recent exposure. Therefore, it is imperative the use of an alternative biological matrix that may provide relevant and important retrospective information about the use of drugs, namely cocaine and morphine which are commonly abused. *Methods*: A qualitative and quantitative method for the simultaneous determination of cocaine and morphine in human hair was developed and validated. After decontamination, hair samples (20 mg) were incubated with a mixture of methanol/hydrochloric acid (2:1) at 65 °C overnight in order to extract the XBs of the matrix. Samples were cleaned-up by mixed-mode solid-phase extraction (SPE), XBs were derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide and then analyzed by gas chromatography/electron impact/mass spectrometry (GC/EI/MS). *Results of the study*: The developed method proved to be specific, accurate and precise across the calibration range (0.25–10 ng/mg), where good linearity was observed for both the analytes with correlation coefficients ranging 0.999 and 0.9993. The coefficients of variation oscillated between 0.18% and 16.16%. The limits of quantification (LOQ) were 0.04 and 0.05 ng/mg for cocaine and morphine, respectively. The proposed GC/EI/MS method can be successfully applied in the screening and quantification of these XBs in real cases, namely in clinical and forensic toxicology.

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P23-07
Tramadol and O-desmethyltramadol quantification in hair samples by GC–MS

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Purpose: Abuse of tramadol and related fatal intoxications has been increasing. Hair analysis is the ideal matrix to evidence cumulative long-term exposure. Besides tramadol, it is important to quantify its main metabolite, O-desmethyltramadol (M1), since when present in hair represents internal exposure and it is much more active than tramadol itself. Until now, there is no validated technique to simultaneously quantify tramadol and M1 in hair. *Methods*: A gas-chromatography/mass spectrometry (GC–MS) method with solid-phase-extraction (SPE) for simultaneous determination of tramadol and M1 in human hair samples was developed and validated. Hair samples (60 mg), were subjected to decontamination with dichloromethane, water and acetone, and then extracted with methanol in ultrasonic bath. Samples were then cleaned-up with solid-phase extraction using mixed-mode MCX cartridges. Derivatization was performed using ethyl acetate and bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1%TMCS) and then analytes were analyzed by GC–MS. *Results of the study*: Validation of the method was performed working with spiked hair samples. The method proved to be selective since there were no matrix interferences. The regression analysis for tramadol and M1 was linear in the range of 0.1–20 ng/mg with detection and quantification limits of 0.0003 and 0.0013 ng/mg for tramadol and 0.0001 and 0.0006 ng/mg for M1 respectively. The present method was further applied to six clinical cases of long-term tramadol administration.

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P23-08
Characterization of crystalline deposits in rat kidney using NMR, LC/MS and MALDI MSI

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Wistar rats administered with a microsomal prostaglandin E synthase 1 (mPGES-1) inhibitor for 1 week, showed severe kidney injury. Clinical pathology indicated renal damage (increased plasma levels of urea, creatinine and potassium) in the high dose rats. The histopathological examination of kidneys mainly showed marked tubular degeneration/regeneration, crystalline deposits in tubules surrounded by inflammatory reaction, dilated tubules with cellular remnants in lumen and presence of crystals in the pelvis. One hypothesis was that toxicity was caused by precipitation of the metabolite bisulphonamide which is formed by hydrolysis of the parent compound, as this metabolite is known to have poor solubility.

The aim of this study was to determine the chemical identity of the crystal deposits, by a combination of analyses using nuclear magnetic resonance (NMR), liquid chromatography/mass spectrometry (LC/MS) and matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI). Analysis was performed by extraction of kidney tissue, manual dissection of precipitated crystals from kidney tissue sections and MALDI MSI of kidney sections. Rats administered with vehicle served as controls in the experiments.

Analysis of crystals that were dissected directly from kidney sections and analysed with LC/MS and NMR, clearly showed that the crystals contained bisulfonamide in all investigated samples. In addition, MALDI MS imaging was used to correlate the spatial distribution of bisulfonamide (m/z 234.98) on kidney sections to histological defined areas. These combined results propose precipitation of bisulphonamide as a possible cause to the kidney toxicity observed in this study.

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P23-09
Overcoming some challenges of the Local Lymph Node Assay (LLNA)

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The LLNA is a validated substitute for guinea pig tests for identifying potential skin sensitizer. By measuring the proliferation rate of the lymphocytes in the auricular lymphnodes, the LLNA provides quantitative data about the skin sensitizing potential of a substance. Although the LLNA is a robust assay and allows a reproducible characterization of the test substance, some challenges occur: (A) the OECD TG 429 only suggests organic solvents and organic-aqueous mixtures, but no aqueous vehicle for testing aqueous soluble test substances is generally approved. (B) As the disposal of 3H is due to its long half life (~12.3 years) an environmental as well as a cost problem, an alternative non-radioactive approach (OECD TG 442B), was established. (A) We investigated that 2% carboxymethylcellulose in water is a suitable vehicle for hydrophilic substances which could be approved by the positive control substance alpha-