pharm-analyt Labor GmbH

Your GxP Partner Lab/CRO for Sensitive and Selective Assays for Drugs and Metabolites in Biological Matrix and Detergents/Excipients in Drug Product/Substance

pharm-analyt Labor GmbH

accompanies

the entire pharmaceutical life cycle

from

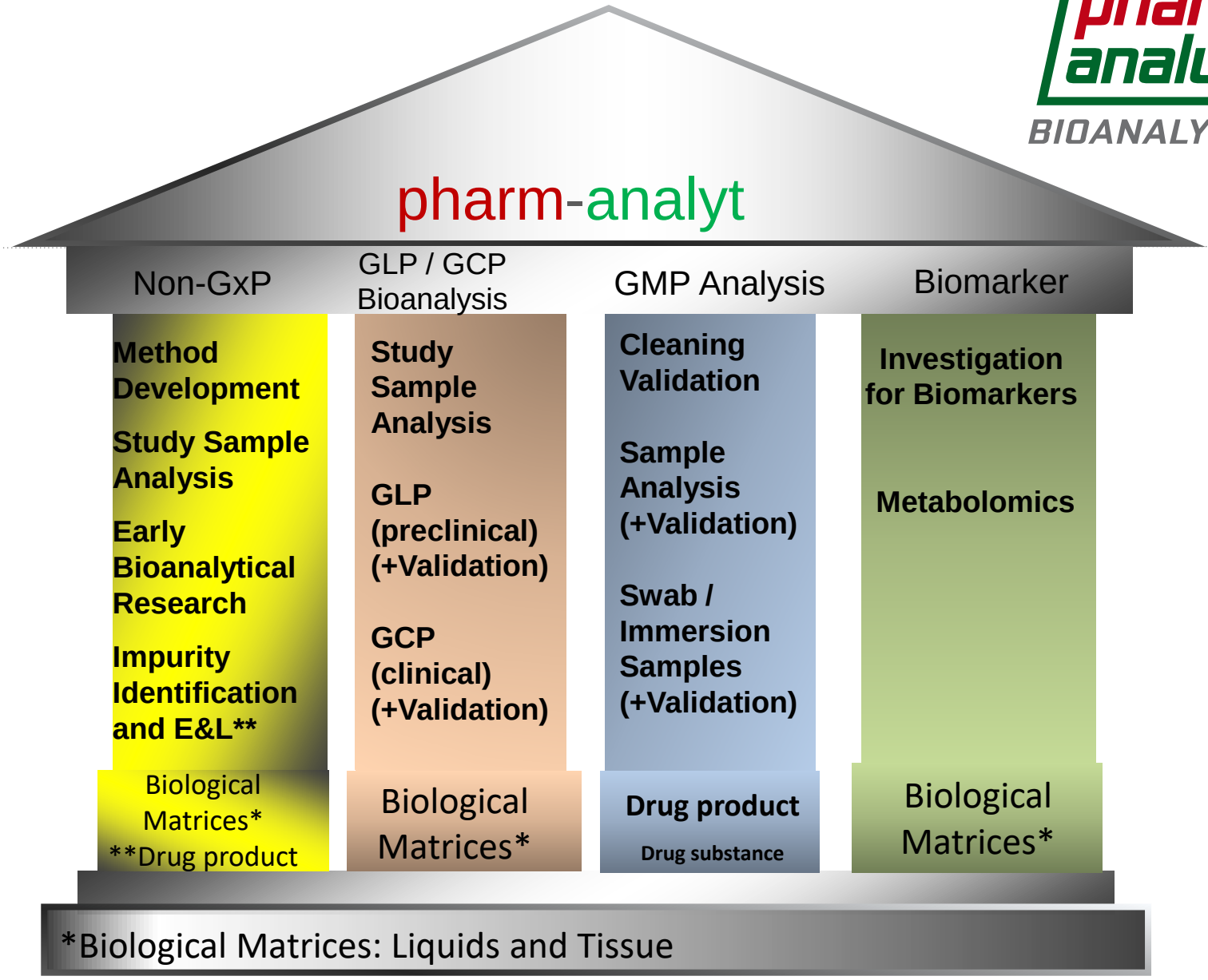
- **early bioanalytical research** to the
- **regulated area (GLP and GCP)** with preclinical and clinical analysis to
- assays for **pharmaceutical production and batch release (GMP)**

Milestones – pharm-analyt

1986	Founded by Hermann Mascher Specialized Bioanalytical Service Provider, now in Baden close to Vienna, Austria, EUROPE
1992	GLP Certification
1998	First HPLC-MS/MS system (AB Sciex API3000), → 25 years of Tandem Mass Spec Experience
2006	GMP Certification
2019	More sensitive Mass Spec for small molecules and peptides with Waters XEVO TQ-XS additionally to API6500 from AB Sciex

pharm analyt
BIOANALYSIS

pharm-analyt



Equipment – HPLC-MS/MS-Systems

HPLC/UPLC: Shimadzu (400 bar), Thermo (600 bar), Waters (1000 bar)

MS Ionisation:
- ESI (electrospray ionization)
- APCI (Atmospheric-pressure chemical ionization)
- UniSpray

MS/MS
SCIEX
QTRAP 6500
+SelexION

GxP

MS/MS
Waters
Xevo TQ-XS

GxP

Ion-Trap-Mass Spectrometer
Thermo Fisher
LTQ Orbitrap XL

Resolution up to 100,000
Mass accuracy 0,00X of MW

Equipment – Other Detectors with HPLC

Thermo Fisher

HPLC UltiMate 3000 Systems with

GxP

GxP

UV Detector
Merck-Hitachi 7400

GxP

Fluorescence Detector
Shimadzu RF-20A

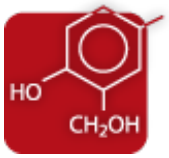
GxP

ELSD Detector
SEDEX 90LT

CRITICAL ASSIGNMENTS

WISELY LEFT TO EXPERTS

QUANTIFICATION



IDENTIFICATION

INVESTIGATION

BIOMARKERS

Could we be of Service?

Core Competencies

- **Polymers in Biological Matrix and Drug Substance/Product**
- **Small Molecules in Biological Matrix**
- **Detergents/Excipients in Pharmaceutical Production (GMP and non-GMP)**
- **Swab/Immersion Samples (GMP and non-GMP)**
- **Impurity ID / E&L**

Core Competencies

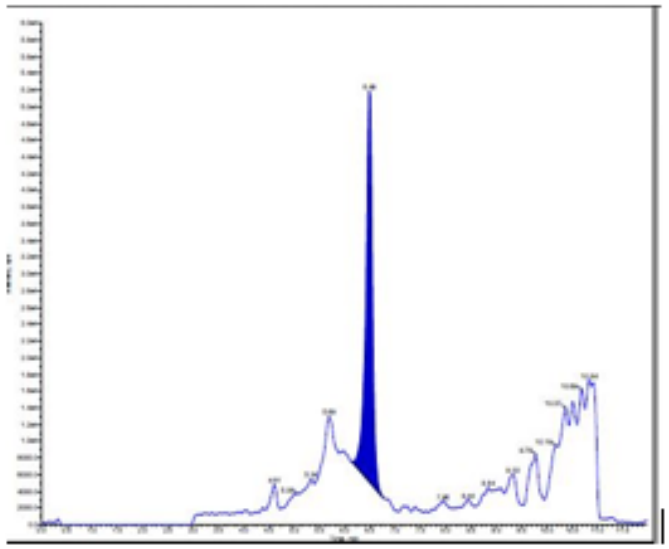
Polymers in Biological Matrix and Drug Substance/Product

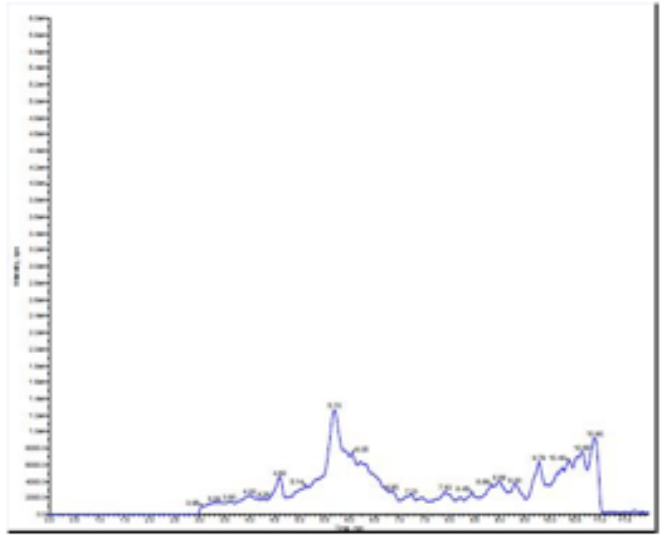
Polyethylene Glycol (PEG)

Free and Total PEG in Liquids (e.g. Plasma, CSF), various Tissues and Pharmaceuticals

Range for PEGs between 4 kDa and 60 kDa
Lowest validated LLOQ 15 ng/mL in Plasma

More than a decade of experience with clients from Biotech to Big Pharma





Free PEG in Plasma *)

Calibration Range
20.0 – 1,000 ng/mL
LLOQ – LLOQ x 50 (=ULOQ)
in 8 calibration standards

Upper Chrom Std at LLOQ
Lower Chrom Plasma blank

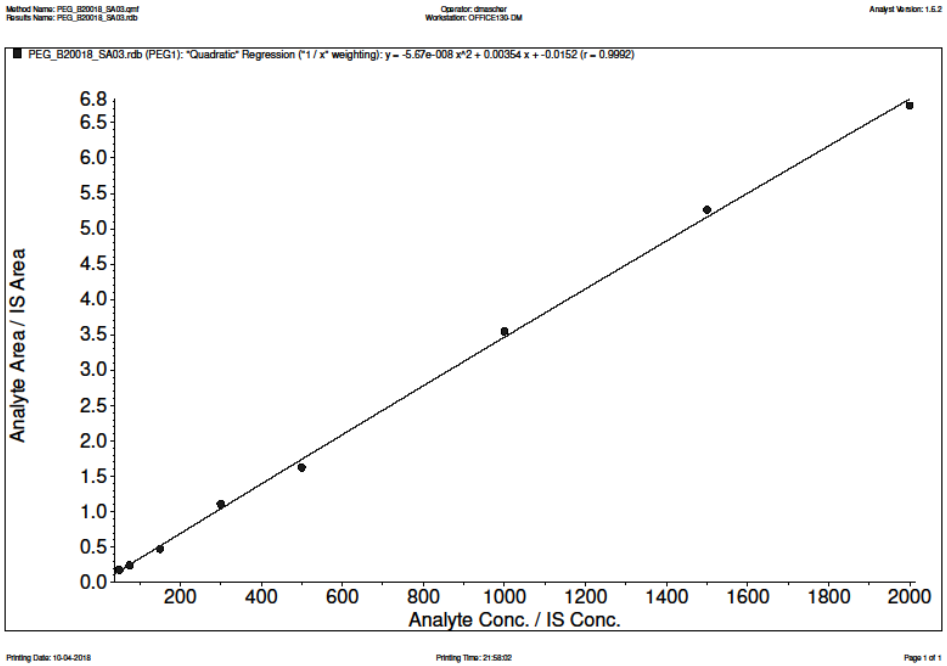
HPLC-MS/MS

Sample Volume for Prep. 50 µL

*) > 30 kDa

Free PEG > 30 kDa in CSF

Calibration Range 50.0 – 2,000 ng/mL
LLOQ – LLOQ x 40 (=ULOQ) in 8 calibration standards



Randomly branched PEGs: No immunogenic response

Calibration Range 15.0 – 1,000 ng/mL for 30 kDa branched PEG

4 available randomly branched PEGs with various chain lengths and various degree of branching

- **RP HPLC-MS/MS** could be used for all 4 of them, either after protein precipitation alone or after protein precipitation followed by liquid-liquid extraction
- **SEC HPLC-MS/MS** could be used for all 4 of them, only after protein precipitation followed by liquid-liquid extraction. Strong matrix effects result if protein precipitation is used alone
- **Randomly branched PEGs** can be a great alternative for pegylation of APIs that were previously pegylated with linear PEGs only. Randomly branched PEGs do not trigger an immune response, making them very unique and far superior to linear PEGs

Other Polymers

Polylactic Acid (PLA), LLOQ 50 ng/mL
with good linearity for calibration range in Plasma

Polyethyloxazoline (PEtOx),
LLOQ 2 ng/mL for a 5 kDa PEtOx in Plasma

Polyethylenimine (PEI)
LLOQ 1 µg/mL in Drug Substance

Poloxamer (POL)
LLOQ 0.7 µg/mL in Drug Substance and Drug Product

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Core Competencies

Small Molecules in Biological Matrix

LLOQ Record FP in 2012 (250 fg/mL plasma)

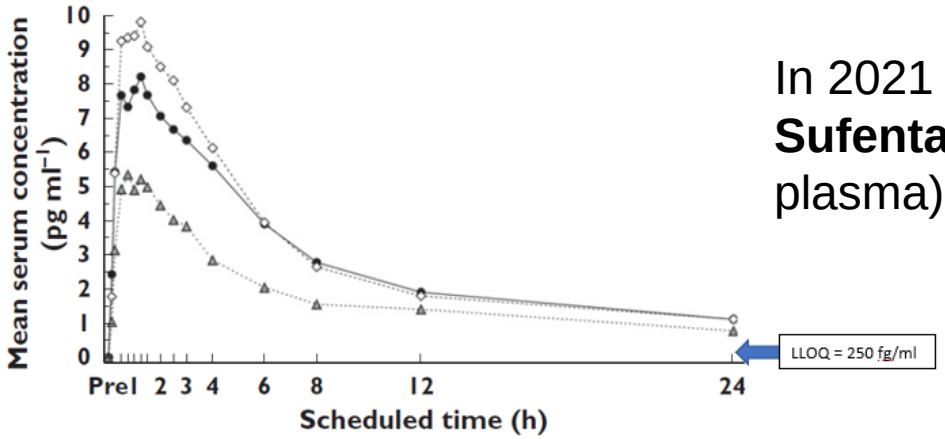


Figure 1

Study 1: Mean fluticasone propionate (FP) serum concentration–time curves after intranasal single dose administration of 200 µg FP delivered by three different nasal spray products; ● MP29-02; ◇ MP29-02-FP-mono (i.e. MP29-02 formulation without azelastine); △ Marketed comparator product FP-BI (Fluticasone propionate Boehringer-Ingelheim/Roxane Laboratories Nasal Spray)

In 2021 record equalled for **Sufentanil** (using only 0.1 mL plasma)

Publication:

Bioavailability and disposition of azelastine and fluticasone propionate when delivered by MP29-02, a novel aqueous nasal spray

Hartmut Derendorf,1 Ullrich Munzel,2 Ursula Petzold,2 Joachim Maus,2 Hermann Mascher,3 Robert Hermann,4 & Jean Bousquet,5

1Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA,

2MEDA Pharma GmbH & Co. KG, 61352 Bad Homburg, Germany, 3pharm-analyt Labor GmbH, 2500

Baden/Vienna, Austria, 4cr.appliance, Rossittenstrasse 15, 78315 Radolfzell, Germany and 5Hopital

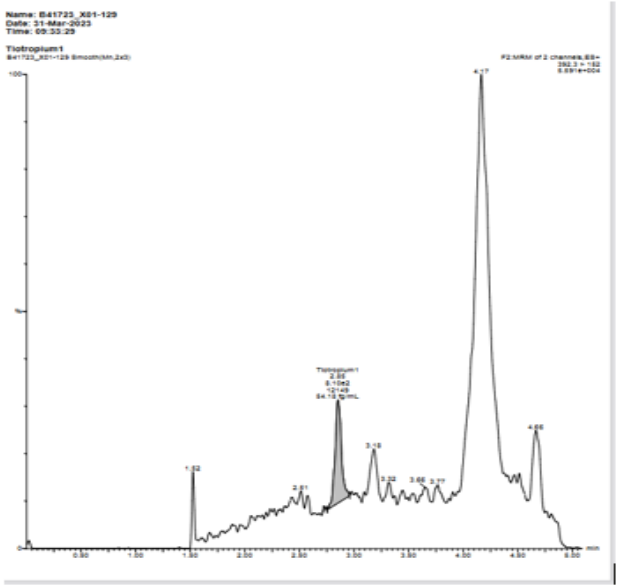
Arnaud de Villeneuve University Hospital, Montpellier, and Inserm CSP1018, France

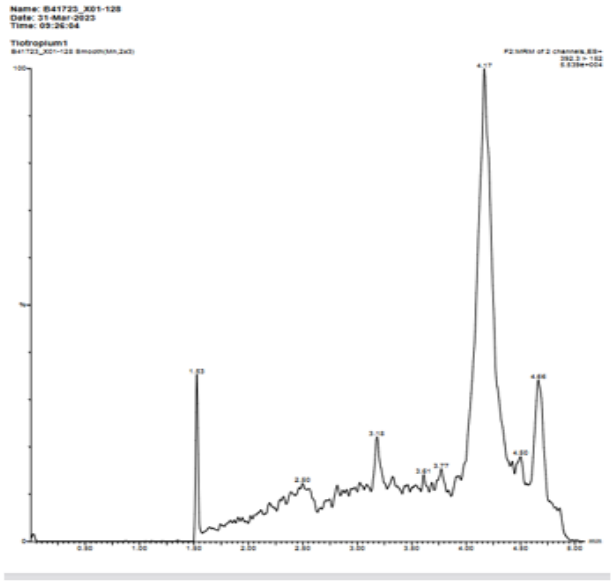
pharm analyt BIOANALYSIS

APPI: Expanding the horizon to very apolar compounds

An APPI source was purchased for the Xevo TQ-XS providing:

- **Improved sensitivity** even for **medium-polar substances** such as budesonide, fluticasone propionate and fluticasone furoate by up to a **factor of 5**
- **Access to the field of (aromatic) hydrocarbons and heteroaromatic compounds** which are either not commonly detectable with other HPLC-MS/MS ion sources or lack in ionization efficiency when using e.g. APCI
- **Easy handling of dopant delivery** (acetone, toluene, anisole...)
- Hence selective ionisation possible due to ionisation energies of common mobile phase constituents and additives being above the delivered energy of the used UV lamp





2023 Record for Tiotropium

Calibration Range
50.0 – 8,000 **fg/mL**
LLOQ – LLOQ x 160 (=ULOQ)
in 8 calibration standards

Upper Chrom Std at LLOQ
Lower Chrom Plasma blank

HPLC-MS/MS

Sample Volume for Prep. 1 mL

Experience with various peptides in plasma in the molecular weight range of 1 to 6 kDa

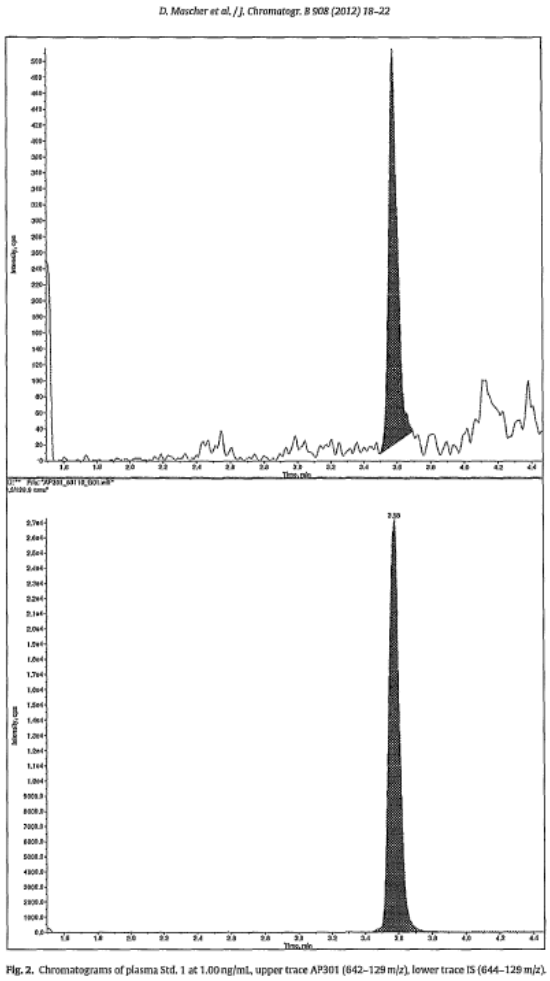


Fig. 2. Chromatograms of plasma Std. 1 at 1.00 ng/mL, upper trace AP301 (642–129 m/z), lower trace IS (644–129 m/z).

Journal of Chromatography B, 908 (2012) 18-22

Sensitive determination of the peptide AP301 – A motif of TNF-α – From human plasma using HPLC-MS/MS

Daniel Mascher et al.

Molecular weight 1923.1 amu for AP301

Upper chromatogram at LLOQ (= 1 ng/mL plasma)

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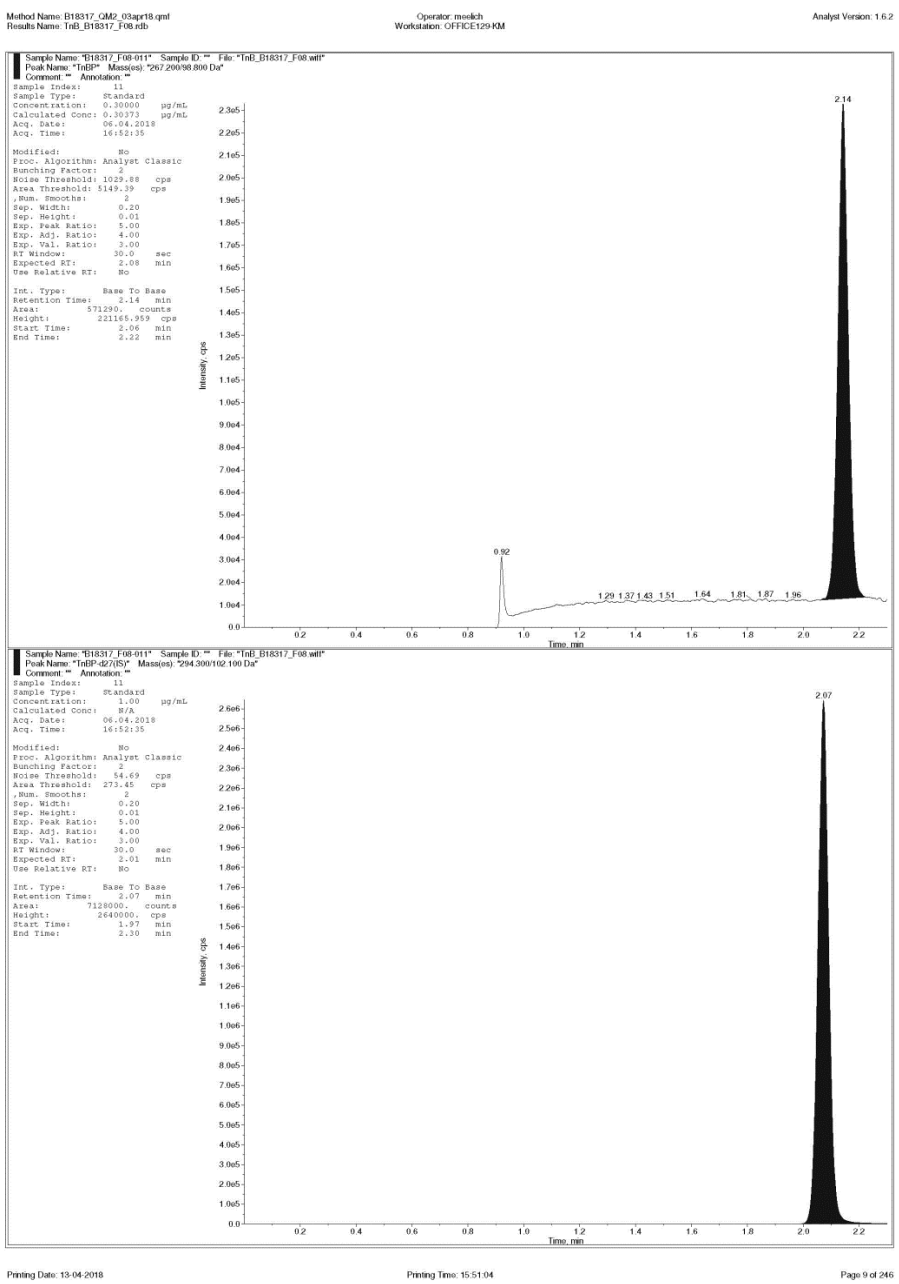
Core Competencies

Detergents/Excipient in Pharmaceutical Production (GMP and non-GMP)

Detergents: Triton, Tween/PS, TnBP, Poloxamer, PEG …
Excipients: Sorbitol, Cyclodextrin…

Fast Turnaround Time (maximum of 4 weeks) with 2 Qualified Persons at pharm-analyt



TnBP in FDP/FC

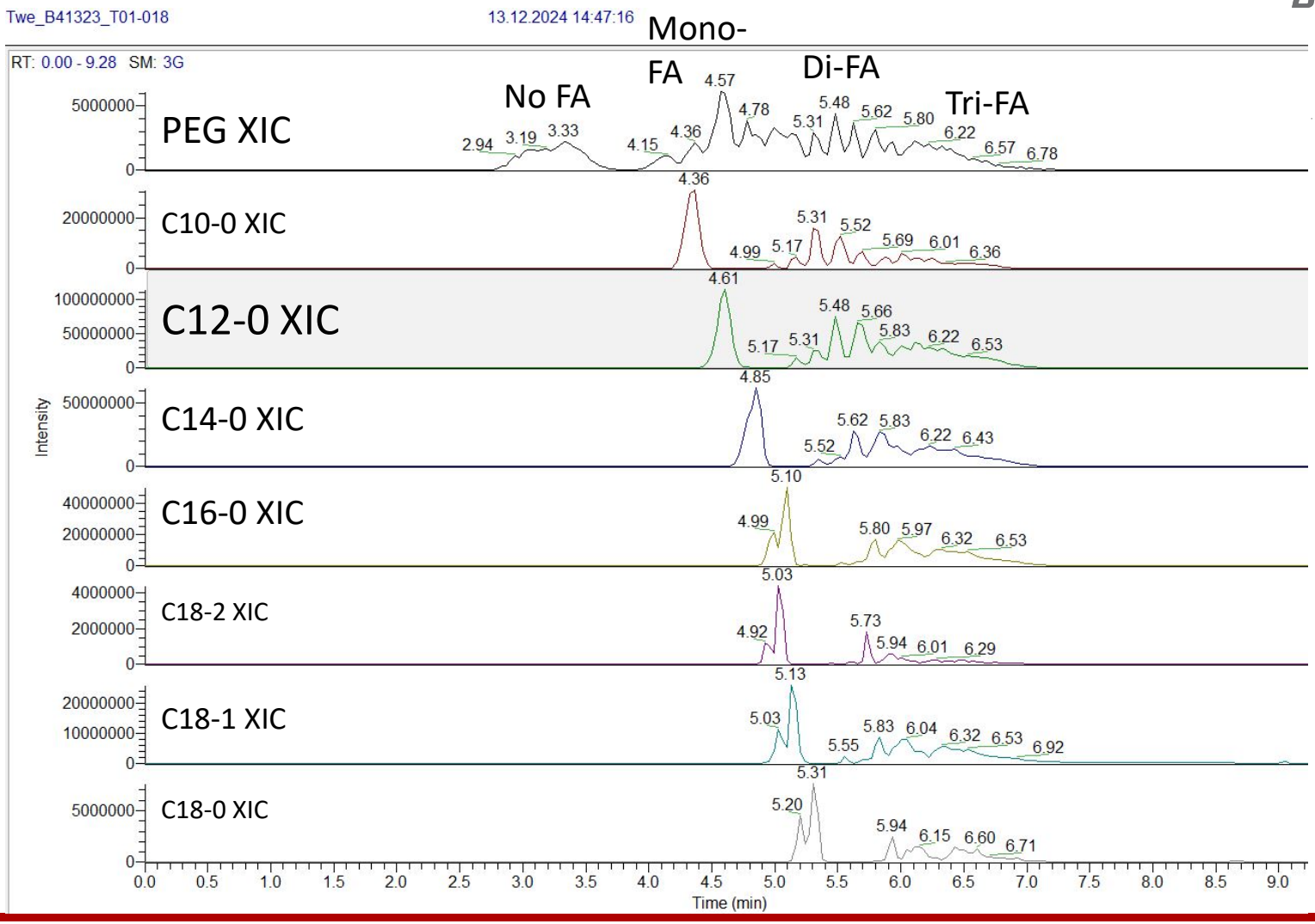
LLOQ 100 ng/mL

Upper Chrom Std at LLOQ

Lower Chrom Internal Standard

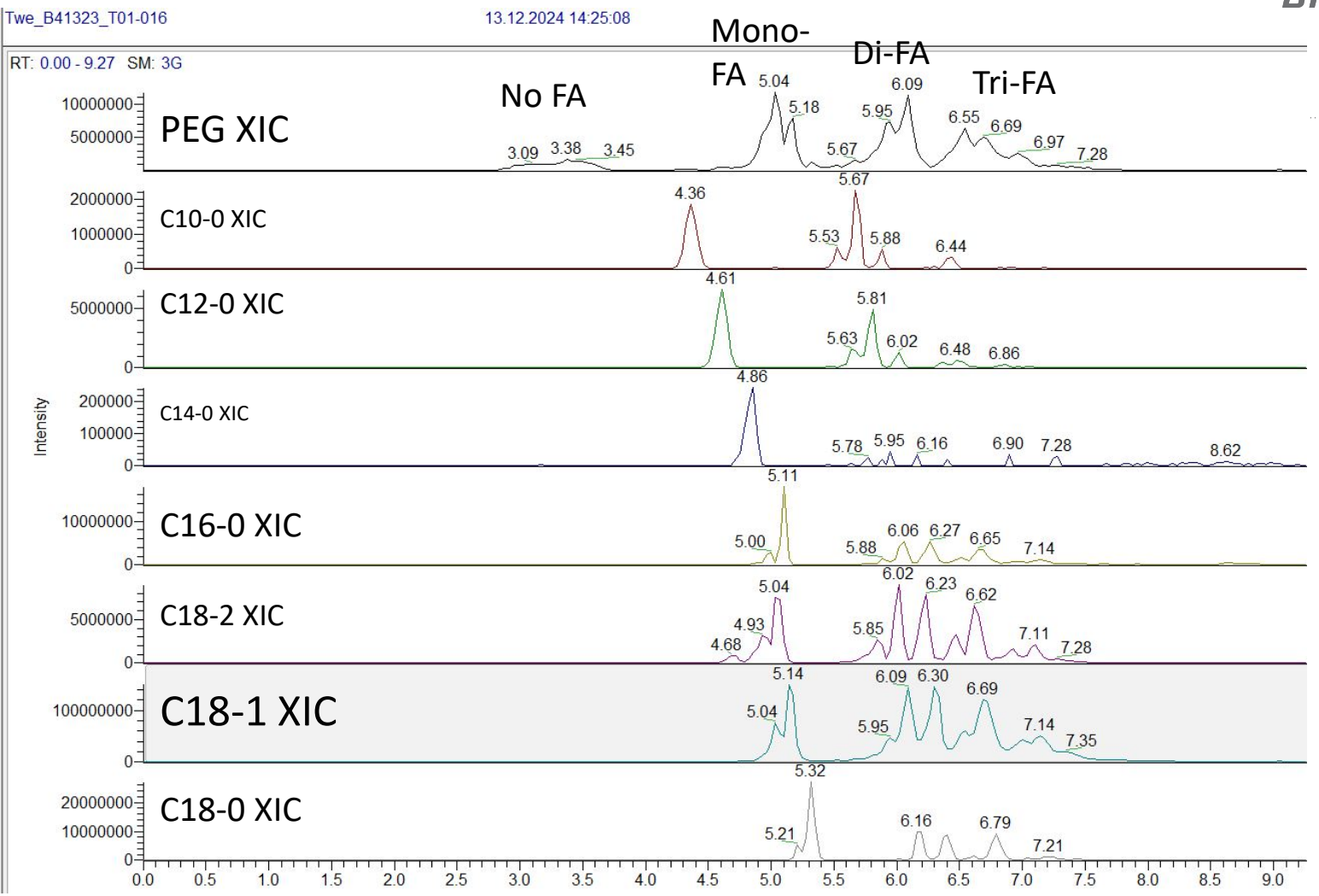
HPLC-MS/MS

Tween 20



Data generated on Orbitrap SID @80V, in order to see respective fragment ions typical for PEGs (upper trace) and some of the fatty acid residues

Tween 80



Data generated on Orbitrap SID @80V, in order to see respective fragment ions typical for PEGs (upper trace) and some of the fatty acid residues

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Core Competencies

Swab/Immersion Samples (GMP and non-GMP)

Analytes with LLOQ in ng/mL (depending on ng/100 cm² criterion of cleaning validation), e.g.:

Cyclosporin A: 4 ng/ml

Testosteron Enanthate: 1 ng/ml

Fulvestrant: 2 ng/ml

Palonosetron: 2 ng/ml

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Core Competencies

Impurity ID with Ion-Trap-Mass Spectrometer (Non-GxP)

You detect in your pharmaceutical product an

- **Unknown peak**
- **unexpected color during manufacturing etc.**

We deliver:

- a proposal for a **molecular formula**
- a proposal for a structure
- or the ID of a **molecular structure**

within 1-4 days incl. exemplary chromatograms

the success of the detection depends on the hypothesis, MS-compatible buffers and the size of the molecule

pharm-analyt has started with **E&L** in 2023 (e.g. tubes, bags)

Inorganic Excursion: Cations and Anions

Determination on a Trinity column with CAD, ELSD and Orbitrap

Anions determined were e.g. chloride, bromide, iodide, nitric acid, nitrous acid, perchloric acid, methane sulfonic acid, sulfuric acid, phosphoric acid and citric acid.

- CAD was more sensitive than ELSD, **LLOQ in low µM range achievable** for some compounds
- **Orbitrap** provided of course **more selectivity, plasma analysis possible** with it**, LLOQ** down to **mid nM range** achievable for some

Cations determined were e.g. lithium, sodium, potassium, cesium, magnesium, calcium, barium, manganese, silver, cobalt, zinc, iron(II), cerium:

- Comparable results as for anions in terms of sensitivity and selectivity
- **Monovalent ions are more readily detectable** and easier in chromatography

Nitrite/Nitrate/Nitrosamine Determination

On XEVO TQ-XS and with Electrochemical Detection (EC)

1. pharm-analyt was involved in NDMA and related substance determination in the first Nitrosamine „Wave“ in 2019. Some products indeed showed critical levels of NDMA in regards to daily intake.
2. In 2022/2023 the second Nitrosamine „Wave“ followed where the actual API specific Nitrosamines got into the regulator’s focus with us also being involved in this phase analytically.
3. Since nitrite is the direct precursor regarding Nitrosamine formation we looked at determination of it.
4. Nitrite is unfortunately a very small molecule not ideal for LC-MS/MS determination with its mass of 46 amu only (poor ion transmission and poor fragmentation). With the XEVO TQ-XS about 200 ng/L could be achieved using UNISpray, however nitrate – which is ubiquitous, in pipette tips, solvents etc. – interferes since it degrades in the ion source of the mass spec to nitrite. So nitrate-free environment would be needed.
5. Nitrite however is perfect when using EC Detection. Nitrate is practically not oxidizable and shows only at about 0.05 %. 50 ng/L nitrite could relatively easily be achieved as LLOQ without much optimization.

PUBLICATIONS – pharm-analyt

Over 100 Publications in Peer-Reviewed Journals

4 Representative Publications of the Last Years

- **Lethal versus surviving sepsis phenotypes displayed a partly differential regional expression of neurotransmitters and inflammation and did not modify the blood–brain barrier permeability in female CLP mice**
  Author: F. Azizian-Farsani, K. Weixelbaumer, D. Mascher, A. Klang, S. Högler, N. Dinhopl, B. Bauder, H. Weissenböck, A. Tichy, P. Schmidt, H. Mascher, M. F. Osuchowski
  Publisher: Intensive Care Medicine Experimental volume 12, Article number: 96 (2024)
- **Letermovir in Paediatric HSCT Recipients**
  Author: V. Strenger, D. Sperl, K. Kubesch, J. Donnerer, W. Schwinger, K. Zach, H. Lackner, M. Benesch
  Publisher: Journal of Antimicrobial Chemotherapy, October 2019, 1093
- **Effect of Particle Deposition Density of Dry Powders…by an In Vitro Test System Simulating Dissolution – and Absorption Rates in the Lungs**
  Author: M. Malmlöf, M. Nowenwik, K. Meelich, I. Radberg, E. Selg, J. Burns, H. Mascher, P. Gerde
  Publisher: European Journal of Pharmaceutics and Biopharmaceutics, 2019, 139, 213-223
- **A FIM Study to Assess Safety and Exposure of Inhaled Single Doses of AP301 – A Specific ENaC Channel Activator for the Treatment of Acute Lung Injury**
  Author: R. Schwameis, S. Eder, H. Pietschmann, B. Fischer, H. Mascher, S. Tzotzos, H. Fischer, R. Lucas, M. Zeitlinger, R. Hermann, Publisher: J. Clin. Pharmacol, 2013, 54, 341-350