30th Scientific Meeting
of the
Society of Hair Testing

Scientific Program

May 14-15, 2026

City of New York
Office of Chief Medical Examiner
New York, NY

NYC
Office of Chief
Medical Examiner

SOCIETY OF HAIR TESTING

Organizing Committee & Scientific Committee

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Dr. Karen Scott
Dr. Craig Chatterton
Dr. Marta Concheiro-Guisan
Dr. Damon Borg

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Dr. Vincent Cirimele
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Dr. Frank Sporkert
Dr. Tina Binz
Dr. Donata Favretto
Dr. Elena Lendoiro

Design and Public Communications

Jennifer Vignone

Welcome to New York City

On behalf of the Board of the Society of Hair Testing, the 2026 SoHT Organizing Committee and my colleagues at the NYC Office of Chief Medical Examiner, welcome to New York City.

The 30th Scientific meeting of the SoHT will involve delegates representing 17 different countries and 10 different US States. Thank you to everyone who chose to present their research, the program is rich with exciting oral and poster presentations and for the first time will have a seminar session on the topic of Drug Facilitated Crime.

I will take this opportunity to thank the Board of the Society of Hair Testing for selecting New York City to host the 30th Scientific meeting of the SoHT. In addition to sharing knowledge, catching up with old friends and meeting new, you will have the best time exploring the City during your stay. New York City is my second home and a wonderful place to stay but it is magical to visit. Some popular attractions are suggested in the program. My personal favorite, take the free Staten Island Ferry ride. Wonderful view of Manhattan from the river and you also pass the Statue of Liberty.
Looking forward to seeing you all in New York City!

Dr. Gail Cooper – Meeting Host
Representing New York City and Scotland

Table of Contents

Scientific Program

Thursday, May 14th, 2026

Scientific Session 1

Chair: Dr. Robert Kronstrand Co-Chair: Dr. Craig Chatterton

9:00 a.m. – 9:15 a.m.	**Welcome Address** Dr. Jason Graham, Chief Medical Examiner, NYC OCME
9:15 a.m. – 9:45 a.m.	**Invited Speaker – Dr. Kristin Roman** "A Case of Homicidal Selenium Poisoning"
9:45 a.m. – 10:00 a.m.	**01 Karen Scott** Postmortem Hair Testing for Fentanyl and Analogs: Experimental, Forensic and Interpretative Aspects
10:00 a.m. – 10:15 a.m.	**02 Frank Sporkert** Investigation of the Incorporation of Tramadol, Acetaminophen and Ibuprofen in Larvae and Flies
10:15 a.m. – 10:30 a.m.	**03 Donata Favretto** Hair Analysis in Psychiatric Patients: Quantification of Drugs of Abuse and Therapeutic Agents in Psychiatric Patients and Comparison with Pharmacological Regimen
10:30 a.m. – 10:45 a.m.	**04 Paula Llabres Piza** Hormonal Profile Differences Associated with COVID-19 Vaccination Revealed by Nair Analysis
10:45 a.m. – 11:00 a.m.	**05 Brooke Fontaine** Hair Glucocorticoid and Endocannabinoid Quantitative Profile as Biomarkers of Stress During Pregnancy
11:00 a.m. – 11:30 a.m.	**Refreshment Break and Poster Session**

Scientific Program

Thursday, May 14th, 2026

Scientific Session 2

Chair: Dr. Vincent Cirimele Co-Chair: Dr. Damon Borg

11:30 a.m. – 11:45 a.m.	06 **Hideaki Okochi** Impact of Segment Length and Environmental Conditions in Segmental Hair Analysis for Adherence Monitoring of HIV Drugs
11:45 a.m. – 12:00 p.m.	**07 Donna Muldoon** Hair and Drug Patch (Sweat) Testing: A Case Review
12:00 p.m. – 12:15 p.m.	08 **Tina Binz** Does CBD-Containing Beard Oil Interfere with THC-Hair Analysis?
12:15 p.m. – 12:30 p.m.	**09 Miriam Blanco-Ces** Further Insights into the Influence of Permanent Chemical Straightening on the Interpretation of Endogenous GHB Hair Results
12:30 p.m. – 12:45 p.m.	**010 Ana de-Castro-Rios – LC-MS/MS** Determination of Cortisol and Cortisone in Human Hair and Application to Alcohol Consumption Assessment in a Driving License Cohort
12:45 p.m. – 1:00 p.m.	**011 Rossella Gottardo** hEtG and Fitness to Drive: The Results of a Two-Year Longitudinal Study at the University of Verona
1:00 p.m. – 2:30 p.m.	**Lunch**

Scientific Program

Thursday, May 14th, 2026

Scientific Session 3

Chair: Dr. Brian Appenzeller Co-Chair: Dr. Karen Scott

2:30 p.m. – 2:45 p.m. **012 Katharina Sander**
Quick Hair Tox: Development of a Rapid Extraction Method for the Detection of Therapeutics and Illegal Substances in Hair

2:45 p.m. – 3:00 p.m. **013 Marija Garvin**
Analysis of Chiral Amphetamine in Hair – Validation and Application

3:00 p.m. – 3:15 p.m. **014 Francisca Corthorn**
Optimization of an LC-MS/MS Method for Cortisol and Cortisone Determination in Hair and Its Application in a Population Study

3:15 p.m. – 3:30 p.m. **015 Camilla Vigato**
Determination of Ethyl Glucuronide in Hair Following Derivatization with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC). Development and Validation of a Method using Liquid Chromatography-High-Resolution Accurate-Mass Orbitrap Mass Spectrometry and Parallel Reaction Monitoring Mode

3:30 p.m. – 3:45 p.m. **016 Sara Casati**
Ethyl Glucuronide in Hair: a Ten-Year Overview

3:45 p.m. – 4:00 p.m. **017 Mégane Moreau**
Improved Extraction of Drugs of Abuse in Hair with Preserved Analyte Integrity

Scientific Program

Friday, May 15th, 2026

DFC Seminar

Chair: Dr. Tina Binz Co-Chair: Dr. Gail Cooper

9:00 a.m. – 9:30 a.m.	**Invited Speakers – Kathleen Baer and Kevin Fulham, NYPD Special Victims Unit** Gender-Based Violence Policy and Planning Unit: The NYPD's Approach for Investigating Crimes of Sexual Violence in New York City
9:30 a.m. – 10:00 a.m.	**S1 Robert Kronstrand** Hair analysis in Drug Facilitated Crimes - Best Practices and Recommendations for Sampling, Analysis, and Interpretation
10:00 a.m. – 10:30 a.m.	**S2 Karen Scott** DFSA? A Cautionary Tale
13:00 a.m. – 11:00 a.m.	**S3 Maria del Mar Ramirez Fernandez** Hair Analysis for Drug-Facilitated Crimes: Bridging Toxicology and Judicial Decision-Making
11:00 a.m. – 11:30 a.m.	**Refreshment Break and Poster Session**

Scientific Program

Friday, May 15th, 2026

DFC Seminar

Chair: Dr. Mário Barroso Co-Chair: Dr. Marta Concheiro-Guisan

11:30 a.m. – 12:00 p.m.	**S4 Craig Chatterton** Operation Paris – the Shannon Matthews Investigation
12:00 a.m. – 12:30 p.m.	**S5 Vincent Cirimele** DFSA Casework
12:30 a.m. – 1:00 p.m.	**Open Forum Discussion with DFC Seminar Speakers**
1:00 p.m. – 2:30 p.m.	**Lunch**
2:30 p.m. – 3:00 p.m.	**Awards Presentation**
3:00 p.m. – 4:30 p.m.	**Business meeting (Members Only)**

List of Poster Presentations

P1 **Global PFAS legislation and hair biomonitoring: do regulatory patterns emerge in human hair?**
Ashef Ameer, Chassity Correa, Ana Miguel Fonseca Pego

P2 **A Derivatization-Free LC-MS/MS Approach for d/l-Methamphetamine Analysis in Hair Samples.**
Collin Stirling, M.S. and Jason Hudson, Ph.D.

P3 **Real NPS Exposure and Complex Drug Mixtures Revealed by Hair Toxicology in the Spanish Mediterranean Region.**
Cristina Hernando, Alberto Cordoba, Maria Estela García, Tamara Khazooz, Elena Hernández, Virginia Lostao, Manuel Perea, Raquel Poveda, Anna Robert, Marta Sánchez, Marta Vázquez, Nuria Sanvicens.

P4 **Methodological trends in hair NPS analysis: a narrative review of analytical performance.**
João Manoel Pinheiro, Alison Castillo Payano, Ana Miguel Fonseca Pego

P5 **The Use of a Novel Quadrupole Time of Flight Instrument for the Quantitative and Sensitive Analysis of Drugs of Abuse in Hair Samples.**
Jonathan P. Danaceau, Gareth Hammond and Lisa J. Calton

P6 **Drug Findings in Pediatric Hair: A 2021 – 2025 Assessment**
Laura M. Labay, PhD and Barry K. Logan, PhD Speakers

P7 **Hair analysis and exposure assessment: a case of fatal methadone Intoxication in a child.**
Salomé Riess, Ruben Goncalves, Fabien Lamoureux, Vincent Cirimele

P8 **Unveiling child exposure to cocaine through hair testing.**
Suzel Costa, Anabela Neves, Mário Barroso, João Franco

P9 **Validation of a method for drug research in hair samples by UHPLC-MS/MS.**
Tomas Leitão, Suzana Fonseca, Mónica Antunes, Suzel Costa, João Franco, Ricardo Bettencourt da Silva

P10 **Case Report: Strychnine Poisoning.**
Zoila Rosario, Kristin Roman, Vincent Cirimele and Gail Cooper

Invited Speaker Biographies

Dr. Kristin Roman

My cases are far more interesting than I am, particularly the cases of death by selenium and strychnine that we will be reviewing here. Challenging and fascinating cases always seem to find me, and I have always eagerly welcomed them. Beginning in 2003, I worked at the Office of Chief Medical Examiner of the City of New York for the majority of my career. I did leave briefly to work for a year in Madison, Wisconsin as a deputy chief medical examiner, but I returned home to OCME for the last 12 years that I was employed as a medical examiner. Additionally, instead of taking vacations, I did per diem medical examiner work in Vermont, Maine and New Hampshire for the last seven years that I practiced forensic medicine, which exposed me to even more interesting cases. After retiring from both the OCME and forensic medicine entirely in 2022, I have changed directions completely. Presently, I work as a veterinary nurse at an animal shelter while simultaneously returning to school to earn an associates degree in veterinary technology. I hope to soon be a licensed veterinary nurse technician, and plan to either return to forensics and specialize in animal forensic medicine, or to indulge my new interest in anesthesiology, and specialize in that. Either way, I look forward to a new career filled with challenging animal cases that will hopefully be as interesting as my human forensic cases were.

Kathleen Baer, Assistant Commissioner, Gender-Based Violence Policy and Planning Unit

Kathleen Collins Baer is the Assistant Commissioner of the NYPD's newly created Gender-Based Violence Policy and Planning Unit. She earned her undergraduate degree from St. John's University and her J.D. from St. John's University School of Law in 2007. In 2008, she joined the Brooklyn District Attorney's Office, where she investigated and prosecuted the sexual and physical abuse of children age 10 and under, including infant fatalities.

In 2010, Kathleen joined the first Sex Trafficking Unit established in any of New York City's five boroughs and helped build the unit from the ground up. In that role, she developed investigative techniques for long-term investigations and prosecutions targeting individuals engaged in sex trafficking and in compelling and promoting prostitution of sexually exploited individuals—while advancing a victim-centered approach to supporting survivors.

Kathleen also served as an assistant adjunct professor at CUNY Hunter College, teaching Women and the Law in the Political Science Department. The course examined the evolving role of women in society and the impact of landmark Supreme Court decisions, covering topics including domestic and gender-based violence, intimate partner violence, sexual violence, and LGBTQ issues.

In 2016, Kathleen joined the Bronx District Attorney's Office, Child Abuse and Sex Crimes Bureau, where she handled investigations and prosecutions involving sexual and physical abuse of children (including infant fatalities), internet crimes against children, and adult sex crimes (including intimate partner sex crimes, acquaintance sexual assaults, and stranger sexual assaults). She began supervising the Bureau in 2019.

Kathleen later joined the NYPD as the first Executive Counsel for the citywide Special Victims Unit, serving as legal advisor to the Chief of Special Victims and to adult special victim and child abuse squads across all five boroughs, including the Human Trafficking Task Force. Promoted to Director of Special Victims Unit in 2024 and most recently to Assistant Commissioner of the newly created Gender-Based Violence Policy and Planning Unit, she leads policy and training initiatives to strengthen trauma-informed, culturally sensitive, offender-focused investigations; expand community outreach; and deepen collaboration with city agency partners to build trust in the communities the NYPD serves.

Sergeant Kevin Fulham, Special Victims Unit

Sgt. Kevin Fulham is a senior law enforcement supervisor with the New York City Police Department and more than a decade of experience in patrol operations, Special Victims investigations, training, and policy development. Sgt. Fulham has extensive experience investigating sexual assaults, child abuse, human trafficking, and domestic violence cases. He currently serves as the Sergeant of the NYPD's Special Victims Training and Liaison Unit, where he oversees citywide training initiatives and develops instruction on trauma-informed and victim-centered practices, investigative methodology, and analytical data-mining. In this role, he also provides policy and compliance guidance to investigative commands and works closely with prosecutors, advocacy organizations, medical providers, and government partners.

DFC Seminar Speaker Biographies

Dr. Robert Kronstrand

Robert Kronstrand is currently the Chief Toxicologist at the National Board of Forensic Medicine in Sweden. He received his BSc degree in analytical chemistry in 1989, and his PhD in human toxicology in 2001, both from the University of Linköping, Sweden. He also holds a part time position as professor in forensic toxicology at Linköping university. Dr Kronstrand has 36 years of experience in the fields of postmortem toxicology, DUID, DFSA and drug testing in various matrices. His research has covered a wide range of topics over the years, including opiate toxicity, analytical toxicology and the incorporation of drugs into hair. Of his more than 130 papers many focus on hair analysis on various contexts.

Dr. Karen S Scott, F-ABFT

Dr Karen Scott is the Director of the Forensic Toxicology and Medical Toxicology Laboratory's in the Division of Laboratory Medicine at the University of Alabama at Birmingham. She has over 30 years of experience in the fields of forensic and clinical toxicology and is published in the areas of post-mortem and human performance toxicology, as well as hair and alternative matrix testing. She is a reviewer for three of the main Forensic Toxicology journals and is on the editorial board for Separations (Forensics/Toxins section).

Dr. Scott has a Bachelor of Science degree in Forensic & Analytical Chemistry from the University of Strathclyde and a PhD in Forensic Toxicology from the University of Glasgow. On completion of her degrees she carried out postdoctoral research in Tokyo, Japan investigating incorporation rates of drugs into hair. Dr. Scott has been a member of the Alcohol Drugs and Impairment Division of the National Safety Council since 2015 and sits on the executive board of this organization. She is also on the editorial board of Clarkes Analysis of Drugs and Poisons and is a member of the OSAC Forensic Toxicology subcommittee.

Dr. María del Mar Ramírez Fernández

María del Mar Ramírez Fernández is a scientific advisor at the Toxicology Unit of the National Institute of Criminalistics and Criminology in Brussels, Belgium (Europe), the Belgian federal forensic institute under the Ministry of Justice. She completed her PhD at the University of Santiago de Compostela (Spain). Since 2004, she has specialized in forensic toxicology, with a primary focus on hair analysis for the detection and interpretation of xenobiotics using advanced UHPLC–MS/MS methodologies. Her work has contributed to strengthening the use of hair analysis as a retrospective tool in drug-facilitated crimes, particularly in documenting exposure to substances such as benzodiazepines, opioids, and illicit drugs. She also addresses key interpretative challenges, including segmental hair analysis and the differentiation between active consumption and external contamination. She is the author of more than 40 peer-reviewed publications and has served on the board of the Society of Hair Testing since 2021.

Dr. Craig Chatterton

Dr. Chatterton is the Chief Toxicologist at the Office of the Chief Medical Examiner (OCME) in Edmonton, AB, Canada and an Assistant Clinical Professor in the Department of Laboratory Medicine & Pathology at the University of Alberta. Dr Chatterton earned his PhD from the University of Liverpool in the United Kingdom prior to taking up employment within the toxicology department of the Forensic Science Service (FSS) in 2001. Dr Chatterton is a board-certified forensic toxicologist (F-ABFT) and chartered chemist.

As a senior reporting officer and service delivery team leader, Dr Chatterton managed the National DUID and Road Traffic Alcohol units of the FSS before moving to Canada in 2011 to join the OCME as Deputy Chief Toxicologist; he was promoted to Chief Toxicologist in 2017.

Dr Chatterton is a member of numerous professional organisations including The Society of Forensic Toxicologists (SOFT), The International Association of Forensic Toxicologists (TIAFT), The Society of Hair Testing (SoHT), The American Academy of Forensic Sciences (AAFS) and The United Kingdom and Ireland Association of Forensic Toxicologists (UKIAFT).

As an active member of these organisations Dr Chatterton has chaired scientific workshops, presented numerous platform, poster and workshop presentations and completed a large number of abstract reviews. Dr Chatterton has published peer-reviewed papers and book chapters on forensic toxicology and drugs in hair analysis, and most recently, post mortem toxicology in Clarke's Analysis of Drugs and Poisons.

Dr. Vincent Cirimele

Vincent Cirimele studied biochemistry and pharmacology at the Louis Pasteur University of Strasbourg and received his Ph.D. in 1996 (thesis entitled “Analysis of Xenobiotics incorporated in Hair”, awarded by the French Academy of Pharmacy in 1998). In 2003, he founded ChemTox and became Scientific Director and CEO of this laboratory in 2009. He was listed as Expert by the Cour d’Appel de Colmar in 2007 and by the Court de cassation since December 2012 for forensic and analytical toxicology, blood alcohol determination, drugs of abuse and doping substances. He is mainly involved in Forensic toxicology investigations and especially in alternative tests such as hair testing. He developed and validated new analytical approach dedicated to xenobiotics detection in hair which leads him to be author and co-author of 12 book chapters and more than 300 papers and oral presentations. He is member of several scientific societies (TIAFT, SFTA, STC) including the Society of Hair Testing (SoHT) since its creation in 1995, and became SoHT board member in 2012.

Oral Presentation Abstracts

O1. Postmortem Hair Testing for Fentanyl and Analogs: Experimental, Forensic and Interpretative Aspects

Nicola Pigaiani[1], Marco Ballotari[2], Anna Bacci[3], Karen S. Scott*[4], Gregory G. Davis[4], Rossella Gottardo[3], Federica Bortolotti[3]

[1.] Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Italy.
[2.] Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, College of Medicine, Gainesville FL, US.
[3.] Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Italy.
[4.] Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, US.

In 2025, synthetic opioids, mainly illicit fentanyl, were responsible for approximately 73,000 overdose deaths in the United States, highlighting the urgent need for enhanced forensic and public health surveillance. In this context, hair testing emerges as a valuable toxicological matrix. It offers extended detection windows and is particularly useful in postmortem investigations, where traditional matrices (e.g., blood, urine) may be degraded or unavailable. This study aimed to assess the feasibility, reliability, and interpretative value of hair analysis for detecting fentanyl and its analogues in postmortem forensic casework. The broader goal was to evaluate the method's potential to support epidemiological mapping of opioid exposure and inform preventive strategies.

Hair samples (n = 250) were collected from all postmortem cases examined by the Jefferson County Coroner/Medical Examiner's Office between January and March 2023. These included a range of manners of death: overdoses, homicides, suicides, traffic fatalities, and natural causes. Standard toxicological analysis was performed on available biological matrices (blood, urine, vitreous humor, bile, or tissues), incorporating immunoassay screening and confirmatory gas chromatography-mass spectrometry (GC-MS).

Hair specimens were analyzed using a newly validated ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method. The method targeted fentanyl, other analogues and adulterants, and was validated per international forensic standards evaluating selectivity, linearity, sensitivity (LOD and LOQ), precision, accuracy, matrix effects, extraction recovery, carryover, and stability.

Hair samples tested positive for fentanyl or its analogues in 129 out of 250 cases (51.6%), including 92 males (71.31%) and 37 females (28.69%), with a mean age of 41.72 years. Manner of death among positive cases was classified as accident (n = 92), homicide (n = 14), natural (n = 10), suicide (n = 7), and undetermined (n = 6). Drug-related deaths (opioids, n = 76; unspecified drugs, n = 13) and firearm-related deaths (n = 19) were most prevalent. Fentanyl was identified in 125 of 129

positive hair samples, and 101 of those also contained analogues. Frequently detected compounds included norfentanyl (n = 83, 66.4%), β-hydroxyfentanyl (n = 42, 33.6%), acetylfentanyl (n = 16, 12.8%), 4-ANPP (n = 83, 66.4%), and despropionyl para-fluorofentanyl (n = 26, 20.8%). Xylazine, a common adulterant, was present in 51 cases (40.8%). Geospatial mapping identified several neighborhoods in Jefferson County with high concentrations of hair-positivity as well as xylazine-positive subjects. Age and cause of death were significant risk factors for hair positivity.

This study confirms that hair is a robust and informative matrix for detecting fentanyl and analogues in postmortem cases. It offers critical advantages in decomposed bodies or cases lacking viable blood or urine. Furthermore, hair testing enables localized surveillance of opioid spread, aiding forensic investigations and public health responses. Routine implementation of hair analysis could substantially enhance understanding and management of the synthetic opioid crisis.

O2. Investigation of the incorporation of tramadol, acetaminophen and ibuprofen in adults and larvae of Lucilia sericata

Frank Sporkert,*, Joëlle Baechler[1], Vincent Varlet[1], Jiri Hodecek[1]

[1] University Center of Legal Medicine (CURML), CHUV-HUG, Lausanne-Geneva, Switzerland

*Corresponding author

Routes of incorporation of drugs are a major issue in hair analysis. We aimed to investigate the adsorption and absorption of drugs on and in larvae and flies. Hair analysis procedures like washing, pulverization, digesting and extraction should be applied to detect selected analgesics.

In three different experiments, larvae and flies of Lucilia sericata had been exposed for several days to tramadol, acetaminophen (paracetamol), or ibuprofen incorporated in 100 g minced beef with concentrations expected after therapeutic administration and overdose. Before extraction or hydrolysis, flies and larvae were washed with methanol. The washing solutions had been analyzed for the target compounds. For the extraction, 3 different protocols had been applied:

A) Tramadol was extracted by micropulverized extraction using methanol as extraction solvent.

B) Acetaminophen was extracted after alkaline hydrolysis by direct liquid liquid extraction (LLE) with ethyl acetate.

C) Ibuprofen was extracted after alkaline hydrolysis, acidification and subsequent LLE with tert-butylmethylether.

Tramadol, acetaminophen and ibuprofen were analyzed by LC-MS/MS using their isotopic labelled homologues as internal standard. The separation was carried out on a Kinetex C18 analytical column (2.5 um 100 A, 100 x 2.1 mm). Tramadol and paracetamol were measured in positive ESI mode, ibuprofen in negative ESI mode. Flies and larvae were analyzed in a single piece. Flies weighed between 6 and 12 mg, with females being significantly heavier. Larvae weighed between 40 and 70 mg.

Tramadol could be detected only in flies exposed to high concentrated meat at concentrations of 23 and 81 pg/mg. Larvae were not analyzed. The washing solution in both experiments did not show any tramadol. Paracetamol was detected in adults exposed to therapeutic concentrations only in a few cases from 16 to 138 pg/mg, and in adults exposed to lethal concentrations from 125 to 862 pg/mg. There was no statistically significant difference between males and females.Ibuprofen was detected in flies exposed to therapeutic concentrations in all cases from 4 to 258 pg/mg, and in flies exposed to lethal concentrations from 281 to 2081 pg/mg. There was no statistically significant difference between males and females. In larvae, concentrations were significantly higher.

Concentrations from 3.2 to 22.2 ng/mg (exposed to therapeutic levels) and 44.7 to 153 ng/mg (exposed to lethal levels) were found.

Analytical protocols similar to hair analyses can be applied to flies and larvae for the detection of therapeutic drugs. Alkaline hydrolysis is the preferred method, since chitin unlike hair does not show swelling properties. For adult flies, adsorption on the chitin surface could not be observed. These findings are in contradiction to the observations in hair, where washing solutions often show the presence of drugs. That can be related to the almost hydrophobic nature of the chitin surface.

Larvae instead show higher concentrations of the absorbed therapeutic drugs which is probably due to their physiological state where food ingestion is the main goal for the development.

Keywords: alternative matrices, flies, larvae, drug incorporation, entomotoxicology, Lucilia sericata

O3. Hair Analysis in Psychiatric Patients: Quantification of Drugs of Abuse and Therapeutic Agents in Psychiatric Patients and Comparison with Pharmacological Regimen.

Alice Costantini, Elisabetta Pozza, Claudio Terranova, Donata Favretto

Forensic Toxicology, Legal Medicine, University Hospital of Padova, Padova, Italy

The analysis of drugs of abuse and pharmaceuticals in hair has gained increasing importance in clinical toxicology, offering significant advantages in terms of detection window and verification of compliance to therapy. This study aimed to evaluate and quantitate drugs of psychiatric interest, drugs of abuse, and new psychoactive substances (NPS) in keratinous matrices in order to be able to compare the results with the patient's clinical profile.

A cohort of 15 subjects hospitalized in psychiatric departments were selected based on medical history. Hair samples (0-3 cm) were collected from the posterior vertex region of the scalp and underwent a standardized decontamination procedure prior to analysis. A recently developed liquid-chromatography-tandem mass spectrometry (LC-MS/MS) method was used to identify and quantify drugs of abuse, therapeutic agents and NPS in a single run.

A total of 15 cases were examined. In 10 out of 15 patients, the presence of drugs corresponded with the compounds listed in their documented pharmacological regimen. Quantification of these analytes was performed using a seven-point calibration curve spanning 10–1000 pg/mg. For the remaining 5 patients, the administered pharmacological treatment was unknown, precluding direct comparison; of these, only one patient tested negative for all target analytes. The psychiatric drugs of interest that were identified included: bupropion, lamotrigine, trazadone, venlafaxine, fluvoxamine, quetiapine, promazine, levomepromazine, clotiapine, haloperidol, citalopram, clonazepam, triazolam, naltrexone, sertraline, carbamazepine, fluoxetine, amisulpride, perphenazine, amitriptyline, risperidone and methylphenidate. Regarding substances of abuse, analysis revealed the following distribution among the 15 patients: 13 tested positive for cocaine and its metabolites, 9 for cannabinoids, 6 for benzodiazepines, 4 for amphetamines and related analogues, 4 for methadone, 3 for opioids, 2 for ketamine, 1 for zolpidem, and 1 for tramadol. Only two patients tested negative for all screened substances. Finally, emerging NPS were detected in two patients, specifically 3,4-MDPHP and methylphenidate.

The results demonstrated that the developed method is highly sensitive, robust and reliable for simultaneous determination of substances of abuse, psychiatric drugs and NPS in hair, providing a valuable tool for retrospective toxicological investigations and therapeutic drug monitoring. Its application in psychiatric populations may provide valuable insights into substance use patterns, ultimately contributing to improved patient management and risk assessment.

O4. Hormonal profile differences associated with COVID-19 vaccination revealed by hair analysis

Paula Llabres[1,2], Vladimir Despotovic[3], Paul Wilmes[4], Guy Fagherazzi[5], Alba Iglesias-González[1], Linda R. Macheka[1], Brice MR Appenzeller[1]

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[5] Deep Digital Phenotyping Research Unit, DoPH, Luxembourg Institute of Health, Strassen, Luxembourg

The hormonal profile reflects individual characteristics and can serve as an indicator of health status. Given the cross-talk between the endocrine system and other systems in the body, such as the immune or nervous system, any alteration or activation of these systems may lead to changes in hormonal levels. Immune system stimulation, whether through exposure to pathogens or vaccination, can therefore influence the hormonal profile. The aim of this study is to investigate the link between SARS-CoV-2 vaccine and hormonal levels in hair.

This cross-sectional study used data from the CoVaLux study in Luxembourg, in which hair samples were collected and 37 hormones were quantified with LC-MS/MS. A total of 143 individuals were included in the analysis: 12 males and 18 females in the vaccinated group, and 41 males and 72 females in the non-vaccinated group. Principal Component Analysis (PCA) and Factor Analysis of Mixed Data (FAMD) were employed to visually explore differences in hormonal profile between vaccinated and non-vaccinated participants. Multivariate linear regression models were applied to assess hormone levels depending on vaccination status. Additionally, advanced methods such as DDR Tree and Latent Profile Analysis were used to identify specific patterns.

Preliminary results show two distinct hormonal profile clusters between vaccinated and non-vaccinated individuals. In males, significant differences were observed for 1 progestogen, 6 corticosteroids, and 1 androgen. In females, 4 corticosteroids and 1 thyroid hormone differed between groups. Overall, the trend indicates that vaccinated participants show lower hormone levels than non-vaccinated individuals.

This study suggests that vaccination is associated with changes in the hormonal profile, potentially mediated by immune system activation and its close interaction with the endocrine system. Further

research is needed to clarify the implications of these changes and the mechanisms underlying immune–endocrine interactions.

Keywords: SARS-CoV-2 vaccine, hormones, hair analysis

O5. Hair Glucocorticoid and Endocannabinoid Quantitative Profile as Biomarkers of Stress During Pregnancy

Brooke Fontaine[1], Alexis Palma[1], Mamta Fuloria[2], Maureen J. Charron[3], Marta Concheiro[1]

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[2] Division of Neonatal-Perinatal Medicine, Department of Pediatrics, The Children's Hospital at Montefiore and Albert Einstein College of Medicine, Bronx, NY, USA
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Chronic stress can lead to long-lasting health problems, such as anxiety, depression, and heart disease. During pregnancy, it can have deleterious health effects such as shortened gestation and a higher risk of neurodevelopmental and cardiometabolic disease across the life course. Glucocorticoids (cortisol) and endocannabinoids (lipid-based neurotransmitters) regulate the human stress response. Hair is an ideal matrix for monitoring stress biomarkers; it offers a long-time window and is not affected by circadian variations. The goal of this study was to determine glucocorticoid (cortisol and cortisone) and endocannabinoid (anandamide, AEA; 2-arachidonoylglycerol, 2-AG; oleoylethanolamide, OEA; palmitoylethanolamide, PEA) concentrations in hair samples from pregnant women throughout pregnancy.

Cortisol, cortisone, AEA, 2-AG, OEA, and PEA were quantified in hair samples employing a previously validated method. Twenty mg of pulverized hair were incubated in 1 mL of methanol:acetonitrile (75:25) in an ultrasound at 55°C for 2h, and analyzed by liquid chromatography-tandem mass spectrometry, using ESI in positive and negative mode. The limits of quantification were 1 pg/mg for cortisol and cortisone, 10 pg/mg for AEA, 20 pg/mg for 2-AG, and 100 pg/mg for OEA and PEA. The hair samples were collected as close as possible to the scalp from a cohort of 35 pregnant women who delivered at Jack D. Weiler Hospital in the Bronx, NY, between 5/11/22-1/26/23 (NICHD; 3R01HD092533-05S1 study), and stored at room temperature in the dark until analysis. A total of 66 3-cm-segments across the three trimesters (32 from the first, 18 from the second, and 16 from the third) were analyzed. Thirteen cases had segments for all three trimesters.

Out of the 66 segments analyzed, glucocorticoids were detected in 62 cases (cortisol in 62, cortisone in 54), and endocannabinoids in all of them (OEA and PEA in all, 2-AG in 53, AEA in none). Regarding glucocorticoid concentrations, cortisol ranged from 1 to >500 pg/mg, median 3.4 pg/mg (n=62), and cortisone from 1-60 pg/mg, median 3.8 (n=54). In the three segments with cortisol concentrations >500 pg/mg, the cortisone concentrations remained around 4-7.8 pg/mg. The median cortisol-to-cortisone ratio was 1.4 (n=51), excluding cases with cortisol >500 pg/mg. Regarding endocannabinoids, 2-AG concentrations ranged from 24 to 341 pg/mg, median 72 pg/mg (n=53), OEA ranged from 178-140,754 pg/mg, median of 1,637 pg/mg (n=66), and PEA from 422-42,267

pg/mg, median 4,417 pg/mg (n=66). AEA was negative in all samples. In the 13 cases with segments from the three trimesters, no significant changes for endocannabinoids were observed throughout pregnancy, and a decrease was observed for cortisol and cortisone. These findings demonstrate that hair analysis enables reliable, longitudinal assessment of glucocorticoids and endocannabinoids during pregnancy, supporting its utility as a non-invasive monitoring tool.

Keywords: cortisol, endocannabinoid, stress biomarker, hair, LC-MSMS

O6. Impact of Segment Length and Environmental Conditions in Segmental Hair Analysis for Adherence Monitoring of HIV Drugs

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In the clinical management of HIV, objective biomarkers for long-term adherence to antiretroviral drug therapy (ART) can circumvent virologic failure and the emergence of drug resistance. While hair drug concentrations have been recognized as a powerful tool for monitoring cumulative drug exposure, the longitudinal stability of analytes within the hair shaft remains a critical variable in data interpretation. This study aims to evaluate the consistency of ART hair concentrations using segmental analysis, specifically investigating the impact of segment length and distance from the scalp on the accuracy of adherence assessment.

Hair samples were obtained from adherent volunteer patients receiving stable ART regimens containing tenofovir (TFV) and emtricitabine (FTC), dolutegravir (DTG), or cabotegravir (CAB) and rilpivirine (RPV), under the Institutional Review Board (IRB)-approved UCSF Shaved Heads Study. For segmental analysis, a hair sample was cut into 1 cm segments from the proximal end closest to the scalp. For long-term stability studies, hair samples 3–6 cm in length were used. For both analyses, hair samples were cut into 1–3 mm pieces with scissors and weighed at approximately 2-5 mg. Each sample was analyzed using a validated LC-MS/MS method approved by the NIH's Division of AIDS Clinical Pharmacology and Quality Assurance (CPQA) program.

At room temperature and protected from light, all drugs were stable in hair: TFV and FTC levels remained stable for more than 5 years, DTG for more than 7 years, and CAB and RPV for more than 3 years. Segmental analysis showed that hair concentrations of TFV (up to 3 cm), CAB (up to 5 cm), and RPV (up to 5 cm) remained constant. In contrast, FTC hair concentration declined and was significantly lower in the 4 cm segment (43.0 ± 25.0%) compared to the proximal 1 cm segment ($p < 0.05$). DTG hair concentration showed a slight decline at the 3 cm segment (89.3 ± 7.6%) compared to the proximal 1 cm segment ($p < 0.05$). Additionally, some samples exhibited extra peaks, as previously reported (Sykes et al., Anal Bioanal Chem, 2018), which increased by up to 9% relative to the DTG peak area over time.

Segmental hair analysis appears to be a useful biomarker for assessing ART adherence over time, although the reliability may be limited for FTC. In this study, DTG showed a slight decline in distal segments, possibly due to light sensitivity, whereas the reason for the more significant FTC decline remains unclear. These findings suggest that analyzing long hair sections (e.g., >4 cm) as an overall measure of FTC may increase the risk of false-negative results and misclassifying adherent patients

as non-adherent. FTC comes co-formulated with TFV, so we recommend focusing on the latter for adherence measurement. Overall, for better diagnostic accuracy, clinical hair testing should focus on the proximal 1–3 cm of hair (preferably 1.0 cm) to minimize the risk of environmental degradation. Since 3 cm represents ~3 months of exposure, we recommend quarterly analysis of hair levels when using these as a research tool in HIV treatment and prevention.

Keywords: HIV, Adherence, Segmental analysis

O7. Hair and Drug Patch (Sweat) Testing: A Case Review

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Hair testing is a well-established monitoring tool, servicing the family law market in the UK. In 2024 our Laboratory introduced Drug Patch (sweat) testing. Hair and Sweat testing are individually robust testing methods that are used to monitor an individual's drug use / abstinence; however, combining them provides a multi-layered approach and a more comprehensive insight. This study presents a real-life example demonstrating this.

The hair samples were sectioned, except in cases of body hair, and weighed. The accredited method involves washing the hair with methanol twice to decontaminate the hair and then homogenising the sample prior to being extracted. They were then analysed using GC-MS/MS or LC-MS/MS. The drug patches were tested by an independent testing laboratory with validated methods using LC-MS/MS.

Hair testing is a powerful and practical way to establish repetitive historic drug use. The hair testing in this case supported that both parents had been using cannabis. However, both ceased using cannabis when the pregnancy was discovered. Due to the way in which hair grows, it can take many months for a donor to return a not detected result following abstinence. This can be even more problematic to demonstrate if a donor does not have scalp hair. Both parents underwent drug patch testing, which was negative, demonstrating their cessation.

The case study demonstrated that both mum and dad had been users in the past, but with both recently ceasing drug use there was a need to demonstrate this to the court quickly to allow the baby to remain in their care once born. Therefore, by undertaking drug patch testing, along-side hair testing, changes in use were demonstrated in a much more realistic timeframe. This combined testing method allowed the baby to be kept in the care of both parents.

Keywords: Hair, Drug Patch, Sweat, abstinence

O8. Does CBD-containing beard oil interfere with THC-hair analysis?

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Cannabidiol (CBD), a non-psychoactive compound derived from Cannabis sativa, has gained increasing attention for its potential therapeutic applications across a range of conditions. As CBD use becomes more widespread, there is growing interest in reliable methods for detecting and quantifying CBD in hair and to investigate its influence on toxicological analysis. This study aimed to investigate the analytical consequences of the application of a CBD-containing beard caring oil for routine toxicological (hair) analysis.

In this single-case study, a full-bearded healthy male volunteer without prior cannabis consumption externally applied a beard caring oil daily over a period of 28 days. The product (Malantis CBD BarberOil) was labelled as containing 0.5% CBD and being free from THC. Beard specimens were collected during the application period on days 0 (before application and after first application), 1, 2, 7, 14, and 21. Additional beard and head hair specimens were collected 1 day, 4 weeks, 8 weeks, and 12 weeks after the last application, respectively. The hair samples were cut into snippets and digested under alkaline conditions followed by a liquid-liquid extraction. Analysis was carried out using an ESI-LC-MS/MS/MS method for THC, CBD, CBN, HHC, THC-A, OH-THC, and COOH-THC. The method was fully validated for the following criteria: selectivity, linearity, limit of detection (LOD), lower limit of quantification (LLOQ), precision, accuracy, matrix effects, and recovery.

The LLOQ were 5 pg/mg (THC, CBD, CBN, HHC, THC-A), 2 pg/mg (OH-THC), and 0.1 (COOH-THC) pg/mg, respectively. Validation criteria for selectivity, linearity, LOD, matrix effects, recovery, accuracy and precision were fulfilled. The initial beard hair sample before application was tested negative for CBD and all other substances. After application of the beard oil, CBD could be detected in all beard samples during the treatment. CBD concentrations were far above the highest calibrator and ranged from approximately 24'000 to 40'000 pg/mg. Surprisingly, also THC, CBN, and THC-A could be detected in all beard hair samples during the treatment. Concentrations ranged from 38 – 73 pg/mg for THC, 16 – 29 pg/mg for CBN, and 17 – 82 pg/mg for THC-A. At the end of the 4 weeks application, CBD could be detected in a head hair sample at 960 pg/mg. After discontinuation of the treatment, CBD could be detected in beard and head hair samples for as long as 8 weeks after the last application. THC could not be detected in the beard hair samples after discontinuation. Furthermore, THC metabolites could not be detected in any of the THC positive samples. The results show that CBD-containing cosmetic products are not free from THC and

therefore can influence toxicological results and their subsequent interpretation. After applying the cosmetic product to the beard, CBD and THC were detected therein, and even the corresponding head hair sample was tested positive for CBD.

These results prove that it is strongly recommended to ask for any cosmetic treatments of hair (not just head hair) before conducting hair analysis and that positive THC results should always be confirmed by analyzing the corresponding THC metabolites (OH-THC and COOH-THC).

Keywords: CBD, beard hair, THC-analysis

O9. Further insights into the influence of permanent chemical straightening on the interpretation of endogenous GHB hair results

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Cosmetic hair treatments are known to affect the interpretation of drug concentrations in hair; however, the effects of permanent chemical hair straightening remain insufficiently characterized. In a previously case report, we described marked analyte-dependent changes in hair concentrations following a thioglycolate-based (alkaline) straightening treatment. The present study extends this work by evaluating the effect of a glyoxylic acid-based hair straightening treatment on endogenous gamma-hydroxybutyrate (GHB) concentrations in hair.

Hair samples were collected from five female participants (two brown, two black, one blond-grey) before and immediately after a commercial chemical hair straightening treatment. Participants reported no consumption of drugs of abuse or medications during the period represented by the analyzed hair segments, and no previous cosmetic treatments other than the straightening procedure. A glyoxylic acid-based formulation was used (Goa Organics). Two strands of equal length were obtained from each participant and segmented into 0.5 cm sections. All segments were analyzed except the proximal 0.5 cm segment, which was excluded to minimize potential external contamination from sweat and sebaceous secretions. Endogenous GHB was determined using a validated LC-MS/MS method previously described by Blanco-Ces et al. [1].

GHB was detected in all hair segments prior to the straightening treatment, with concentrations ranging from 0.346 to 1.109 ng/mg (median: 0.589 ng/mg) across the five participants. Following glyoxylic acid-based straightening, heterogeneous effects were observed. In one participant, GHB became undetectable in all segments after treatment (pre-treatment median: 0.555 ng/mg). In two participants, increased concentrations were observed, with mean increased changes ranging from approximately 1.2 to 1.5-fold (pre-treatment medians: 0.882 and 0.609 ng/mg vs. post-treatment medians: 1.134 and 0.931 ng/mg, respectively). In another participant, a mixed pattern was observed. Only 10 out of 16 segments remained detectable after treatment (the 6 proximal segments were undetectable), despite all segments being positive before straightening (pre-treatment median: 0.559 ng/mg). Among the detectable segments, five showed a decrease (0.8-fold) and five an increase (1.2-fold). In the remaining participant, a moderate decrease (0.8-fold) was observed (pre-treatment median: 0.580 ng/mg vs. post-treatment median: 0.500 ng/mg). These findings differ from those previously reported after alkaline permanent straightening based on

ammonium thioglycolate, where marked and consistent increases in GHB concentrations (6-43-fold) were observed in the single participant studied. No clear association between hair color and the observed changes were identified. The heterogeneous patterns observed suggest that the impact of hair straightening on endogenous GHB depends on the chemistry of the treatment and may involve both increases and loss of detectability.

Glyoxylic acid-based permanent hair straightening can alter endogenous GHB concentrations in hair in different ways, including increases, decreases or even loss of detectability, with potential implications for result interpretation. However, due to the limited number of samples analyzed, additional studies are needed to better characterize these effects and to assess their impact on other analytes. Overall, these findings highlight the importance of considering cosmetic hair treatments when interpreting GHB concentrations in hair samples.

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2. **Keywords:** Gamma-hydroxybutyrate (GHB); Hair; Cosmetic Treatment; Chemical Straightening; Glyoxylic acid

O10. LC-MS/MS determination of cortisol and cortisone in human hair and application to alcohol consumption assessment in a driving licence cohort

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Measuring cortisol and cortisone in human hair has proven to be an effective method for assessment of chronic glucocorticoid exposure. While blood, urine, or saliva matrices show momentary or short-term fluctuations, hair provides a retrospective insight into cumulative hormone secretion over weeks to months. This approach is particularly useful for investigating long-term dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, which plays a crucial role in the pathophysiology of several metabolic, cardiovascular, and neuropsychiatric disorders.

This research aimed to develop and validate an LC–MS/MS method for the determination of cortisol and cortisone in hair, and subsequently apply it to a cohort of subjects undergoing driving suitability assessment, with varying levels of ethyl glucuronide in hair (hEtG) (hEtG<5 pg/mg, hEtG=5-30 pg/mg, hEtG>30 pg/mg), in order to explore potential correlations between glucocorticoid levels and hEtG concentration.

Hair samples (3 cm) were collected, decontaminated and pulverized, following SoHT guidelines (1). Subsequently, 20 mg of hair were incubated with 1 mL methanol for 30 min, evaporated to dryness, reconstituted with 50 µL of mobile phase, and injected into the LC-MS/MS system. LC-MS/MS analysis was performed using an ACQUITY UPLC H-Class system coupled to a Xevo TQ-XS triple quadrupole mass spectrometer (Waters Corp., Milford, USA). Chromatographic separation was performed with a CORTECS T3 analytical column, using 0.1% formic acid and acetonitrile as mobile phase. As cortisol and cortisone are endogenous compounds, the entire validation process was carried out using two surrogate analytes,13C-labeled cortisol and 13C-labeled cortisone, and based on "Standard Practices for Method Validation in Forensic Toxicology" guidelines (2). The validated method was applied to 227 individuals from a cohort of driving licence assessment subjects who had been previously tested for hEtG.

Chromatographic elution of both analytes was achieved in 3.5 min, with a total chromatographic run of 11 min. The method was fully validated in terms of limits of detection (0.1 pg/mg) and quantification (0.5 pg/mg), linearity (0.5-250 pg/mg), imprecision (%CV <19.0%) and accuracy (82.2-100%). Moreover, selectivity showed no endogenous or exogenous interferences. Matrix effect were

<19.3%, recovery >91.9% and total process efficiency >92.1%. No carryover was observed, and autosampler stability was confirmed after 72 h at 6ºC (%loss < 20%).

Analysis of real specimens showed that hair cortisol levels increased significantly with alcohol consumption and were positively associated with hEtG concentrations ($p<0.05$), supporting an association with HPA-axis activation and dysregulation. In contrast, cortisone showed inconsistent or weak associations ($p=0.891$). Furthermore, absolute cortisol and cortisone levels were also influenced by biological and external factors such as sex, cosmetic hair treatments, pigmentation, and age. On the contrary, the cortisol/cortisone ratio emerged as the most robust marker found in this study, showing a strong association with alcohol intake ($p<0.001$), independently of major biological covariates.

These findings support the added value of integrating hair glucocorticoid analysis into alcohol consumption assessment, with the cortisol/cortisone ratio providing a more reliable and less confounded biomarker than individual analytes. This approach may enhance the interpretation of chronic alcohol exposure and its physiological impact in forensic and clinical settings.

Keywords: hair analysis, cortisol, cortisone, stress, hypothalamic–pituitary–adrenal axis, ethyl glucuronide

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O11. hEtG and fitness to drive: the results of a two-year longitudinal study at the University of Verona

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According to Italian legislation, DUI offenders with a blood alcohol concentration higher than 0.5 g/l have their driving license suspended and, prior to restoring their driving privileges, must be examined on the basis of anamnestic data and toxicological analyses, including ethyl glucuronide in hair (hEtG). The present study aimed to evaluate, according to a longitudinal perspective, hEtG data obtained from people undergoing toxicological analyses to assess their fitness to drive, after having their license withdrawn for driving under the influence of alcohol.

The study included n=697 individuals (621 male and 76 female) referred by the Verona CML from September 2023 to September 2025. hEtG determinations were performed at the Forensic Toxicology Laboratory of the University of Verona using a highly sensitive LC-MS/MS method. Hair sampling procedures were carried out according to SoHT guidelines. The subjects were classified on the basis of hEtG concentrations, taking into consideration the cut-off proposed by SoHT (< 5pg/mg, group A; <30 pg/mg, group B; ≥30 pg/mg, Group C). Excessive alcohol intake (hEtG ≥30 pg/mg) was considered cause for unfitness to drive. Over the course of 2 years, each subject has undergone 1 to 4 hEtG determinations, depending on anamnestic-clinical data and previous hEtG results.

As per group A, hEtG proved to be an effective marker for identifying individuals at low risk of alcohol-impaired driving, since most subjects in this population showed hEtG < 5 pg/mg at evaluations following the first.

Most individuals with hEtG ≥ 30 pg/mg (Group C) showed a decrease in hEtG levels in subsequent samples, demonstrating that they benefited from detoxification treatments initiated after the first hEtG result.

Finally, individuals with 5 < hEtG < 30 pg/mg at the second and/or third determination showed heterogeneity, with some showing similarity to the group A and others to the group C. Overall, the analysis of hEtG results brought then to identify two main populations rather than three. Individuals with hEtG ≤ 5 pg/mg (abstinent or occasional drinkers) and those with hEtG > 5 pg/mg (regular alcohol consumers).

The study demonstrated the fundamental role of hEtG as a biomarker in identifying alcohol abuse conditions that may pose a risk for driving under the influence of alcohol, and in monitoring the

effectiveness of detoxification programs to which CML-referred individuals may be directed. These findings support the inclusion of hEtG in CML protocols, to be used with timing and application tailored to individual cases.

Keywords: hair, ethylglucuronide, driving licence regranting

O12. Quick Hair Tox: Development of a Rapid Extraction Method for the Detection of Therapeutics and Illegal Substances in Hair

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Delirium is a common and serious complication in intensive care units, characterized by disturbances in attention, reduced consciousness, and altered perception of reality. It is associated with increased healthcare costs, higher mortality, and worse long-term outcomes. Unintentional discontinuation of prescribed psychotropic medication or illicit substances during hospitalization can contribute to delirium. Therefore, rapid clarification of a patient's medical and substance use history is essential, but sometimes—especially in neurocritical care—inaccessible due to the patient's comatose state. A recent pilot study demonstrated that hair analysis can provide objective insights into medication and substance use prior to hospital admission and can also identify endogenous compounds as predictors for delirium (1). However, current methods for hair sample preparation are time consuming and thus unsuitable for acute clinical settings. To facilitate the use of routine forensic hair analysis in clinical applications, we aimed to develop a rapid and reliable hair sample preparation method for 119 substances—including opioids, stimulants, benzodiazepines, Z-drugs, antidepressants, neuroleptics, and analgesics—using the Biotage® Lysera bead-mill homogenizer. Our goal was to obtain same-day hair analysis results, thereby creating a feasible and clinically applicable diagnostic tool.

For the validation of this method, 20 mg of authentic hair was used per sample. A one-step extraction was performed with 400 µl extraction agent (1-mM aqueous ammonium formate containing 0.1% formic acid/methanol (1:1, v/v)) and 100 µl internal Standard. Each sample was extracted for 3x3 minutes with a one-minute dwell time between cycles, resulting in a total extraction time of eleven minutes. Purification was achieved by centrifuging the samples for 10 minutes through a 0.45 µm PVDF centrifugation filter. Substances were analyzed using an LC-MS/MS system (Shimadzu Prominence HPLC system coupled to a SCIEX QTrap 6500+) with positive electrospray ionization in scheduled MRM mode. The method was fully validated in terms of calibration model, selectivity, recovery, accuracy, precision and matrix effects.

A quadratic calibration model was identified as the most appropriate approach for most analytes. The method demonstrated high selectivity, enabling reliable differentiation of the respective target analytes from potential interfering substances. Recovery rates exceeded 50% for all analytes. Accuracy was successfully validated for two quality control levels. Precision was confirmed for most analytes, with only a few exceptions at the lower concentration range. Matrix effects were generally acceptable, with only a small number of analytes exceeding ±30%.Furthermore, hair samples collected as part of the pilot study (1) were reanalyzed using the new sample preparation method, yielding consistent and comparable results. This confirmed the reliability and equivalence of the new approach.

The validated method presented here significantly accelerates the hair analysis workflow, reducing the total processing time from several days to less than 24 hours by markedly shortening the extraction step. This substantial time gain makes the method suitable for clinical applications. Faster hair analysis enables clinicians to identify potential substance use or undisclosed medications more rapidly and adjust treatment plans accordingly. Ultimately, the implementation of this method can contribute to reducing mortality, improving patient outcomes, and lowering healthcare costs in neurocritical care.

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Keywords: Delirium; Rapid toxicology screening; LC-MS/MS quantification

O13. Analysis of chiral amphetamine in hair – validation and application

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In Norway, amphetamine is usually prescribed as pure dexamphetamine (S-enantiomer) or as lisdexamphetamine, a prodrug that is converted to dexamphetamine in the body after ingestion. Illegal amphetamine is generally racemic, consisting of equal parts of R- and S-Amphetamine. To distinguish the use of prescribed medical amphetamines from the use of illegal amphetamines, chiral analysis is used. Such an analysis in hair will be able to reveal any intake of illegal amphetamines back in time. Amphetamine is one of 21 analytes in the routine hair screening method (achiral) in our lab and is often detected. In many cases we observe concentrations above our calibration range (2.5 ng/mg). The chiral method was developed as an extension to the screening method with the addition of one more Std and QC to cover the higher concentration range. The method was validated using ultra-high performance supercritical fluid chromatography-tandem mass spectrometry (UHPSFC-MS/MS). The applicability of the method was assessed by analyzing amphetamine positive authentic hair samples both during the validation and after the method has been implemented in the routine.

Hair samples were cut into 2 cm segments, weighed (20 mg) and finely cut into snippets using a pair of scissors. 2-propanol was added to all the segments for a 10-minute wash in a shaking water bath. After removing the washing solutions, extraction buffer and internal standard were added to each tube. The samples were incubated at 37 ºC (±1 %) overnight. The next day, tubes were centrifuged and the supernatant was pipetted over to a different tube for evaporation in vacuum centrifuge. The samples were then reconstituted with 100 µL 5% methanol, mixed and transferred to vials with inserts. Separation was performed on Waters Acquity UPC2 system using Chiralpak AD-3 column. Analytes were eluted with a 4-minute isocratic gradient with 6.5% mobile phase B. Injection volume was 0.3 µL. Analytes were detected both by using Waters Xevo TQ S and TQ-S µ tandem quadrupole mass spectrometer in positive electrospray ionization and multiple reaction monitoring (MRM) mode.

The five-point calibration curve was linear in the range of 0.05 to 20 ng/mg, with coefficient of correlation (R) of 0.9999 for both R- and S-Amphetamine. Coefficient of variation for within-day precision was < 2.0% for all QC levels and both enantiomers, while for between-day precision it was < 2.2%. Accuracy for all QC levels and both enantiomers was < 6.0%. The newly developed chiral method has shown good compliance with the routine screening method in hair. Amphetamine was found in all analyzed samples with more than 60 % of the samples containing equal amounts of R- and S-enantiomer.

A specific and robust UHPSFC-MS/MS method for quantification of R- and S-amphetamine in hair has been developed and validated. This method has been successfully implemented in our routine laboratory since January 2026.

Keywords: Chiral amphetamine, supercritical fluid chromatography, method development, validation

O14. Optimization of an LC-MS/MS Method for Cortisol and Cortisone Determination in Hair and Its Application in a Population Study

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Hair cortisol is a useful non-invasive biomarker for retrospective assessment of long-term hypothalamic-pituitary-adrenal axis activity. Simultaneous measurement of cortisone may provide complementary information and support interpretation in population-based research. However, the determination of both analytes in hair remains analytically challenging due to their low concentrations and the complexity of the hair matrix.

A preliminary LC-MS/MS method for cortisol and cortisone in hair was optimized with emphasis on sample preparation and analytical performance. The original protocol included a simplified washing procedure and a 90-minute sonication extraction. The optimized workflow incorporated sequential washing with water and isopropanol, extended air-drying, and a 4-hour sonication step under controlled temperature conditions prior to evaporation and reconstitution. Cortisol-d4 was used as the internal standard. The optimized analytical workflow was applied to 150 hair samples collected from 29-year-old adults from Chile's oldest birth cohort (Santiago Longitudinal Study) in collaboration with the Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile.

Compared with the initial workflow, the optimized method showed improved chromatographic performance, including better peak definition and signal quality for both cortisol and cortisone. These changes supported more reliable detection and integration of low-level analytes in hair extracts. When applied to the population samples, the method enabled the simultaneous determination of cortisol and cortisone across the analyzed cohort. A subset of samples showed detectable cortisone in the absence of quantifiable cortisol, highlighting the value of measuring both analytes in hair. Detailed concentration distributions across the cohort are presented.

The refined LC-MS/MS method provides a reliable approach for the simultaneous determination of cortisol and cortisone in hair. The optimized sample preparation workflow improved analytical performance in a complex biological matrix and supported application in a population-based study. These findings support the feasibility of dual glucocorticoid measurement in hair for large-scale research settings. Funding: FONDECYT-1210283, ACT210006.

Keywords: hair cortisol, cortisone, LC-MS/MS, method optimization, hair analysis

O15. Determination of ethyl glucuronide in hair following derivatization with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC). Development and validation of a method using liquid chromatography-high-resolution accurate-mass Orbitrap mass spectrometry and parallel reaction monitoring mode

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The determination of ethyl glucuronide (EtG) levels in hair (hEtG) has been established as one of the most reliable biomarkers for long-term alcohol consumption. In a recent paper we described a novel analytical approach to determine EtG in biological fluids, including hair [Frison G et al. Rapid Commun Mass Spectrom. 2026; 40(4):e70000]. This involves the derivatization of EtG and D5-EtG with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) in aqueous solution, and the analysis of the derivatives obtained using liquid chromatography-high-resolution accurate mass Orbitrap mass spectrometry (LC-HRAM-Orbitrap-MS).

Here we present the development and validation of a method to determine hEtG levels, following EDC derivatization, using LC-HRAM-Orbitrap-MS and parallel reaction monitoring acquisition mode.

Hair samples were externally decontaminated and pulverized. 30-mg hair aliquots were sonicated for 2 hours after addition of 800 µL of deionized water/MeOH (7:1, v/v) and D5-EtG. The mixtures were centrifuged and the supernatants taken to dryness. The residues were derivatized with 50 µL of 10 mM aqueous EDC solution at 25 °C for 30 minutes. The reaction mixtures were reduced to dryness, reconstituted with 50 µL of methanol:LC mobile phase A, and 5-µL aliquots injected into the LC-HRAM-Orbitrap-MS instrument.

LC-HRAM-Orbitrap-MS determinations were performed using a Thermo Scientific Exploris 120 MS system, and a Phenyl Hexyl analytical column (100 × 2.1 mm, 2.6 µm) at 40 °C. MS acquisition was performed in parallel reaction monitoring (PRM) mode, with mass resolution 30,000; AGC Target 1x105; maximum IT 100 ms. Precursor ions m/z values were 378.2235 (EDC-EtG) and 383.2549 (D5-EDC-EtG); product ions m/z values were 307.186, 261.145, 198.076 (EtG-EDCA); 250.128, 204.087, 168.066 (EtG-EDCB); 312.218, 261.145, 198.076 (D5-EtG-EDCA); 255.160, 204.087, 168.066 (D5-EtG-EDCB); quadrupole resolution was 0.4 m/z, collision energies were from 15 to 25 eV.

The method was fully validated by assessing limits of detection (LOD) and quantification (LOQ), linearity, intra and inter day (intermediate) precision, bias (trueness), matrix effects, recovery, carry-over, and measurement uncertainty. The LOD and LOQ were 1 pg/mg and 3 pg/mg, respectively. The calibration model showed good linearity over the 0–300 pg/mg range (R^2 = 0.990). Across the 30–300 pg/mg range, precision in repeatability conditions was 4.0%, intermediate precision was 6.9%, and bias was 6.0%, with both precision and bias showing expected increases (up to about 24% and 49%, respectively) as concentrations approached the LOD. Recovery values obtained from different hair samples ranged from 90% to 120%. No carry over was observed after injections of samples spiked at 400 pg/mg. The combined standard measurement uncertainty, derived from matrix variability, intermediate precision, and bias, was 9.2% within the validated range. A guard band of 4 pg/mg at the 30 pg/mg threshold was established to support forensic interpretation. Proficiency tests confirmed the suitability of the method for routine forensic analysis.

The developed and validated method combines the benefits of a simple, fast, reproducible, inexpensive, and safe chemical derivatization with those of HR-MS, i.e. high analytical specificity and sensitivity. The method meets the hEtG cut-offs established by the SoHT and is routinely applied in our laboratory for clinical and forensic toxicology cases.

Keywords: Ethyl glucuronide, hair analysis, chemical derivatization, liquid chromatography - mass spectrometry, orbitrap, forensic toxicology

O16. Ethyl glucuronide in hair: a ten-year overview

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This 10-year retrospective study evaluates hair ethyl glucuronide (EtG), a direct metabolite of ethanol, in a large Northern Italian cohort (N=68,221 samples collected between 2013 and 2022). The analysis aimed to assess long-term alcohol consumption by examining the distribution of EtG according to age, gender, recidivism, referral context, sampling region, hair length, and the impact of seasonality and the COVID-19 pandemic.

Hair EtG concentrations were determined using HPLC-MS/MS, and samples were classified according to the Society of Hair Testing (SoHT) recommended cut-offs: EtG≤5 pg/mg (consistent with abstinence), 5<EtG<30 pg/mg (indicative of repeated alcohol consumption), and EtG≥30 pg/mg (indicative of chronic excessive alcohol consumption). A descriptive sensitivity analysis using SoHT cut-offs was performed to ensure international comparability.

The percentage of hair samples classified as repeated or chronic excessive alcohol consumption was 13.6% (N=9,281) and 5.6% (N=3,818), respectively. EtG concentrations varied significantly by gender, age and referral context. Statistically significant differences were also observed among head, chest, axillary, and pubic hair samples. Higher EtG concentrations were detected in the proximal 3-cm segment of head hair compared to the 3-6 cm segment ($p = 2.7353 \times 10-42$), as well as across seasons ($p = 1.4591 \times 10-14$), with higher values during colder months. No measurable short-term population impact of COVID-19 was detected ($p = 0.0980$); however, a significant long-term effect was observed ($p = 4.3000 \times 10-6$).

Overall, despite considerable variability of EtG concentrations, this study provides robust evidence supporting the reliability of hair EtG testing and offers a comprehensive overview of long-term alcohol consumption trends in a monitored Italian population.

Keywords: EtG, Hair Analysis, Addiction

O17. Improved Extraction of Drugs of Abuse in Hair with Preserved Analyte Integrity

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Hair drug testing is a reliable and practical approach to toxicological analysis. Hair samples are not only easy to collect but also are more resistant to tampering than other matrices such as urine and can be stored at room temperature for a long period of time. These advantages make hair an ideal matrix for long-term substance use monitoring. Previous methods used for the analysis of drugs of abuse (DoA) in hair have relied on an acid digestion to extract compounds from the hair matrix, using reagents such as trifluoroacetic acid (TFA) or hydrochloric acid (HCl). However, acid digestion methods, such as 0.5% TFA in methanol, have been shown to cause degradation of certain benzodiazepines, including temazepam and oxazepam. In contrast, the present method preserves analyte integrity while enabling efficient extraction of a broad range of compounds. This work proposes a high-throughput method for screening 35 DoA in hair, combining a novel sample preparation approach with laser diode thermal desorption tandem mass spectrometry (LDTD-MS/MS).

For the previous extraction method, hair (10 mg), cut into small pieces, were transferred in a vial. 2 mL of TFA (0.5% in methanol with ISTD) were added and samples digested at 60ºC for 1hr 45mins. After 15 minutes of sonication, samples (500 µL) were mixed with 200 µL of a solution of KH2PO4 (1 mM) / BSA (100 µg/mL) in water. An 8 µL aliquot was spotted onto a 96-LazWell plate, dried, and analyzed by high-throughput LDTD-MS/MS. For the new extraction method, hair samples (20 mg) were pre-washed with methanol, pulverised with metal beads, and extracted with 1 mL acetonitrile and 50 µL internal standard. After 1 hour at 60 ºC and 15 minutes of sonication, 100 µL of extract was mixed with 30 µL of a desorption solution. A 6 µL aliquot was spotted onto a 96-LazWell plate, dried, and analyzed by high-throughput LDTD-MS/MS (less than 10 seconds per sample). Two panels were used to ensure adequate data points per peak.

Both extraction methods were compared to highlight similarities and differences, and the following criteria were used to assess both methods. Linearity obtained was higher than 0.99 ($R > 0.99$) using a 3-point calibration (1X, 2X, 5X cutoff). Precision and accuracy were assessed in triplicate, expressed as %CV and %Bias. Extracted samples were stable for 1 hour dry and 24 hours wet at room temperature. QC samples (0.5X and 2X cutoff) were correctly classified across two runs. Cross-validation with LC-MS/MS and LDTD-MS/MS (n=40) showed substantial to near-perfect agreement (Kappa: 0.78–1.00). All results met validation criteria, except for benzodiazepines in the first method presented. Real samples were evaluated.

Pulverized hair, followed by acetonitrile extraction with heat and sonication, enables the analysis of a broad range of analytes, including benzodiazepines, without degradation, unlike previous acidic digestion methods.

Conflict of Interest Disclosure: I have a financial relationship with Phytronix Technologies as a salaried employee.

Keywords: Drugs, Hair, High-throughput, LDTD-MS/MS, Pulverised Hair

Poster Presentation Abstracts

P1. Global PFAS legislation and hair biomonitoring: do regulatory patterns emerge in human hair?

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Per- and polyfluoroalkyl substances (PFAS) are increasingly regulated worldwide, with many jurisdictions restricting legacy compounds such as PFOA and PFOS under frameworks including the Stockholm Convention and regional chemical regulations. Despite these efforts, PFAS remain highly persistent in the environment, raising questions about how rapidly regulatory actions translate into measurable changes in human exposure. The objective of this study was to explore whether differences in PFAS regulatory frameworks across countries are reflected in detection frequencies, using hair as a matrix for human biomonitoring.

A targeted literature review of PFAS in hair studies published between 2012 and 2026 was conducted. A total of 12 peer-reviewed were identified, covering populations from Asia and Europe. Detection frequencies for commonly reported PFAS were extracted and compared across jurisdictions. These results were interpreted together with newly generated hair biomonitoring data from a New York City population. To facilitate comparison, countries were grouped into four regulatory tiers based on the strength and scope of PFAS regulation. Tier 1 (strongest regulation) included European Union countries operating under REACH chemical legislation and EU POP regulations implementing the Stockholm Convention (Spain, Belgium, Italy). Tier 2 included countries implementing Stockholm Convention restrictions combined with national chemical management frameworks (China, South Korea). Tier 3 comprised jurisdictions with emerging or sector-specific PFAS regulations (India, Hong Kong). Tier 4 represented countries with mixed federal and regional regulatory frameworks, such as the United States.

Hair biomonitoring results showed substantial variability across regulatory tiers. In Tier 1 EU countries, detection frequencies for legacy PFAS were generally lower but remained substantial. In Spain, PFOS and PFOA were detected in 46% and 34% of samples, respectively, while Italian studies reported PFOA detection frequencies of 36-40% and PFOS frequencies of 38-64%. However, widespread detection of replacement PFAS was observed; for example, PFBS was detected in 100% of Belgian samples, suggesting a shift toward alternative compounds. In Tier 2 countries, legacy PFAS were frequently detected despite regulatory controls. In China, PFOA was detected in 68-100% of samples across multiple studies, while PFOS detection ranged from 83-100%. In South Korea, PFOA was detected in 87% of samples. Tier 3 jurisdictions showed

moderately high detection frequencies, with PFOA detected in 59% of samples in India and PFNA and PFOA detected in 70% and 63% of samples in Hong Kong, respectively. New hair data from New York City (Tier 4) revealed a distinct exposure profile, with PFHpA detected in 100% of samples, while PFOA and PFNA were detected in 31% and 8%, respectively, indicating widespread detection of shorter-chain PFAS alongside legacy compounds in the United States. Overall, countries with stronger PFAS regulatory frameworks tended to show lower detection frequencies of legacy PFAS in hair; however, these compounds remained widely detectable even in highly regulated jurisdictions.

These findings highlight the long environmental persistence of PFAS and suggest that regulatory actions may shift exposure patterns toward replacement PFAS rather than eliminate human exposure. Integrating human hair biomonitoring with regulatory surveillance may provide an additional framework for evaluating the real-world impact of PFAS policies.

Keywords: PFAS, hair biomonitoring, legislation, forensic toxicology

P2. A Derivatization-Free LC-MS/MS Approach for d/l-Methamphetamine Analysis in Hair Samples

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Differentiation of d- and l-methamphetamine in hair is critical for accurate interpretation of drug testing results. Conventional gas chromatography-mass spectrometry (GC/MS) methods require derivatization with reagents such as N-Trifluoroacetyl-L-prolyl chloride (TPC), introducing additional cost, complexity, and workflow dependencies. This study describes the validation of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that eliminates derivatization while maintaining reliable enantiomeric separation utilizing a chiral analytical column.

Analysis was performed using an Agilent 1290 Infinity I LC coupled with an Agilent 6460 Triple Quadrupole operating in positive electrospray ionization mode. Separation of d- and l-methamphetamine was achieved by isocratic elution using a Poroshell 120 Chiral-V column, with methamphetamine-d11 used as the internal standard.

The method was validated by assessing precision and accuracy of achieving targeted ratios of d- and l-methamphetamine, carryover effects, and interference. Method performance was also evaluated using 40 authentic hair samples, primarily head hair with inclusion of alternative hair sources, and compared to a validated GC/MS method. Results demonstrated agreement for methamphetamine enantiomer detection between methods. No l-methamphetamine was detected using the LC-MS/MS method in samples where the GC/MS method reported an average of 9% l-methamphetamine. Additionally, the LC-MS/MS method achieved improved chromatographic separation with an approximate 3-minute reduction in retention time.

This LC-MS/MS method provides a streamlined and reliable approach for methamphetamine enantiomer differentiation in hair, offering practical advantages in workflow, analytical performance, and instrument efficiency.

Keywords: hair testing, derivatization, workflow efficiency, chiral, d/l-methamphetamine

P3. Real NPS Exposure and Complex Drug Mixtures Revealed by Hair Toxicology in the Spanish Mediterranean Region.

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New Psychoactive Substances (NPS) have emerged as legal alternatives to controlled drugs and represent an increasing challenge in forensic toxicology. Hair analysis constitutes a well-established matrix for documenting chronic drug exposure over extended periods and provides objective evidence of actual substance use, complementing indirect indicators such as drug seizures or self-report.

This retrospective study evaluates 27 forensic cases collected over a two-year period (2024-2025). The inclusion criterion was the detection of NPS. All cases involved individuals accused of drug trafficking. The legal basis for conducting the analysis is to support their defense, trying to demonstrate that the individuals involved are consumers rather than distributors.

Hair samples were analyzed using protocols capable of detecting a wide range of synthetic stimulants, dissociatives, cannabinoids, and classical controlled substances. Hair samples were collected by a coroner, processed in our laboratory using a workflow based on sample washing, pulverization, incubation, organic solvent extraction, Gas Chromatography–Triple Quadrupole Mass Spectrometry and UHPLC- Q-exactive Orbitrap. The epidemiological study was based on information supplied by the coroner.

This study presents an analysis of NPS and poly consumption patterns identified through toxicological screening in 2024 and 2025. Detected substances were classified by chemical family to assess prevalence, co occurrence, and distribution.

A shift in potency and diversity of NPS has been detected in 2024/2025. While 2024 patterns were dominated by established cathinones and dissociatives, in 2025 there is a marked increase in complex mixtures involving potent alpha pyrrolidines. Market information from European drug monitoring agencies was used to contextualize the findings. The rise of alpha pyrrolidine stimulants may reflect market adaptation or regulatory pressure. The comparison of analytical results indicates evolving trends in NPS availability, with increasing potency and chemical diversity. The frequent co detection of NPS and classical drugs indicates that poly drug use is a predominant pattern within the cohort.

There are frequent discrepancies between hair findings, seizures, and self reported substances. One possible explanation is the marketing of mixed products or those sold under vague names. Another factor is the broader detection window of hair analysis, whereas seized material reflects only a single moment.

Hair testing provides reliable evidence of actual exposure, including substances users may not report or may be unaware of. It should also be noted that non detection in hair does not exclude consumption, particularly for emerging NPS with limited data on incorporation, stability, and detectability. Further research is needed to better characterize these processes.

Keywords: NPS, cathinone, pyrrolidine, phenetylamine, pyrovalerone, ketamine

P4. Methodological trends in hair NPS analysis: a narrative review of analytical performance

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The rapid emergence of new psychoactive substances (NPS) poses significant analytical challenges in forensic toxicology, particularly in the analysis of hair as a biological matrix. This review evaluates methodological trends in analytical approaches for hair-based NPS detection, emphasizing extraction protocols and performance variability. From 587 identified records, 52 studies were selected after screening. These covered four major NPS classes: synthetic cannabinoid receptor agonists (SCRAs), opioids, cathinones, and phenethylamines. Hair sample weights ranged from 10 to 100 mg across all classes, with a predominant median of 20 mg for SCRAs, opioids, and phenethylamines, and 22.5 mg for cathinones.

Decontamination procedures were highly heterogeneous: SCRAs primarily used acetone or a methanol-water-methanol sequence (n=3, each); opioids favored dichloromethane (n=4); cathinones preferred dichloromethane followed by methanol (n=3); and phenethylamines typically required water-acetone combinations (n=5). Notably, 38% of the studies (n=20) failed to specify pulverization techniques, often citing vague descriptors such as "cut to small fragments". This oversight is critical, as particle size reduction directly dictates the surface area-to-volume ratio and, consequently, extraction efficiency. Methanol emerged as the primary extraction solvent for SCRAs (54%, n=12), opioids (33%, n=6), and cathinones (20%, n=3).

In contrast, phenethylamines were frequently extracted using acidified methanol (HCl) in varying proportions (26%, n=5). Extraction times showed a bimodal distribution: while 1 hour was the most frequently reported specific duration (n=14 across groups), prolonged protocols ranging from 18 to 24 hours (including 'overnight' incubation) remained a significant trend for complex drug screenings.

Analytical detection was primarily performed via LC-MS/MS. While the mean run time was 15 minutes, significant extremes were observed: a 5.5-minute high-throughput method and a 41-minute comprehensive multi-class drug screening. Analytical sensitivity varied across drug classes, with the lowest Limit of Detection (LOD) achieved for opioids at 0.05 pg/mg (LOQ of 0.1 pg/mg). For both SCRAs and cathinones, the maximum LOD sensitivity reached 0.1 pg/mg, with corresponding LOQs of 0.1 pg/mg and 1.0 pg/mg, respectively. Phenethylamines exhibited the highest thresholds, with an LOD of 1.0 pg/mg and an LOQ of 2.0 pg/mg.

Overall, the data demonstrates a robust analytical landscape. These values underscore the high-performance capabilities of current LC-MS/MS methods in detecting trace amounts of these substances in hair matrices; however, they also reveal a critical lack of methodological standardization across studies. Variability in decontamination, extraction conditions, and pulverization techniques continues to impact reproducibility. These findings highlight the importance of continued methodological refinement and validation, as differences in physicochemical properties across NPS classes often require tailored extraction and analytical strategies.

Keywords: New Psychoactive Substances (NPS); Hair Analysis; Sample Preparation; LC-MS/MS; Forensic Toxicology.

P5. The Use of a Novel Quadrupole Time of Flight Instrument for the Quantitative and Sensitive Analysis of Drugs of Abuse in Hair Samples

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The detection and quantification of drugs in hair present significant analytical challenges, particularly with respect to sensitivity and matrix effects. Tandem quadrupole mass spectrometers are typically employed for targeted analyses due to their high sensitivity and the specificity afforded by multiple reaction monitoring (MRM). However, high resolution mass spectrometry (HRMS) instruments offer additional advantages, including enhanced selectivity for compounds with similar m/z values and improved discrimination against background interferences. The objective of this study was to expand upon our previous work (1), in which a panel of drugs in hair was analyzed using a tandem quadrupole mass spectrometer. In the present study, a novel quadrupole time of flight (QTof) mass spectrometer was evaluated for the accurate identification and quantification of a panel of commonly encountered drugs of abuse. The developed method readily met the confirmation cutoff criteria recommended by the Society of Hair Testing (SoHT).

Decontaminated hair samples were pulverized, weighed into vials (20 mg), and incubated in a solvent mixture for 2 hours. Samples were subsequently extracted using mixed mode cation exchange solid phase extraction plates (Waters Oasis™ MCX), as previously described (1), with one modification. The final eluate was evaporated to dryness and reconstituted, rather than diluted as in the earlier method. LC–MS/MS analysis was performed using a Waters ACQUITY™ Premier UPLC system coupled to a Waters Xevo™ MRT mass spectrometer operated in Tof MRM mode, in which precursor ions are fragmented and analyzed by the time of flight analyzer, leading to highly specific quantitation.

The modified extraction procedure enabled a 7.5 fold reduction in final sample volume (100 µL versus 750 µL) without compromising extraction recovery or overall method performance, resulting in a corresponding increase in analytical sensitivity. Calibration curves encompassed the cutoff concentrations recommended by the Society of Hair Testing and the European Workplace Drug Testing Society. Quantitative results demonstrated acceptable accuracy and precision for all analytes.

A sensitive and reliable method was developed for the quantitative analysis of drugs of abuse in hair using Tof MRM on a novel high resolution time of flight mass spectrometer. The method demonstrated excellent accuracy and precision and met SoHT cutoff criteria, highlighting the applicability of high resolution mass spectrometry for targeted forensic hair analysis.

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P6. Drug Findings in Pediatric Hair: A 2021 – 2025 Assessment

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Hair testing is frequently used in investigations of potential drug exposure in children. Characterizing drug findings in pediatric hair provides insight into drug trends within this population. This study assessed drug findings in hair samples collected from pediatric patients (ages <1-16) between 2021 and 2025 to characterize patterns of substances detected in this population. Out of 605 cases tested, 340 showed positive findings, which were subsequently compiled and categorized by age group: neonate (≤28 days; n=12), infant (29 days - <1 year; n=6), toddler (1-3 years; n=126), preschool (4-5 years; n=59), school-age (6-12 years; n=108), and adolescent (13-16 years; n=29). These groupings were used to examine patterns of drug detection across developmental stages.

Drugs and metabolites identified across pediatric age categories included stimulants, opioids, prescription and over-the-counter medications.

Stimulants were among the most frequently detected compounds, with methamphetamine, often with metabolite amphetamine, and cocaine, with or without benzoylecgonine, identified in 66% and 31% of cases, respectively, across all age groups. The cocaine adulterant, levamisole, was detected in one toddler case.

Opioids were detected in 23% of cases across all age groups, including fentanyl, morphine, oxycodone, methadone, hydrocodone, tramadol, and codeine. The heroin-specific metabolite 6-MAM, often co-detected with morphine, was observed less frequently (3.5%) and identified across multiple pediatric age groups (neonate, infant, toddler, preschool, and school-age), but not in adolescents.

Additional substances included recreational drugs such as MDMA (2.6%) and MDA (0.88%), as well as nicotine (4.4%), which was detected beginning in the preschool age group and continuing through adolescence. A variety of therapeutic and over-the-counter medications were also identified, including antihistamines (diphenhydramine, hydroxyzine, doxylamine), antidepressants (fluoxetine, bupropion), anticonvulsants (carbamazepine), and cough/cold or stimulant-related compounds (d-/l-methorphan, ephedrine/pseudoephedrine, caffeine, and phentermine).

Stimulants and opioids were the most consistently detected drug classes across all age groups. Poly-drug exposure was common, with 76% of findings involving two drugs and 78% involving three. Co-

positivity was highest in toddlers, with 19 instances in which cocaine was detected alongside methamphetamine and amphetamine.

Age stratification provides important context for interpreting pediatric hair drug findings. Differences in developmental stage can indicate distinct exposure pathways, such as in utero exposure in neonates versus environmental exposure in younger children, while also helping distinguish these findings from potential intentional use in adolescents. When sufficient sample length is available, hair segmentation may further aid in evaluating patterns of exposure over time. Continued monitoring and investigation of these exposures may help inform prevention strategies and public health initiatives aimed at reducing pediatric drug exposure, as well as support family safety initiates through increased caregiver education, safe drug storage practices, and early intervention in at-risk environments.

Keywords: pediatric; childhood drug exposure; child and family medicine

P7. Hair analysis and exposure assessment: a case of fatal methadone intoxication in a child

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An 11-month-old child was found dead at his home. The family was on record with social services for drug use, alcoholism, and unsanitary living conditions.

Based on the pathological and toxicological findings, the cause of death was determined to be the result of methadone overdose, leading to pulmonary asphyxia. Given the child's age, this exposure implies the involvement of a third party.

The judge ordered a hair test to determine what substances the child may have been exposed to, in order to better understand the child's environment.

Hair samples, collected post-mortem, were analysed according to a validated method. Hair strands were twice decontaminated in methyl chloride, cut in 3*3 cm segments starting at the root, finely sliced in shorter pieces (<1mm), incubated with methanol and deuterated IS, sonicated for 15-minute; centrifuged; evaporated and dried extract solubilisation with mobile phase.

Screening for cannabinoids was carried out on GC-MS/MS, screening for amphetamines, opiates, cocaine and psychoactive drugs was carried out on HPLC-MS/MS.

Several substances were found at significant concentrations in the child's hair in the proximal, median and distal hair segments. Concentrations systematically increased from the root to the tip end. For the controlled substances, cocaine, 6-MAM, morphine, methadone, THC and their metabolites were identified. Some medications were also found in high concentrations, such as oxazepam and tramadol.

For methadone, defined as the substance implicated in the death, the concentrations were 202pg/mg in the proximal, 861pg/mg in the median and 1556pg/mg distal segments, and EDDP concentrations were 92; 116 and 338pg/mg, respectively.

These concentrations are significant and increase from the proximal to the distal segment. These findings suggest significant environmental contamination: the child has been repeatedly exposed to

methadone, but accidental or non-accidental ingestion cannot be excluded. Some cases report parents using methadone to calm down their children1.

Analysing hair samples from young children makes the interpretation of results particularly challenging2,3 contamination via breast milk, asynchronous growth and porosity are all factors that must be taken into account when determining whether the presence of these molecules is due to contamination of the hair (contamination of the hair through touching caused by sweat or residues on the hands) or systemic absorption of these substances (hand-to-mouth contact, food contamination).

Analysis of hair washes may have helped to clarify the distinction between contamination of the hair itself and contamination through passive ingestion.

In this case, the hair analysis did not provide clear corroboration of the toxicological analyses carried out on body fluids but revealed significant environmental exposure for a range of narcotic substances. This demonstrates once again the complementary nature of analysing all relevant biological matrices.

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Keywords: children, methadone, fatal, overdose

P8. Unveiling child exposure to cocaine through hair testing

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Toxicological testing is a key component of child protection and family court proceedings, particularly when there are concerns about possible drug exposure in children. Hair analysis has become an important diagnostic tool in suspected child abuse and neglect cases, providing complementary information that supports the detection and management of potential maltreatment. The presence of drugs of abuse in a child's hair may result from several mechanisms, including passive environmental exposure, accidental ingestion, or intentional administration.

A 5-year-old boy was admitted to the emergency department with a 48-hour history of visual hallucinations and episodic agitation. In the waiting room, he stated that his mother had "creatures behind her and on her head." He became very agitated and frightened; however, when calm, he showed no drowsiness or fluctuations in his level of consciousness. He also presented with rhinorrhoea and had been taking oxolamine, a medication not known to be associated with hallucinations. The family denied any possible access to prescribed medication or illicit substances. Urine samples were collected on admission and again 24 hours later. Blood and urine toxicological screening by immunoassay tested positive for cocaine, opiates, and methamphetamine.

Due to child protection concerns regarding possible ongoing exposure to substances of abuse within the home environment, this study aimed to document and clarify the circumstances of cocaine intoxication and exposure through hair analysis performed on all family members.

Three-centimetre hair segments were collected from the child, both parents, and the siblings, and cut into small pieces. Twenty milligrams of hair were incubated overnight in methanol at 55 °C. An aliquot (2 µL) was injected into an LC-MS/MS system. For child custody cases, no hair-washing step was included in the analytical procedure in order to improve the detection of environmental exposure.

Analysis of the parents showed low blood concentrations of benzoylecgonine in the mother. The mother tested positive for benzoylecgonine, cocaine, norcocaine, ecgonine methyl ester (EME), and cocaethylene in urine and hair samples. The father tested positive for benzoylecgonine in urine and for benzoylecgonine, cocaine, norcocaine, and EME in hair samples. The siblings' hair samples tested negative (below the cut-off), whereas urine samples were positive only for benzoylecgonine (<10 ng/mL), suggesting environmental contamination or passive exposure. The child tested positive for benzoylecgonine and norcocaine in blood, and for benzoylecgonine, cocaine, norcocaine, and EME in urine collected at admission. In the hair sample, cocaine (2140 pg/mg), benzoylecgonine (104 pg/mg), cocaethylene (6 pg/mg), EME (18 pg/mg), and norcocaine (54 pg/mg) were quantified. These findings suggest that the child had been directly exposed to or had ingested cocaine, rather than being subjected solely to environmental contamination or passive exposure. Even more, as during the medical evaluation, the child reported that one of his favourite games involved "playing with smoke" with his father, who blew smoke into his mouth. When asked how this was done, the child described a flour-like substance that was burned with a lighter.

Keywords: Cocaine, Hair, UHPLC-MS/MS

P9. Validation of a method for drug research in hair samples by UHPLC-MS/MS

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Hair has become one of the most relevant and widely used alternative biological specimens in the investigation of drug-facilitated sexual assault (DFSA) cases. This relevance mainly arises from the delay in reporting the crime to authorities, often caused by the amnesic effects of the drugs involved, which renders blood and urine samples useless while hair remains suitable for detecting drug use. Among the drugs most frequently detected in this context, in addition to alcohol, benzodiazepines are particularly prevalent, as they are among the most prescribed medications worldwide. In this context, developing highly sensitive multi-analyte methodologies is crucial, as these cases frequently involve single exposure events. The aim of this work was the development and validation of a simple method for the detection of substances related to DFSA cases, with particular emphasis on benzodiazepines and their metabolites, allowing its implementation in routine toxicological analysis.

Hair samples (20 mg) were washed, dried, pulverized, and then spiked, in 2 mL Precellys® tubes, with working solutions of the analytes and internal standards. This validation involved 30 compounds, with calibration curves established between 1–250 pg/mg and 5–250 pg/mg, depending on the analyte. The extraction procedure consisted of direct extraction with 500 µL of methanol using a ball mill, followed by centrifugation. An ultra-high-performance liquid chromatography coupled with triple quadrupole linear ion trap tandem mass spectrometry (UHPLC-QTRAP-MS/MS) was used for analyte determination, operating in MRM mode with two transitions monitored for each analyte.

The method was validated in accordance with ANSI/ASB Standard 036, evaluating selectivity, linearity, limits of detection (LOD), lower limits of quantification, precision, accuracy, matrix effects, autosampler stability and carry-over. The LODs were established at 1 and 5 pg/mg, depending on

the analyte. This validated multi-compound method allows for the simultaneous detection of additional substances, such as drugs of abuse and other pharmaceuticals. Finally, the method was successfully applied in a routine context, using both in vivo and post-mortem hair samples.

This analytical method proved to be simple, fast, eco-friendly, and suitable for routine application, representing a valuable and sustainable alternative compared with other existing approaches.

Keywords: Benzodiazepines, drug-facilitated sexual assault, UHPLC-MS/MS

P10. Case Report: Strychnine Poisoning

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Strychnine is an alkaloid associated with potent central nervous system activity as a stimulant and convulsant. Although historically used medicinally at low dose to improve muscle tone, its use is banned in many countries and restricted in the United States of America to use as a rodenticide. Poisoning in humans is rare with no deaths reported in the 2024 Annual report of the National Poison Data System ® (NPDS). [1] A total of 75 cases involving strychnine exposure were reported in 2024, with 37 cases (49%) deemed unintentional and 8 (10%) intentional.

The case presented involves the death of a 48-year female by suicide following the intentional ingestion of strychnine. Multiple matrices were collected at autopsy and then tested for common drugs of abuse and specifically targeted for strychnine. Head hair was segmented, washed and tested for strychnine.

Strychnine was detected and quantified using GCMS and GC-NPD in femoral blood (1.5 mg/L), heart blood (1.9 mg/L), vitreous humor (1.8 mg/L), bile (14 mg/L), liver (6.4 mg/kg), brain (4.3 mg/kg) and gastric contents (102 mg/kg). Head hair was collected at autopsy with a length in excess of 12 cm. The hair was initially cut into two 6 cm segments from the proximal end and washed. The hair was then cut into smaller 2cm segments and strychnine quantified using LCMSMS. The ULOQ for strychnine in hair/hair washes was 1000 pg/mg. The hair wash associated with the first 0-6cm segment was positive for strychnine at an estimated concentration of 1217 pg/mg and 993 pg/mg for the second 7-12cm segment. All six 2cm segments were positive for strychnine at concentrations far exceeding the ULOQ. The estimated concentrations ranged from 18,000 to 29,491 pg/mg. In comparison, hair strychnine concentrations were reported in three cases where adulteration of cocaine was suspected and ranged from 250 to 850 pg/mg. [2]

Strychnine detected in the biological fluids and tissues confirmed acute strychnine poisoning. Segmental hair analysis combined with the analysis of hair washes is a useful tool when interpreting hair drug concentrations and the assessment of chronic use/exposure. In this case, consistently high concentrations across all six segments and strychnine positive washes indicate external contamination. Decomposition fluids were unlikely to contribute to external contamination of the hair as the deceased self-admitted themself to the hospital less than 8 hours prior to being pronounced dead. However, sweat and sebum would be a contributory factor. In this case, chronic use cannot

be excluded but sweat and sebum contamination from the acute poisoning provides the most likely explanation for the findings.

This is the first reported case of strychnine detected in head hair from a strychnine poisoning related death.

Keywords: strychnine, poisoning, segmental analysis

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Things to See and Do

Here are some suggestions for getting around New York City.

Apps

Seesaw

Free app that shows all the current gallery exhibits and how to get there

Current Exhibits and Sights

Metropolitan Museum of Art
Raphael: Sublime Poetry
The Magical City: George Morrison's New York

Museum of Modern Art
Elizabeth Murray: Painters Progress
Naufus Ramírez-Figueroa: Lugar de Consuelo (Place of Solace)

Guggenheim Museum
Carol Bove

Acquavella Galleries

Matisse: The Pursuit of Harmony

Statue of Liberty: Liberty Enlightening the World

9-11 Memorial and Museum: 9-11 Memorial and Museum

Shopping Guide to NYC: Shopping in New York City

Metropolitan Museum of Art

Located at 5th Avenue and 82nd Street, the MET is the home of over 5,000 years of art from all over the world.

Hours

Sunday	10 a.m. – 5 p.m.
Monday	10 a.m. – 5 p.m.
Tuesday	10 a.m. – 5 p.m.
Wednesday	Closed
Thursday	10 a.m. – 5 p.m.
Friday	10 a.m. – 9 p.m.
Saturday	10 a.m. – 9 p.m.

Admission

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Seniors (65 and over): **$22**
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The Museum of Modern Art and MoMA PS1, celebrates creativity, openness, tolerance, and generosity. Their inclusive places—both onsite and online—invite diverse cultural, artistic, social, and political positions. MoMA is committed to sharing the thought-provoking modern and contemporary art.

11 West 53 Street, Manhattan
New York, New York, 10019

Hours

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Tuesday: 10:30 a.m.–5:30 p.m.
Wednesday: 10:30 a.m.–5:30 p.m.
Thursday: 10:30 a.m.–5:30 p.m.
Friday: 10:30 a.m.–8:30 p.m.
Saturday: 10:30 a.m.–5:30 p.m.
Sunday: 10:30 a.m.–5:30 p.m.

Admission

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Children 16 and under: **Free**
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Greenwich Village

Wandering its tree-lined streets—you'll quickly notice that Greenwich Village feels more like a small European town than part of a big city.

Stop by Washington Square Park to watch street performers under the iconic arch, then explore the area's mix of historic brownstones, indie shops, and cozy cafés.

The neighborhood has deep roots in art and music, so don't be surprised if you stumble upon live jazz or a tiny gallery tucked into a side street. It's the kind of place where getting a little lost is part of the experience.

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