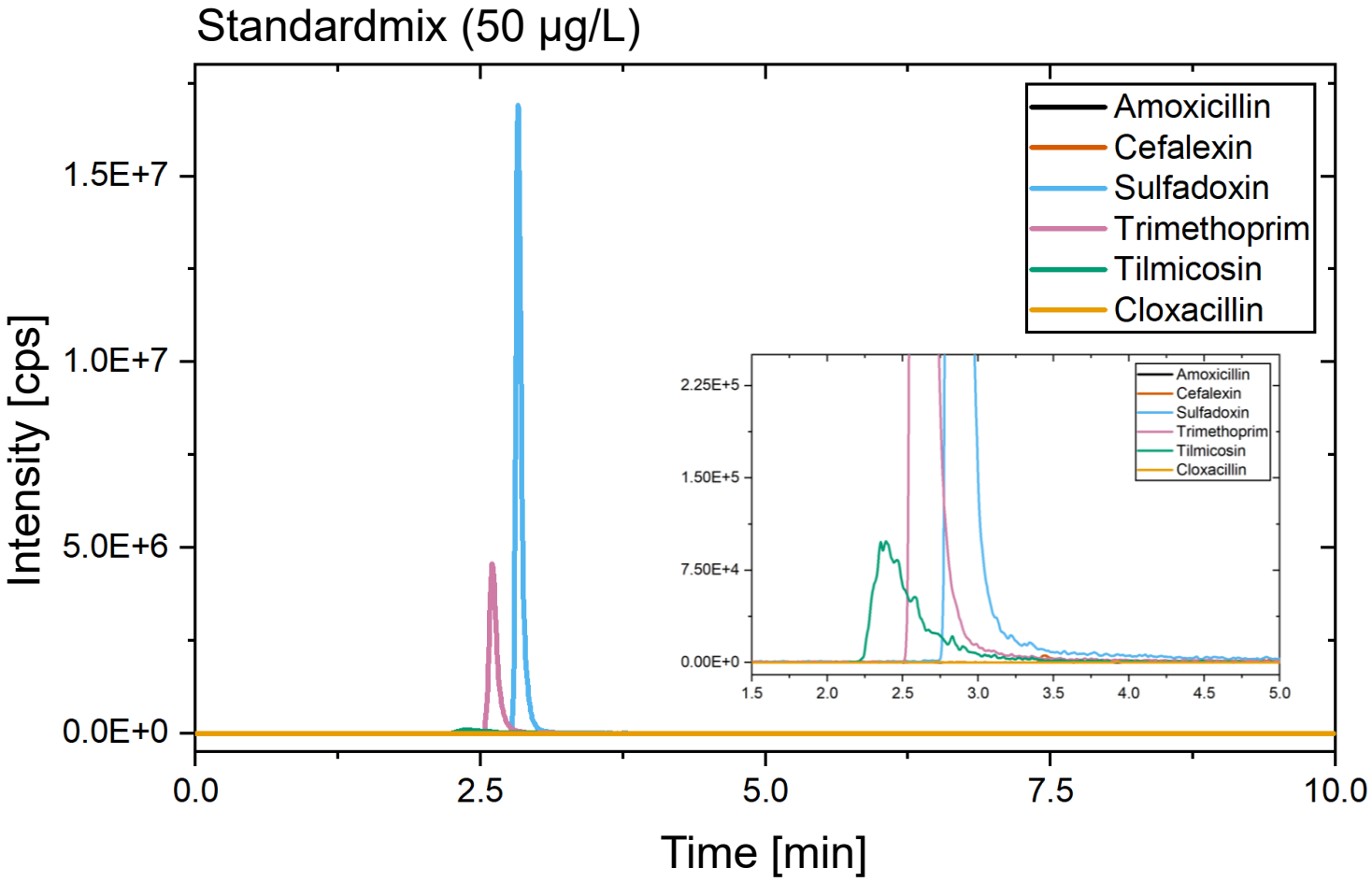
MS/MS optimization



Analyte	ESI mode	Parent ion [*m/z*]	Product ion [*m/z*]	CE [eV]
Amoxicillin	+	366.00	208.10	15
Cefalexin	+	348.00	157.90	12
Sulfadoxine	+	311.00	156.00	18
Trimethoprim	+	291.05	230.10	25
Tilmicosin	+	869.60	174.20	47
Cloxacillin	+	436.10	276.90	?

SFC-MS/MS; standard measurement

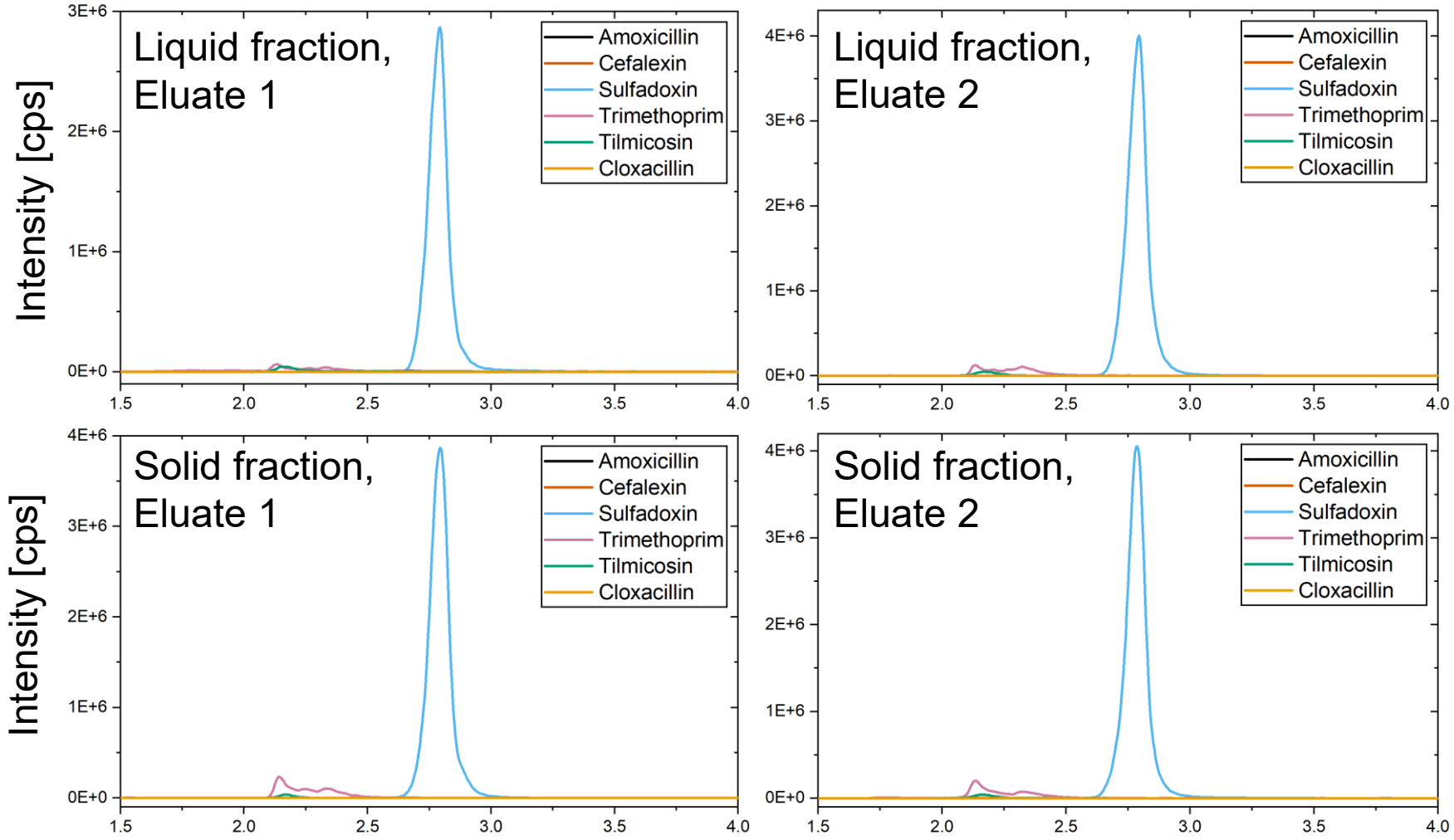




- Higher modifier percentage doesn't elute amoxicilline and cefalexin
- Cloxacillin peak missing & loss of baseline separation

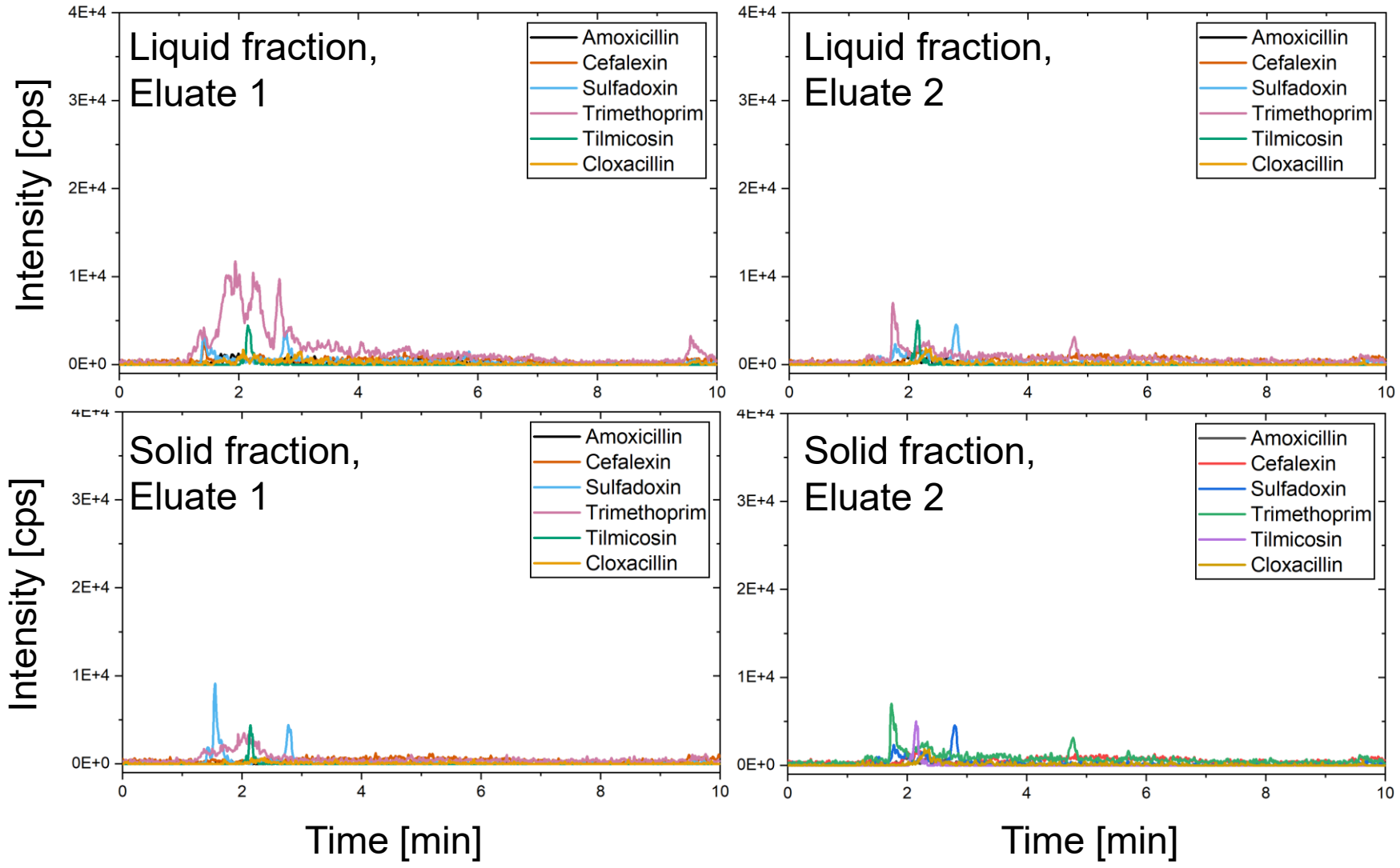
SFC-MS/MS; spiked eluates

Spiking SPE eluates with Standardmix (50 µg/L)



- Analytes affected differently by matrix → matrixed-matched calibration required

SFC-MS/MS; Eluates



- Preliminary analysis revealed no significant analytical response

Column screening - parameter

PyE:
Pyrenylethyl group

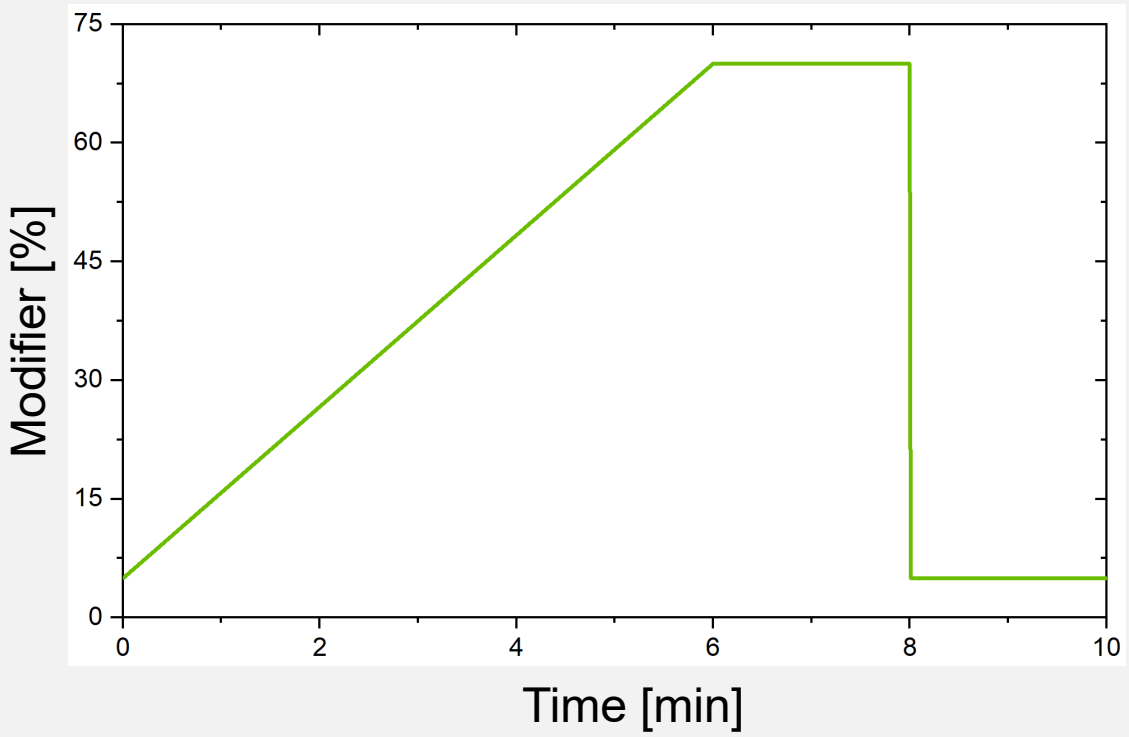
PolyBT:
Polybutylene terephthalate
(coated on silica gel)

HyP:
3-hydroxyphenyl group

Py:
Pyridinyl group

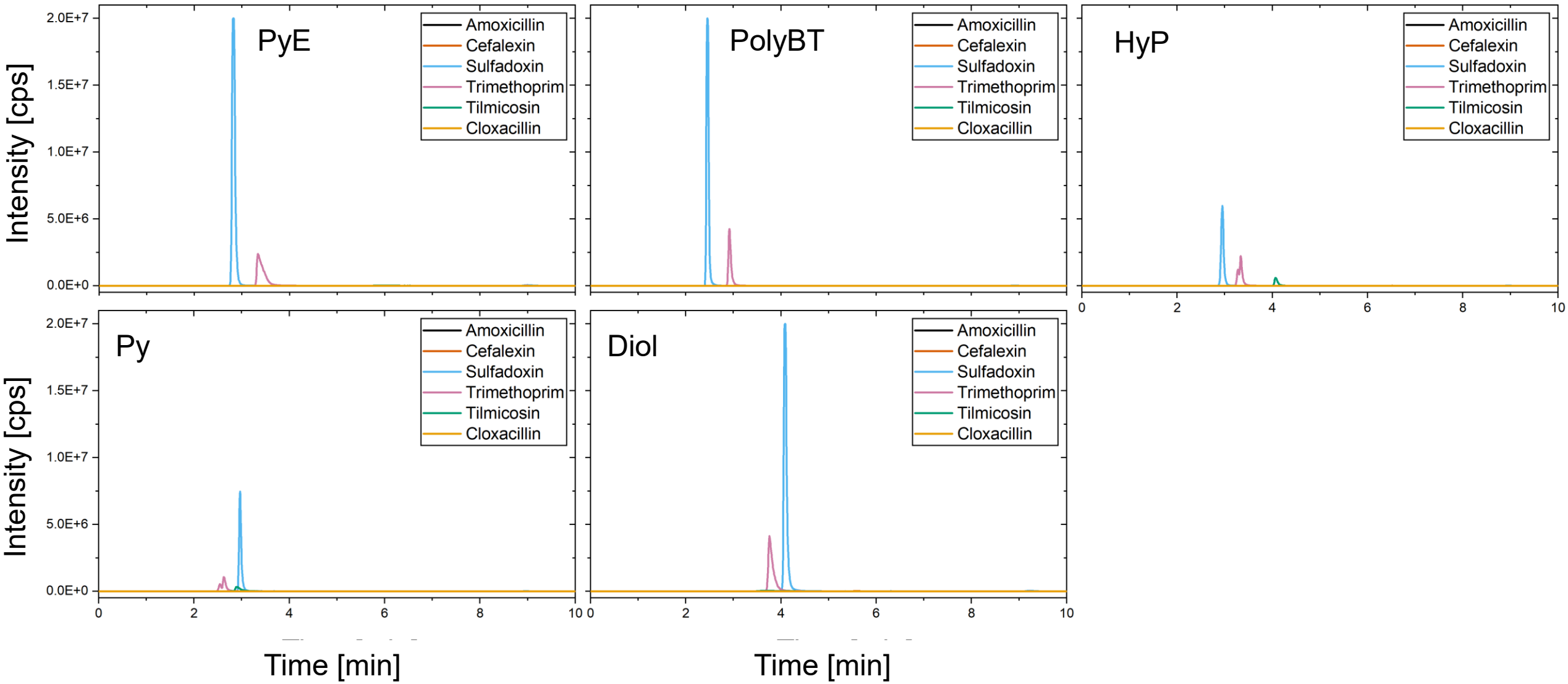
Diol

Non-polar → Polar

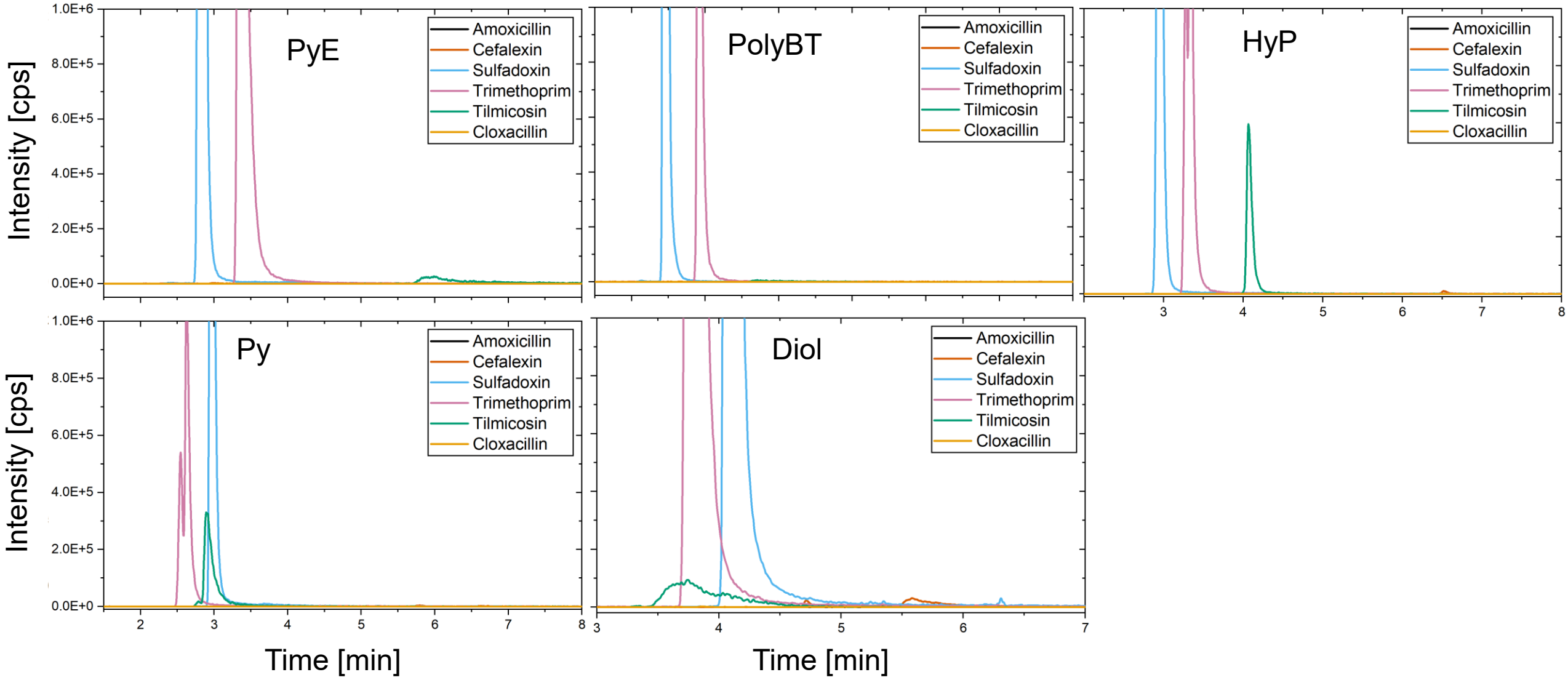


Flow rate: 1 mL/min,
PolyBT 1.5 mL/ min

Column screening - results



Column screening – results (zoom in)



Conclusion & Outlook

- ✓ SFC-UV/VIS: Rapid separation of four antibiotics
- ✗ Amoxicillin and Cefalexin could not be eluted within the scope of the method
- 🔍 Check for ionization of ß lactam antibiotics
- ! Analytes affected differently by matrix → matrix-matched calibration required
- ✓ 3-hydroxyphenyl- functionalized column as promising alternative to diol column

→ Further method optimization SFC-MS/MS, including screening of additional columns

→ Transferring method to additional antibiotics

→ Evaluating and optimization of extraction

→ Comparison to LC-MS/MS (greenness)

Interreg
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Thank you for your attention!