NORSK SELSKAP FOR MASSESPEKTROMETRI

18. Norske symposium i massespektrometri

Digitalt
25. januar 2021

Norsk Selskap for Massespektrometri (NSMS)
Norwegian Society for Mass Spectrometry

Stine Göransson Aanrud, Hanne Devle, Susana Villa Gonzalez, Terkel Hansen,
Jan Arild Hustad, Lene Hop Johannessen, Leon Reubsaet, Åse Marit Leere Øiestad,

Innholdsfortegnelse

NORSK SELSKAP FOR MASSESPEKTROMETRI

Hjertelig velkommen til “NSMS goes digital!”

Velkommen til det 18. norske seminar i massespektrometri. Dette blir et unikt møte siden vi møtes digitalt. Det blir annerledes på flere måter: det blir kortere enn vanlig, ingen (after)ski, ingen utstilling, ingen kakao foran peisen. Allikevel kan vi by på et spennende program med fokus på fag, med presentasjoner og postere samt nyheter fra våre leverandører.

Dette er tredje gang jeg er med på å arrangere dette møte og det har vært en fin utfordring. Vanligvis kunne vi i arrangementskomiteen “surfe” litt på det gode grunnarbeidet som er gjort av de som har arrangert møtet før, dette kunne vi ikke denne gangen. I august/september 2020 tok beslutningen om å gjennomføre det 18. norske seminar i massespektrometri digitalt. Det var ingen lett beslutning siden vi ikke kunne spå framtiden da. Nå er vi veldig glad for at vi gikk for denne beslutningen og at vi har fått så mange påmeldinger.

Programmet er begrenset til én dag med foredrag og postere. Vi har fått til en god blanding av temaer med foredragsholdere fra hele landet. Leverandørene har mulighet til å vise det siste som er av interesse for massespektrometrister.

Vi sees på Zoom til dette 18. norske seminar i massespektrometri.

Vel møtt!

Léon Reubsaet
På vegne av arrangementskomiteen

Monday 25/1/2021

Time	Speaker	Title
0900-0910	Leon Reubsaet	***Opening***
0910-0940	**Andreas Thomas**	New challenges in doping controls
0940-1000	Per Ole Gundersen	Retrospective screening of new psychoactive substances in data files from forensic post mortem samples analysed by LC-QTOF-MS
1000-1015		***Digital break***
1015-1035	Fredrik Hansen	Electromembrane extraction of streptomycin from biological fluids prior to hydrophilic interaction liquid chromatography – tandem mass spectrometry
1035-1055	Sandra Huber	Automated sample preparation and atmospheric pressure GC-MS/MS as a powerful tool for analysis of legacy POPs in human serum and plasma
1055-1200		The last news from our vendors. Short and exciting elevator pitches.
1200-1245		***Digital break and poster session***
1245-1315	**Alexandros Asimakopoulos**	Supercritical fluid and liquid chromatography methods for rapid trace analysis of emerging pollutants and biomolecules
1315-1345	**Nicholas Warner**	Advantages and Challenges in GC-Orbitrap high resolution mass spectrometry for trace analysis of legacy and emerging contaminants
1345-1400		***Digital break***
1400-1430	Tuula Nieman	An Introduction to the National Network of Advanced Proteomics Infrastructure (NAPI)
1430-1450	Christine Olsen	Disulfide bond reduction upfront liquid chromatography mass spectrometry for protein analysis
1450-1510	Joakim Samuel Jestilä	GCxGC QTof for the Analysis of Complex Environmental Samples
1510-1520	Léon Reubsaet	***Closing of meeting***

Tusen takk til leverandørene av utstyr og tjenester til arbeid innen massespektrometri som har bidratt med deltagelse i årets møte.

NORSK SELSKAP FOR MASSESPEKTROMETRI

Kjære MS-kollega!

Norsk Selskap for Massespektrometri (NSMS) er en nasjonal forening for de som har interesser innen massespektrometri. Vi er et frittstående selskap, men er medlem av International Mass Spectrometry Foundation (IMSF). NSMS ble stiftet 6. april 1973, og har som formål å styrke det faglige miljøet innen MS i Norge. Dette gjør vi blant annet ved å arrangere møter for å bidra til økt faglig kontakt og samarbeid, både nasjonalt og internasjonalt.

Vi avholder nasjonale seminarer i MS annethvert år. Her kombinerer vi det faglige med det sosiale. På den måten får vi en fin og hyggelig atmosfære rundt det hele, og nye kontakter dannes mens gamle pleies. Det aller første møte ble holdt på Ustaoset høyfjellshotell i 1982. Etter Ustedalen og Geilo kom møtet til Hafjell der det 10. norske seminar i massespektrometri ble holdt. Hafjell har siden da vært vert inntil 2019. Fra 2023 blir det sannsynligvis et annet hotell vi skal være på.

I tillegg ønsker vi å gi yngre forskere muligheter til å delta i nasjonale og internasjonale MS-møter gjennom reisestipend via Georg Hvistendahl minnefondet. Til det 17. norske seminar i massespektrometri fikk 10 studenter bruke seg av denne muligheten. Vi håper at vi kan utvikle og utvide denne muligheten framover slik at enda flere kan oppleve gleden av å delta på et faglig MS-møte.

Tirsdag 26. januar 2021 avholder NSMS sin generalforsamling digitalt (Zoom). Vi oppfordrer alle våre medlemmer til å delta. Informasjon om dette kommer på våre hjemmesider.

Mvh
Léon Reubsaet
Leder NSMS

Abstrakt

Foredrag
NSMS 18

New challenges in doping controls

Andreas Thomas, Mario Thevis

Institute of Biochemistry / Center for Preventive Doping Research, German Sport University Cologne, Germany

Abstract

Modern mass spectrometry is frequently used in doping control analysis in all accredited laboratories all over the world due to its potential to provide ultimate reliable and specific results. Although the list of prohibited substances of the World Anti-Doping Agency (WADA) comprises a plethora of drugs with highly variable physico-chemical properties (small molecules, peptides, proteins, RNA etc.) the doping control laboratories aim (if possible) to identify these drugs or their metabolites by means of mass spectrometry. Noteworthy, all kinds of mass spectrometers (e.g. high/low resolution instruments, such as Orbitrap, Time-of-Flight, Triple Quadrupole, etc.) and different couplings (mainly LC and GC) are utilized in this field. Within this presentation, several recent aspects and challenges are presented, which will focus on mass spectrometric topics. Especially, the search for new and relevant peptidic metabolites by means of a sophisticated stable isotope-labeled reporter ion screening [1] and also first steps towards the detection of gene-doping attempts with CRISPR/Cas9 [2] will be included.

References

[1] Thomas A, Thevis M., Identification of metabolites of peptide-derived drugs using an isotope-labeled reporter ion screening strategy. **Clin Chem Lab Med.** 2020 Apr 28;58(5):690-700. doi: 10.1515/cclm-2019-1009.

[2] Paßreiter A, et. al., First Steps toward Uncovering Gene Doping with CRISPR/Cas by Identifying SpCas9 in Plasma via HPLC–HRMS/MS, **Anal. Chem.** 2020, DOI: 10.1021/acs.analchem.0c04445

Retrospective screening of new psychoactive substances in data files from forensic post mortem samples analysed by LC-QTOF-MS

Per Ole M. Gundersen[a], Sebastian Broecker[b], Lars Slørdal[c], Olav Spigset[b,a], Martin Josefsson[d,e]

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New psychoactive substances (NPS) are emerging in the illegal drug market, which has led to major challenges for analytical laboratories. Keeping screening methods up to date with all relevant drugs is hard to achieve and the risk of missing important findings in biological samples is a matter of concern. A strategy for extended retrospective data analysis of data files acquired by LC-QTOF-MS was developed. Diagnostic fragment ions from synthetic cannabinoids (n=251), synthetic opioids (n=88), and designer benzodiazepines (n=26) were obtained from the crowdsourced database HighResNPS.com and converted to a personalized library containing spectra in a format compatible with the analytical instrumentation. Data files from the analysis of 1314 forensic post mortem samples with an Agilent 6540 LC-QTOF-MS performed in our laboratory from January 2014 to December 2018 were retrieved and retrospectively processed with the new personalized library. Potentially positive findings were grouped into category 1 (most confident) and category 2 (less confident) depending on the information available in the files. Five new findings of category 1 were identified: flubromazepam in two data files from 2015 and 2016, respectively, phenibut (4-amino-3-phenylbutyric acid) in one data file from 2015, fluorofentanyl in one data file from 2016, and cyclopropylfentanyl in one data file from 2018. Retention time match with reference standards further strengthened these findings. One category 2 finding of phenibut was considered plausible after the checking of retention times and signal-to-noise ratios, whereas 34 other potentially category 2 findings were refuted after a closer evaluation. This study showed that new compounds can be detected retrospectively in data files from LC-QTOF-MS using an updated library containing diagnostic fragment ions.

Electromembrane extraction of streptomycin from biological fluids prior to hydrophilic interaction liquid chromatography – tandem mass spectrometry

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Streptomycin is an aminoglycoside antibiotic used against gram-negative bacteria, such as tuberculosis. The chemical structure is composed of amino-modified glycosides. Streptomycin is thus highly polar, with a log P value of -7.6, and log D of -12.0 at physiological pH. Efficient and selective sample preparation from complex biological samples is therefore challenging. In the present presentation, purification of streptomycin from urine and human plasma using electromembrane extraction (EME), prior to hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry (HILIC-MS/MS), is proposed. EME is a technique based on electrokinetic migration of charged analyte species across a hydrophobic supported liquid membrane (SLM). The hydrophobicity of the SLM enables selective extraction of non-polar substances, but it represents a great challenge when the analyte of interest is polar. To enable extraction, the SLM chemistry therefore has to be modified. In addition, it may be difficult to get selective extraction of a polar analytes from a polar matrix.

The presentation will feature a discussion on optimization of experimental parameters for different biological matrices, as well as the clean-up of proteins, phospholipids, and other endogenous metabolites that are undesirable to introduce in the HILIC-MS system. Further, the applicability of EME for high-throughput sample preparation prior to HILIC-MS/MS will be discussed.

Automated sample preparation and atmospheric pressure GC-MS/MS as a powerful tool for analysis of legacy POPs in human serum and plasma

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+UiT - The Arctic University of Norway, Department of Community Medicine, N-9037 Tromsø, Norway

Legacy persistent organic pollutants (POPs) are still of interest in the research and regulatory community due to their continued presence in the environment. These chemicals are biomagnifying through food chains years after regulations on production and use. Although most of these compounds are phased out and banned from the market worldwide, DDT use is still permitted in parts of the southern hemisphere and Asia for malaria control, creating current emissions sources. The application of other legacy POPs, as for example chlordanes, PCBs and PBDEs, has stopped due to harmful side effects on environmental and human health. However, the emission of these legacy POPs have still not come to an end yet. Recent studies identified regions in Africa and Asia known to be destinations for electronic waste as new emission sources for PCBs[1] and probably PBDEs. Other sources were identified in arctic regions where the impact of climate change is contributing to emissions of old burdens hidden by permafrost, snow and ice. The Arctic Monitoring Assessment Programme addressed in its Human Health report from 2015 a need for continuous biomonitoring of the legacy POPs, as well as prospective studies for a better understanding of health effects related to POP exposure[2].

Analysis of DDTs/metabolites, PCBs and PBDEs is typically done by GC-EI-MS. Enhanced sensitivity for other halogenated legacy pesticides and PBDEs is achieved by applying NCI. Introduction of GC-MS/MS enhanced the analysis for environmental applications by minimizing background and matrix response on the analytical signal using MRM. The combination of atmospheric pressure ionisation (API) with GC-MS/MS offers the advantage for ionisation at atmospheric pressure conditions which results in reduced fragmentation. Ions are generated through charge transfer or protonation processes. Mass spectra are dominated by $M^{+\bullet}$, $[M+H]^+$ or $[M-H]^+$, offering extensive capabilities for selection of precursor ions for targeted quantification in MRM. An automated high through put sample preparation method for analysis of legacy POPs (i.e. organochlorine pesticides (OCPs), PCBs and PBDEs) in human serum and plasma by atmospheric pressure (AP) GC-MS/MS was developed. Sample preparation was performed on a Tecan Freedom Evo 200 liquid handler equipped with a Te-Vac solid phase extraction station and a BioShake microplate shaker according to Huber et al[3]. Instrumental analysis of the legacy POPs were performed on an APGC coupled to a Xevo TQ-S/XS triple quadrupol mass spectrometer. Fast and ultra-sensitive methods for analysis of POPs were developed and validated and will be presented together with a demonstration for applicability in real samples from the Tromsø region[3].

References:

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Supercritical fluid and liquid chromatography methods for rapid trace analysis of emerging pollutants and biomolecules

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Nowadays, there is a constantly increasing demand by public and private stakeholders for rapid multi-residue targeted analysis in the context of environmental chemistry. A portofolio of such methods based on supercritical fluid and liquid chromatography tailored to mass spectrometry is now established and currently available at the University of Science and Technology (NTNU) for environmental monitoring and biomonitoring of emerging pollutants and biomolecules. Analytical approaches and settings configured for rapid analysis of novel and legacy perfluorinated contaminants, plasticizers (e.g., phthalates metabolites and bisphenols), personal care products (e.g., benzophenone UV-filters), flame retardants (e.g. brominated and organophosphates), and hormones (e.g., steroid and thyroid), will be shown as case studies. The presentation will focus on both the instumental and relevant sample preparation aspects of the analytical methods, which when appropriately combined, they provide the desired rapid analytical determination (fit-for-purpose). On this aspect, the benefits of supercritical fluid and ultra performance liquid chromatography, will be thoroughly presented and explained in light of the research output during the past year (2020).
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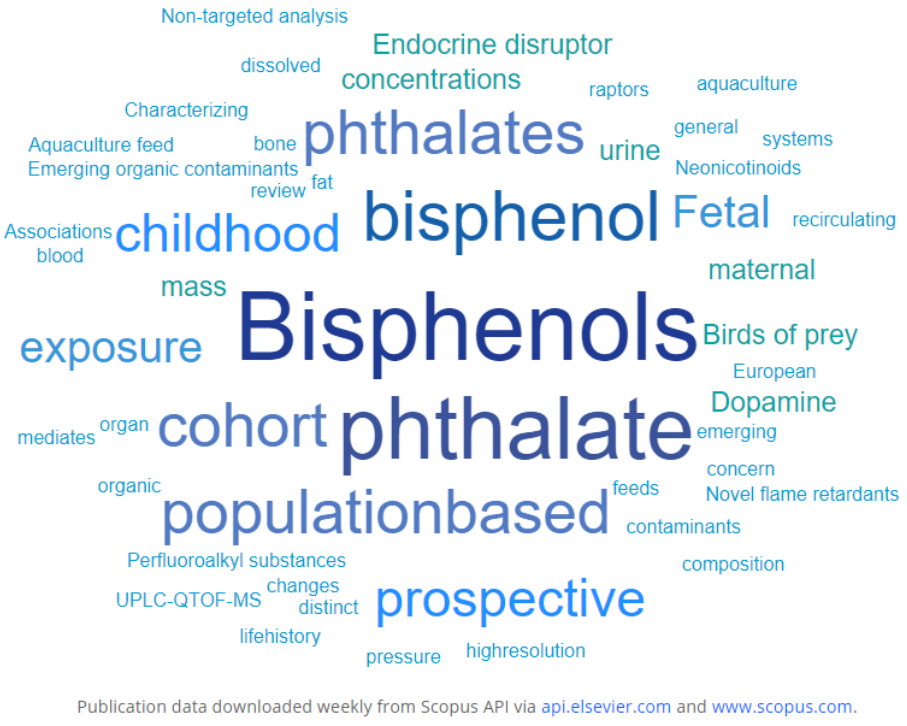

Infographics ('A picture is worth a thousand words') presenting the research out-put of the EnvironChemisty lab (NTNU) during the past year (2020); https://www.ntnu.edu/chemistry/research/envchem/asimakopoulos-group#/view/tags)

Advantages and Challenges in GC-Orbitrap high resolution mass spectrometry for trace analysis of legacy and emerging contaminants

Nicholas A. Warner[1,2]

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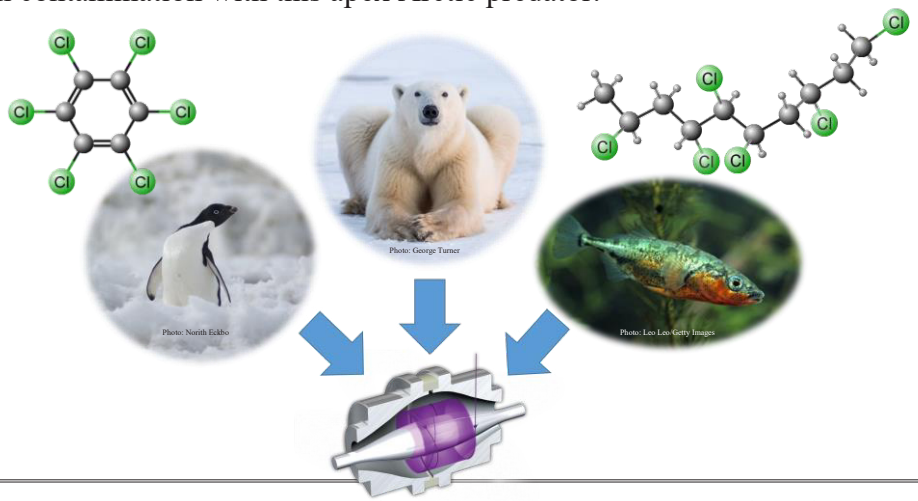
Ultra-trace analysis of environmental contaminants has relied on gas chromatography high resolution mass spectrometry (GC-HRMS) for several decades providing selectivity and sensitivity in complex environmental matrices. In most cases, mass resolution between 10 000 to 20 000 has been utilized for data acquisition. With the recent merging of Orbitrap technology to GC, even higher mass resolution can be achieved (60 000 to 120 000). However, does higher resolution capabilities provide any advantage towards environmental contaminant analysis? Several sample matrices (penguin blood, polar bear fat, fish muscle) were used to evaluate the performance of GC Orbitrap towards the analysis of persistent organic pollutant (POPs) and the emerging contaminant class of chlorinated paraffins (CP).

Several POPs where detected in penguin blood from Antarctica (Crozet Islands) using full scan analysis at 60 000 mass resolution. However, detection was hindered by co-extracted sample matrix at sub part per trillion concentrations. Application of targeted single ion monitoring (TSIM) dramatically reduces co-extracted matrix influence, improving detection frequency while conducting simultaneous full scan acquisition for suspect contaminant screening.

Chlorinated paraffins are of growing environmental concern due to their high production/usage, bioaccumulative properties and toxicity. Comprising over 10 000 different polychlorinated alkanes with varying chain lengths and degrees of chlorination, increased mass resolution is needed to handle this complexity. Mass resolution of 60 000 or greater can provide mass separation between CP homolog groups as well as co-extracted POPs. Fish muscle analysis from both remote and anthropogenic impacted lakes showed ion suppression of CP homolog groups by co-extracted POPs. Separation of CPs from co-extracted POPs can aid in detection and analysis quality, particularly in samples from remote regions where concentrations of emerging contaminants may be lower compared to POP concentrations. Short chain chlorinated paraffins (SCCPs) were detected in polar bears fat biopsies. Fractionation of sample extract helped reduce the influence of co-extracted POPs on CP homolog groups. However, suppression of CP signal could still be observed with certain homolog groups as traces of co-extracted contaminants were still observed in the CP fraction due to the high contamination with this apex Arctic predator.



An Introduction to the National Network of Advanced Proteomics Infrastructure (NAPI)

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Proteomics is the 'large scale study of proteins'. Proteins represent the actual functional molecules of the cell, and the fundamental importance of proteome level information in biomedical research is widely accepted. Proteomics research relies heavily on expensive, high-resolution mass spectrometry (MS) instrumentation operated by expert users.

The National network of Advanced Proteomics Infrastructure (NAPI) is a national platform that drives progress in MS-based proteomics across Norway, ensuring that researchers have access to the latest cutting-edge instruments, analytical techniques and bioinformatics tools. The overarching goal of NAPI is to provide a nationally coordinated expertise platform for proteomics research and knowledge transfer in Norway.

NAPI is funded by the Research Council of Norway, and officially launched in 2020. NAPI partners include proteomics core facilities in Oslo, Bergen, Trondheim, Tromsø and Ås, as well as research groups with a strong focus on proteomics technology development. NAPI therefore combines the proteomics expertise available across Norway, streamlining collaborations and accelerating the development of new techniques.

NAPI will focus on the main fields in MS-based proteome analysis, providing expertise in quantitative proteomics, characterization of post-translational modifications (PTMs), analysis of protein interactions, and proteome data analysis. NAPI will also establish and make available novel proteomics techniques such as metaproteomics, imaging-MS, antibody arrays and epigenomics/epitranscriptomics/modomics in Norway.

In this talk we will introduce the NAPI network and provide an overview of our short and long term objectives. We will provide a brief overview of the new NAPI-funded MS instruments that have/will be installed at proteomics core facilities, and how these will expand the analytical possibilities available to the Norwegian research community. We will also summarise the organisation of NAPI, and explain how researchers can communicate and collaborate with us to meet the mass spectrometry/proteomics needs of their projects.

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Microfluidic devices containing human cells or organoids (3D multicellular tissue derived *in vitro* from human pluripotent stem cells) constitute an exponentially growing technology for online analysis of cell responses and human organ functionality [1, 2]. Human pancreas-on-a-chip (PoC) is a relatively new technology, intended for the study of the endocrine tissue, the islets of Langerhans, which are responsible for regulation of the blood glucose levels [3]. These pancreatic islets consist of five major cell types, where the β-cells are responsible for the production of insulin [4]. For patients with type 1 diabetes, where the β-cells are destructed and unable to produce insulin, a common treatment is β-cell replacement therapy. Such therapy includes donor transplantation of either the entire pancreas or isolated islets.

Proteins, which are important compounds regarding quality of donor tissue or cells and the state of the isolated and purified islets may be studied in real-time analysis [3]. Normally, liquid chromatography with electrospray ionization mass spectrometry (LC-ESI-MS) is employed to measure the proteins in complex cell samples. Prior to analysis, the proteins must be digested to peptides, as peptides are far more easily detected and identified with MS. Immobilized enzyme reactors are becoming more common for inclusion of online protein digestion, however, a remaining bottleneck to achieving complete online analysis is that time-consuming reduction and alkylation of the disulfide bonds need to be executed prior to analysis.

Therefore, a platform consisting of a photochemical system upfront an LC-MS system has been set up for achieving online reduction of the disulfide bonds [5]. Insulin has been used as a model protein as it consists of two relatively short chains, A and B, connected by two disulfide bonds. Additionally, there is an internal disulfide bond in chain A. The protein was dissolved in a solution of water and isopropanol (*1+1*, *v/v*) added 1% of acetone as the photoinitiator. As insulin was irradiated by UV-light at 254 nm, the MS revealed that intact insulin (present as *m/z* 1452 and *m/z* 1162) was broken into two chains (A chain with *m/z* 1191 and B chain with *m/z* 858), due to the reduction of the disulfide bonds. The set-up may be used for fast measurements of small proteins with post-translational modifications without protein digestion.

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An investigation of reductive CO_2 activation by tandem mass spectrometric techniques

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The selective reduction of CO_2 to commodity chemicals or fuels is an attractive prospect. In many cases, the selectivity of a chemical process is determined by outcome via trial and error, rather than by design. Consequently, new processes in chemical industry involve time-consuming research and development phases prior to piloting. Tandem mass spectrometry is a suitable tool to screen potential important catalysts and their reactivity on a small scale, especially when combined with quantum chemical methods.[1] Moreover, it enables study of reactive intermediates—the critical intermediary steps of a process—not only the products. In this regard, the reduced forms of CO_2 are conceived as key intermediates in its reduction, yet unstable with respect to electron detachment. Atomic metal anions can both reduce and stabilize the resulting anionic CO_2 moiety in bimolecular reactions.[2] These reactions lead to the formation of reactive MCO_2^- complexes, further undergoing C—C bond forming reactions, an important step towards complex organic chemicals. Thus, we aim to aid in the development of processes utilizing CO_2 through the characterisation of these intermediates.

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Abstrakt

Postere
NSMS 18

New annotation tools for advanced 4D-Lipidomics workflows

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The annotation of lipids can be demanding due to the large number of structural variations. The mass spectrometry-based identification typically relies on characteristic fragments from headgroups and side chains obtained from MS/MS experiments. Depending on the quality of the MS/MS data, the depth of the structure elucidation can cover different levels like molecular formula level, chain composition level, etc. While the matching of MS/MS spectral libraries gives a broad and quick overview on the lipid content, the annotation level can be too detailed. The presented tools avoid this risk of over annotation and simplifies the automatic identification of lipid features by using selected fragmentation rules. The result visualization as Kendrick Mass Defect (KMD) Plots allows for a simple validation.

The total lipid extracts from Liver, Brain and *E. coli* (Avanti Polar Lipids) were investigated using **mobility-enhanced LC-MS/MS** data acquired on a **timsTOF Pro**. LC-MS/MS data of the lipid extracts were acquired as triplicate injections (technical replicates) in positive and negative **PASEF mode**. **Even from single runs, an almost comprehensive MS/MS coverage was achieved.** The raw data were processed with **MetaboScape® 2021** using four-dimensional feature extraction. All important qualifiers such as exact mass, isotopic pattern quality, retention times, MS/MS spectra and CCS values were extracted automatically for all specified adducts and neutral losses by the **T-ReX® 4D algorithm**. To increase the confidence for lipid ID, data of both polarities were merged.

The conclusions were:

- The presented rule-based lipid class annotation allows for a **reliable and confident annotation** of lipids from 24 subclasses
- Mobility-enhanced PASEF LC-MS/MS data generates **comprehensive MS/MS coverage** from a single injection, enabling lipid annotation with higher confidence
- **CCS-Aware Kendrick Mass Defect** plots simplify the verification of lipid classes and the search for non-annotated candidates
- Acquired **CCS values** can be matched to predicted values or public repositories with high accuracy
- The presented **4D-Lipidomics™ workflow** enables deep profiling of lipid extracts from different sources

Optimization of concatenation of fractions in comprehensive two dimensional liquid chromatography bottom-up proteomics

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Two dimensional liquid chromatography (2D-LC) has long been a commonly used technique in bottom-up proteomics, and is as relevant today as ever [1, 2]. To maximize the number of protein identifications, analytical chemists have strived to obtain 2D-LC systems with a high degree of orthogonality, which is needed to reach the required high peak capacities. A system utilizing reversed-phase chromatography (RP) in both column dimensions with a large difference in pH has proven to yield a higher peak capacity for peptides, even with a lower degree of orthogonality compared to other combinations [3].

The present study is based on Reubsaet, *et al*. [4]. The study is a comprehensive offline RP-RP 2D-LC system with high pH (>9) in the first dimension and low pH (3) in the second dimension. The method's purpose is to maximise protein identifications from one trypsinated protein sample. To achieve maximum identifications, Reubsaet incorporated the concept of concatenation of fractions. In practice, concatenation is applied through collecting the column output from the first dimension in eight numbered vials, where the 1^{st}, 9^{th}, 17^{th}, … fractions are collected in the first vial, the 2^{nd}, 10^{th}, 18^{th}, … are collected in the second, etc. [4].

In the present study, the fractionation scheme is examined by changing the total number of vials in which samples are concatenated, the number of fractions in each vial, and the collection time for each fraction. As the method demands high precision and is very time-consuming, automatization is practically essential. Automatization is achieved with the spider fractionation scheme, proposed and shown by Kulak, *et al*.[5]. Here, a 10-port injector connects the first dimension column to each sample vial, and switches between them automatically, reducing the strain on the operator drastically, while removing human errors.

The optimization of the concatenation will lead to higher numbers of identified proteins in proteomics.

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Discovering the major metabolites of the novel fentanyl analogues 3-methylcrotonylfentanyl, furanylbenzylfentanyl and 4-fluorocyclopropylbenzylfentanyl for forensic case work

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Background/aim: The highly potent opioid analgesic fentanyl and its analogues are involved in an increasing number of overdose deaths worldwide. New fentanyl analogues are continuously emerging, and there is a lack of knowledge concerning the metabolism of these compounds. The determination of fentanyl analogues can be challenging due to their low circulating concentrations and rapid and extensive metabolism, making metabolite identification necessary for confirming drug intake. The aim of this study was to discover and elucidate the structures of the major metabolites of the three novel fentanyl analogues 3-methylcrotonylfentanyl (3-MCF), furanylbenzylfentanyl (FBF) and 4-fluorocyclopropyl-benzylfentanyl (4-FCBF), which were all seized at European borders in 2018.

Methods: 3-MCF, FBF or 4-FCBF was incubated with human liver microsomes and human hepatocytes for up to 4 hours. The metabolites formed were separated by ultra-high performance liquid chromatography using a C_{18} column employing solvent gradient elution with a mobile phase consisting of ammonium formate and methanol. The compounds were detected by quadrupole time-of-flight mass spectrometry.

Results: The major metabolites of 3-MCF were formed by *N*-dealkylation, carboxylation, oxidation or hydroxylation of the 3-methyl-2-butene, and hydroxylation of both the 3-methyl-2-butene and the piperidine ring. FBF was metabolized through *N*-dealkylation, amide hydrolysis with/without subsequent hydroxylation at the *N*-phenyl, and dihydrodiol formation at the furan ring. 4-FCBF metabolism was dominated by *N*-dealkylation and *N*-oxidation at the piperidine ring.

Discussion: The metabolism of 3-MCF and 4-FCBF appears to be similar to that of fentanyl, which is dominated by *N*-dealkylation. FBF metabolism resembles that of furanylfentanyl, which is dominated by amide hydrolysis and dihydrodiol formation at the furanyl ring system. The major metabolites of the three fentanyl analogues differed slightly in human liver microsomes and hepatocytes regarding type of biotransformation and ratio of metabolites formed. However, the two *in vitro* models also formed shared metabolites for each compound which can be employed for accurate detection of the parent compounds.

Conclusions: In the present study we successfully discovered and elucidated the structures of the major metabolites of 3-MCF, FBF and 4-FCBF which could be used as markers to confirm intake of these compounds in forensic case work.

Integrating 4D peak picking of LC-TIMS-MS/MS data into GNPS feature based molecular networking for 4D Metabolomics and 4D Lipidomics analysis

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As throughput of metabolomic and lipidomic analyses continuously expands, effective workflows for analyzing the resulting datasets are of increasing importance. Molecular networking in recent years has become a vital tool in the metabolomics community as it quickly allows the identification of compounds with similar fragmentation patterns, which are often structurally related. While this approach mainly focusses on the fragment spectra, important information can be deduced from the precursor spectra, i.e. intensity, accurate mass, isotopic pattern as well as **Collision Cross Sections (CCS)** of the analytes which provide crucial information about the analytes concentration and identity.

Herein, we present a workflow to integrate analyte information for untargeted profiling from the software **MetaboScape** into GNPS feature based molecular networking.

The unique workflow of this study allows the integration of three- and four-dimensional **Time aligned Region complete eXtraction (T-ReX) peak picking** results as well as annotation workflows of MetaboScape with GNPS feature based molecular networking.

The nodes in the resulting molecular network are enriched by useful information about the precursor ions like the intensity in individual samples, molecular formula, annotation, CCS values, group mean intensity and maximum intensity. The information are important indicators to assess distribution of a specific analyte between sample groups (by group mean).

Additionally, the maximum intensity can help to determine if a purification of the analyte is feasible, i.e. to perform structure elucidation via NMR or assess its biological activity. Likewise, interpretation of the resulting molecular network is greatly simplified by displaying generated molecular formulas instead of precursor masses as node labels. The additional metadata greatly eases the interpretation of resulting networks.

A key functionality is that ion mobility separation is taken into account, allowing for analysis of similar fragmenting isomers, which would otherwise be handled as a single node.

Combination of targeted and non-targeted workflows for the identification of pollutants in river water using a passive sampling method

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Many pollutants are ubiquitous in surface waters because of continuous discharges from municipal wastewater treatment plants and we still do not know which pollutants are reaching the environment, the size of the problem for exposed fauna, nor what effects, if any, of that exposure may be. Investigative monitoring of water bodies failing ecological standards as set out in the Water Framework Directive is now a requirement of all European Union member states. In this study we test the combination of a passive sampling device in combination with targeted and non-targeted workflows on a QTOF MS for monitoring surface water.

Chemcatcher® passive samplers were deployed for four weeks in the River Clun, Wales, UK, whose WFD status is classified as poor, to determine which chemicals may be responsible. The extracts obtained were analyzed using an impact II (Bruker) LC-QTOF-MS followed by targeted and non-targeted processing.

Targeted data analysis for the identification of 'known unknowns' was performed with **TASQ** software using a combined database containing pesticides and drugs. Non-targeted data analysis for the identification of 'unknown unknowns' was performed with **MetaboScape**.

As expected, pollution in the river water due to discharges from municipal wastewater treatment plants varies significantly depending on the location and season.

- The passive sampling device allows the end user to obtain a more representative picture of pollutants that may be present in the aquatic environment than other sampling techniques.
- TASQ allows rapid screening and quantitation of 'known unknowns' using databases with more than 3000 compounds.
- MetaboScape enables a de-replication workflow for the identification of 'unknown unknowns' using an Analyte List from TASQ, Smart Formula, Compound Crawler, MetFrag and Spectral Library Search.

Determination of CYP activity in liver organoids using liquid chromatography-mass spectrometry

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The use of animal testing in pharmaceutical drug development comes with biological and ethical issues that need to be approached. Drug development consists of several steps where the early steps involve in vivo and in vitro testing. Human liver microsomes are the gold standard of in vitro studies of the liver enzymatic drug metabolism (CYP enzyme activity). The limitation, however, is that in vitro models lack the complexity that an organ hold [1]. A development that has the potential of dealing with these limitations and reducing animal in vivo testing, is the use of organoids as an in vitro model. Organoids are three-dimensional tissue models typically derived from adult and pluripotent stem cells [2]. They are expected to become a key tool in biology and drug discovery, as they may mimic human physiology to a greater extent than traditional cell cultures and even animal models.
It is not yet known exactly how these organoids work, so to evaluate the quality and relevance of liver organoids, their metabolic activity must be mapped. To study this, drugs with known metabolic profile can be utilised.

In this present study, a method has been developed to determine the CYP activity in liver organoids generated by three well studied, and common drugs. The drugs are fluoxetine, tolbutamide, and phenacetin, which have the metabolites norfluoxetine, 4-hydroxytolbutamide, and acetaminophen, respectively. A reversed phase liquid chromatography method has been established for determination of the concentration of drugs and their metabolites in microsomes, and will be used for determining CYP activity in liver organoids.

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The benefits of a LC-MS/MS interface with hot surfaces

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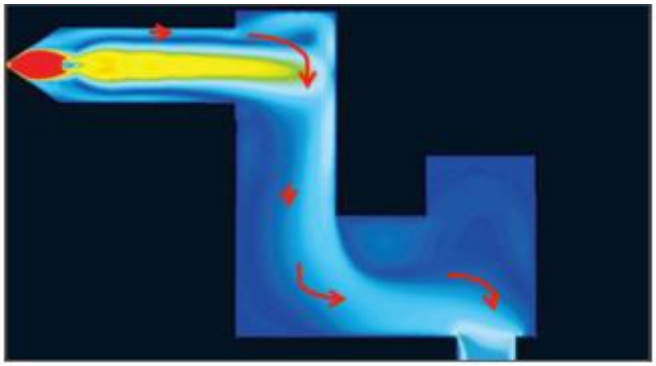
PerkinElmer Norge AS, *PerkinElmer LAS GmbH, Rodgau, Germany, **PerkinElmer Espana SL

Ions that are formed in an ion source in LC-MS/MS are normally sampled into the triple quadrupole mass spectrometer through a small aperture. Usually ion beam are guided and



shaped in the vacuum part of the mass spectrometer by means of electrical fields. Along the pathway through the vacuum part, ions and neutral can be accumulate on the inner surfaces around the ion path. This resulting in increased background, signal fluctuation, disturb the electrical fields of the optics causing thus drift and require user maintenance. In addition, all ion transport through electrical or magnetic fields always induce mass bias effects, i.e. ion scattering and discrimination between high and low mass ions.

These effects are especially problematic for high throughput applications such as screening of biomolecules and contaminants from biological samples.

To address these challenges, we will present here our innovative StayClean™ hot-surface induced desolvation (HSID™) technology which eliminates the deposition on the surfaces by means of continuous hot cleaning laminar flow of sampling gas without user intervention.

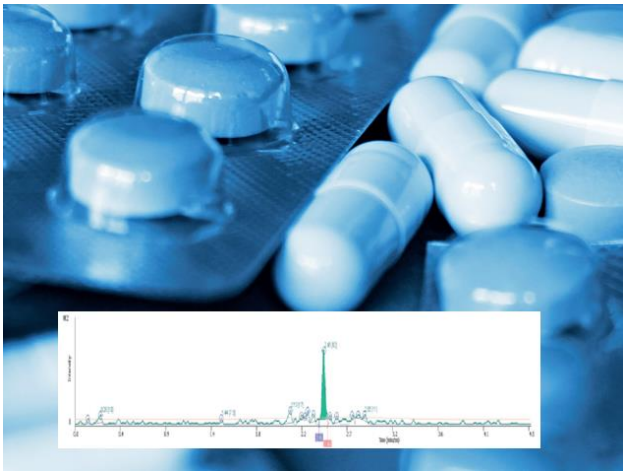
This results in dramatically increased stability, greatly reduced drift and minimized need for cleaning compared to traditional LC-MS/MS technologies which is especially beneficial for the analysis for challenging matrices such as natural samples and biological fluids.

"Dilute and Shoot" Method for Determination of Synthetic Opioids in Urine using QSight triple quadrupole LC-MS/MS

Daniel Fliegel, Derrek Mattern* and Ignazio Garaguso*

PerkinElmer Norge AS, Norway, *PerkinElmer LAS GmbH, Rodgau, Germany

The use and deaths from synthetic opioid have increased significantly in recent years, with the largest increases attributed to fentanyl and its analogues[1] [2]. Due to their high potency, very low quantity of the drugs are used indeed, the amount of these compounds in forensic samples is often in the sub fg/µl range. This poses an analytical challenge as high sensitive methods and instrumentation with high sensitivity of detection. Forensic and toxicology samples are often from urine which is the matrix of choice as it can be collected easily and in large volumes. However, the variations within the urine matrix can adversely affect chromatographic separation and a reliable LC-MS/MS determination.



Moreover, the number of compounds and the number of samples to be analysed by toxicology laboratories is constantly growing. Therefore, rapid methods for sensitive determination of these drugs is essential for forensic and toxicology studies.

In this work, we present a simple "dilute and shoot" method using the QSight® 220 triple quadrupole LC-MS/MS system for a sensitive and reliable determination of fentanyl and synthetic opioids in urine.

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Sensitive Determination of PFAS using QSight 420 triple quadrupole LC-MS/MS system with direct injection

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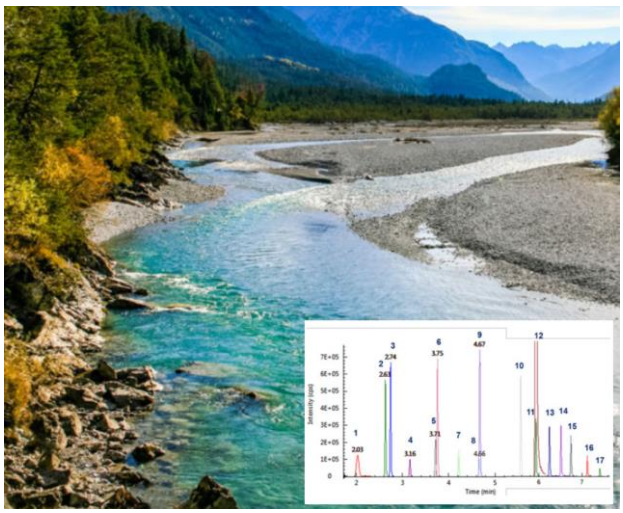
PerkinElmer Norge AS, Norway, * PerkinElmer, Inc. Woodbridge, Ontario, Canada; ** PerkinElmer LAS GmbH Rodgau, Germany

Perfluorinated compounds (PFCs) Per- and polyfluoroalkyl substances (PFAS) are human-made chemicals representing a diverse group of synthetic fluorinated organic compounds which have been used in surfactants, fire-retardants, non-stick cookware coatings, and coatings for paper packaging for over half a century. [1, 2] Fluorinated compounds recently also were gaining increased attention in the Nordics due to their use and now ban in ski wax.

PFASs have received lots of attention because they are highly stable and resistant to degradation in the environment. Reports of their occurrence in tap water, food or even human blood have led to concerns of their effect on human body as pollutants.[3-6] Hence, analysis of PFASs in biological and environmental matrices is critical to understanding their fate, persistence and toxicity.



LC-MS/MS is the analytical technique of choice for the measurement of PFASs in biological and environmental samples due to its selectivity and sensitivity. Typically, determination of low ng/l levels of PFAS requires either a highly sensitive mass spectrometer, or a sample preparation technique that includes a concentration step. The use of solid phase extraction (SPE) procedures before LC-MS/MS analysis is a popular methods for PFASs extraction and concentration from aqueous environmental matrices. However, with the advancement and availability of highly sensitive mass spectrometers, a trend towards developing a high throughput analytical method for the determination of PFAS by direct injection, without SPE, has increased.
In this study, we present a rapid and sensitive LC-MS/MS method with direct sample injection. The results indicate that this simple LC-MS/MS workflow provides an excellent sensitivity and specificity for the analysis of PFASs in water sample.

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Releasing antimicrobial peptides through nanocarriers to fight resistant bacteria – Studies with HPLC-MS

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Discovered in 1949, polymyxin E1 – conventially referred to as colistin (see Figure 1) – is a strong antimicrobial peptide which demonstrates a high lethality towards gram-negative bacteria such as *P. Aeruginosa* [1]. However, only briefly after being put into use, widespread reports of nephro- and neurotoxic side-effects led to the discontinuation of colistin in a pharmaceutikal context [2]. Colistin has become increasingly relevant once more with the widespread rise of antibiotic resistance, currently used primarily as a drug of last resort applied in salvage therapy [3]. As such, there is a rising interest in negating the observed neuro- and nephrotoxic effects of colistin.

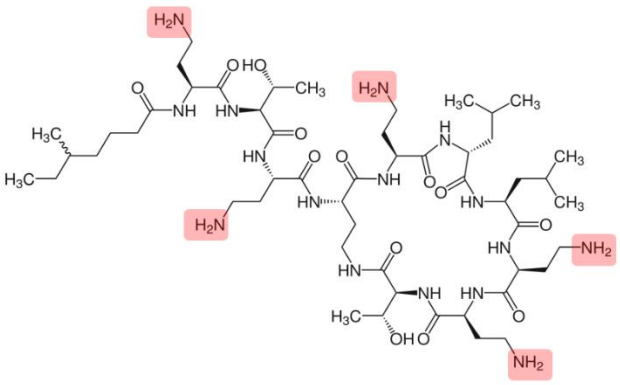

Figure 1. Molecular structure of polymyxin E1 – otherwise known as colistin.

One proposed way of doing so is by the use of nanocarriers, liposomes that can slowly release encapsulated drugs to the surrounding tissue, avoiding 'burst release', in which much of the drug is effectively administered to local tissue where administered [4]. Colistin consists of a highly polar, cyclic heptapeptide, bound to an exocyclic peptide, which is in turn bound to a fatty acid, 6-methyloctanoic acid. Colistin's mode of action is thought to be detergent-like to some extent against gram-negative membranes - however, the kinetics between colistin and (pegylated) liposomes remain unknown, and are the primary focus in this work.

In this work, the release kinetics of liposomally encapsulated colistin is assessed using LC-ESI-MS/MS in MRM mode. Mobile phase A consisted of H_2O + 0.1% formic acid, whereas mobile phase B consisted of acetonitrile + 0.1% formic acid. A C18 column from VWR (100mm x 2.1mm ID, 3µm particles) was used. Isocratic elution was conducted with 17% B for 3 minutes, followed by a 90% B washing step for 0.5 minutes, and a reconditioning of the column for an additional four minutes. Average retention time for polymyxin E1 was 1.7 minutes. A further goal is to study the toxicity of liposomally encapsulated colistin compared to free, non-encapsulated colistin using kidney *organoids*; microscopic microcellular structures and commuinities resembling those in the real human body, with the ultimate goal of presenting a method viable for reducing the toxic side-effects presented by colistin: our last line of defense against multidrug-resistant bacteria [5].

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Uncovering hidden compositional changes in breath profiles using untargeted chemometric workflows

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Volatile organic compounds (VOCs) emitted in breath have great potential for use in non-invasive disease diagnosis. This is largely due to the discovery of so-called 'biomarkers', which provide indicators of normal or abnormal states.

In large scale clinical trials, hundreds of samples may be collected across multiple sites (e.g. clinics or hospitals) over the course of many weeks. During this biomarker discovery phase, an incorrect identification can compromise the validity of an entire trial, meaning that both robust analytical techniques and confident data mining are required.

Thermal desorption (TD) coupled with GC–MS is known as the 'gold standard' for breath analysis, due to its ability to capture a complete breath profile with high sensitivity. Here, we combine TD with advanced separation and detection by GC×GC–TOF MS to gain greater insight into sample composition.

However, data acquisition is just the beginning – the information-rich chromatograms must then be transformed into meaningful results. Here, we demonstrate the use of a powerful data mining and chemometrics platform to automatically find the significant differences in complex datasets and to create statistical models to predict the class of future samples.

Firstly, chromatographic alignment accounts for retention time drift over the course of the study and minimises the risk of false hits. Next, feature discovery is performed on the raw data to find significant changes across sample classes. In metabolomics matrices, the diagnostic compounds are rarely of high abundance - by adopting a raw data approach, trace peaks are not overlooked. Additionally, the use of raw data enables automated workflows to be adopted, minimising laborious pre-processing steps and speeding up analytical workflows.

We will demonstrate how these innovative tools can allow automated untargeted workflows to be adopted, minimising laborious pre-processing steps and accelerating analytical workflows.

Determination of sterols in liver organoids using liquid chromatography-mass spectrometry (LC-MS)

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Abstract

To investigate and model disease development, research models resembling the complexity of human biology to a high degree is essential. Organoids is a research model predicted to surpass current well-established models like cell lines and animal models regarding this, as organoids has a self-organizing 3D cell nature creating an organ structure achieving this resembling [1]. Organoids are used as e.g. disease models to map biomarkers. Non-alcoholic fatty liver disease (NAFLD) is a liver disease caused by obesity and poor lifestyle habits, leading to scarred liver tissue as excess fat accumulates in the liver. In the worst-case scenario, the patient is in need of a critical liver transplant (cirrhosis). NAFLD lacks a less invasive diagnostic method, as the only diagnostic tool per today is liver biopsy which can be harmful to an already fibrotic liver. Hence, biomarkers to detect in e.g., blood samples are of interest to identify for an easier and an earlier diagnosis. Oxysterols (OHCs) (endogenous metabolites of cholesterol) are biologically active molecules proposed to be a potential biomarker for NAFLD as they are involved in some of the same biological pathways seen in NAFLD [2].

Low abundant oxysterols in few-cell cell samples (a hepatic organoid can be 200 μm in size containing ~1500 cells) requires a highly sensitive LC-MS system calling for the employment of nanoLC columns (≤ 100 μm ID) prior to detection with a triple quadrupole MS operated in MRM mode. NanoLC columns have the ability to increase the systems sensitivity 784 times, downscaling from 2.1 mm ID to 75 μm ID [3]. Separation of the isomers using a 2.1 mm ID column has been most successful on 2.5 μm core-shell Super Phenyl Hexyl (SPH) particles [4], after derivatization to enhance ionization efficiency in electrospray ionization (ESI). The columns are not commercially available in nanoLC format, hence nanoLC columns (75 μm ID) have been packed in-house with these particles using different slurry solvents (80% ACN, 90% ACN and 100% acetone) to investigate their performance.

100% acetone was discovered as the slurry solvent giving higher possibility of better performing columns, regarding plate number, asymmetry and retention factor. Additionally, 5 μm phenyl-hexyl particles are tested to have a smaller retention factor than 2.5 μm SPH particles, proposing them to be well suited as a trap column. The fully developed analytical nanoLC-ESI-MS method will be applied on liver organoids for quantification of OHC isomers.

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Determination of 54 contaminants of emerging concern in surface water samples from Sør-Trøndelag, Norway

Kristine Vike-Jonas[a], Susana Villa Gonzalez[a], Alexandros G. Asimakopoulos[a]

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Bisphenols, benzophenones, benzothiazoles, benzotriazoles, parabens, triclocarban and polyfluorinated contaminants present endocrine disrupting potential and are considered environmental contaminants of emerging concern. A solid phase extraction (SPE) protocol was applied for the simultaneous extraction of 54 of these emerging contaminants from surface seawater and freshwater. Analysis of the samples was performed by four rapid (≤5 min) multi-residue UPLC-MS/MS methods, all using the same SPE extract. Overall, 33 water samples were collected from 15 different locations in and near Trondheim, Sør-Trøndelag, Norway (63°25'49.76"N, 10°23'42.22"E.). Of the 54 contaminants analyzed, 30 were detected with each analyte group represented. The sum concentrations of parabens and their transformation products (∑(8)Parab) ranged from 68.4 to 511 (median 166) ng/L. The sum concentrations of benzothiazoles (∑(9)BTHs) and benzotriazoles (∑(7)BTRs) ranged from 0.78 to 70.1 (median 9.09) and from 6.49 to 880 (median 5.07) ng/L, respectively. The sum concentrations of bisphenols (∑(9)Bisph) and benzophenones (∑(5)BzPs) ranged from 1.59 to 60.5 (median 11.3) and from 1.32 to 28.0 (median 5.45) ng/L, respectively. The sum concentrations of the polyfluorinated compounds (∑(15)PFCs) ranged from 1.21 to 648 (median 88.4) ng/L, while triclocarban ranged from 0.97 to 195 (median 2.00) ng/L. 27 compounds demonstrated reproducibility (relative standard deviation; RSD %) ranging from 2.31 to 9.89 %, 14 compounds ranged from 12.0 to 19.2 % (RSD %), while the remaining 11 compounds ranged from 20.1 to 34.3 %. The method lower limit of quantification (MLLOQ) ranged from 0.06 to 8.85 ng/L for 35 compounds, 11.6 to 99.0 ng/L for 14 compounds, and 139 to 576 ng/L for the remaining 4 compounds. 34 chemicals demonstrated absolute recoveries ranging from 81.5 to 105 %, 13 compounds ranged from 40.5 to 77.0 %, and 8 compounds ranged < 34 %. From the water samples, the most detected analytes were benzophenone 2 (BzP-2), bisphenol S (BPS), vanillic acid (OH-MeP) and benzotriazole (BTR) at 97.0%, 90.9 %, 84.8 %, and 78.8 % detection rate, respectively. ∑PFCs were detected in 87.9 % of the samples, but no single PFC was detected in more than 39.4 % of the samples (perfluorooctanesulfonic acid; PFOS). Contamination loads were spaced evenly throughout samples, with no areas showing significantly higher or lower contamination loads.

Silica-based narrow inner diameter open tubular liquid chromatography columns for use in proteomics

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In the world of proteomics, which is the study of proteins and peptides, the samples are usually small and/or with low analyte concentration. To analyse this type of samples it is beneficial to use liquid chromatography (LC) columns with narrow inner diameter (ID) coupled with an electrospray mass spectrometer (ESI-MS). The use of narrow columns in an LC-ESI-MS system makes it possible to achieve high sensitivity [1, 2], due to less radial dilution of the analytes [3]. Such narrow columns can be porous layer open tubular (PLOT) columns, either organic polymer based [2] or silica-based [1, 4, 5].

A silica-based PLOT can be made by sol-gel synthesis [5]. In the present study, both 10 µm and 5 µm ID silica-based PLOT columns have been prepared according to Hara *et. al.* [4, 5] In this preparation method, a solution of tetramethyl orthosilicate (TMOS), polyethylenglycol (PEG), urea and 0.01 M acetic acid is prepared and filled into pre-treated silica capillaries, with 10 µm or 5 µm ID, either by a pressure bomb system or with a pump. The polymerization into a porous layer structure (silica skeleton) takes place in a water bath at 25 °C, and a following heat treatment at 80 °C makes the mesopores in the silica skeleton. After the porous layer has been formed, functionalization with C18 is preformed to obtain a reversed phase PLOT column.

The columns made are used in a nano LC ESI-MS system for ultrasensitive analysis.

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Restructuring of central carbon metabolite pools in *Streptomyces coelicolor* strains during antibiotic production

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Introduction: The presented work relates to our efforts to develop optimized *Streptomyces* 'Superhost' strains to act as microbial cell factories for a more efficient discovery of clinically important novel bioactive natural products by using Systems and Synthetic Biology approaches [1,2]. *S. coelicolor* A3(2) is a well-known microorganism to produce different types of antibiotics and is considered suitable to create 'Superhost' strains because of the available knowledge of genome sequence [1,2]. One important experimental input to this task is deciphering the participation of important metabolic pathways for antibiotic production and generation of high-resolution quantitative metabolite profiles of the *S. coelicolor* host production strains.

Methods: *S. coelicolor* A3(2) M145, its non-antibiotic producing mutant strain *S. coelicolor* A3(2) M1152, and chloramphenicol (CP) producing *S. coelicolor* A3(2) M1581 (a derivative of *S. coelicolor* A3(2) M1152) were used in this study [1]. Microorganisms were cultivated in controlled bioreactors using a medium designed to provide either L-glutamate or phosphate limitation [2]. D-glucose (U-13C6) and L-glutamate (U-12C6) were used in the ^{13}C-isotope-labeling experiments. The intracellular metabolite pools were quantified using several MS/MS-based methods; i.e. four UPLC-MS/MS methods each for amino acids, organic acids, CoAs, and NADs, and a capIC-MS/MS method for nucleotides, sugar phosphates, and other phosphometabolites [3]. The isotopologues of metabolites were analyzed using a capIC-MS/MS.

Results and discussion: Both L-glutamate or phosphate limitation triggered antibiotics production at a defined time in *S. coelicolor* A3(2) M145. *S. coelicolor* A3(2) M1152 had a prolonged CO_2 evolution phase as compared to wild type strain. ^{13}C-isotope-labeling experiments showed the degree of participation of carbon from L-glutamate and D-glucose in metabolic pathways and synthesized antibiotics. L-glutamate enters metabolic pathways through the tricarboxylic acid cycle (TCA) and transfers its carbon into the glycolytic pathway and then into the pentose phosphate pathway. Isotopic patterns show metabolites flooding situation in different metabolic pathways especially in case of non-antibiotic producing strain as along with D-glucose, L-glutamate carbon re-enters into the TCA cycle from the glycolytic pathway.

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