

using an organic solvent while the liquid phase was pre-concentrated by solid phase extraction using OASIS® MCX cartridges. Both matrices were derivatized and further analysed by GC–MS. **Results:** The method was validated according to the International Conference on Harmonization (ICH), considering the following parameters: selectivity, linearity, limit of detection (LOD),

limit of quantification (LOQ), accuracy, precision, and recovery [5]. For both matrices, raw sewage and the SPM, the method was linear ($R^2 > 0.99$ and $R^2 > 0.98$ respectively) and LOQs varied between 10 ng/L to 20 ng/L. **Conclusion:** The validated method will allow to assess the consumption patterns at community level, as well as occurrence, spatial distribution, and the EF of the target chiral PADs.

Keywords: synthetic cathinones; amphetamines; suspended particulate matter; enantioselectivity; wastewater treatment plants.

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POSTER 104

Synthetic cannabinoids affect the expression of autophagic mediators ATG5, BECLIN-1, RAB7A and LC3 in brain-derived NG108-15 cells

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Resumo

Introduction: Autophagy is a lysosome-dependent intracellular degradation pathway required for various physiological processes, playing a housekeeping role in removing misfolded or aggregated proteins and clearing damaged organelles. Modulation of autophagy by synthetic cannabinoids (SCs) has been reported but it is not fully understood, with most of the studies presenting conflicting results. Our group recently showed that 11 out of 14 SCs tested were able to increase autophagy, as indicated by the higher number, compared to control, of autophagosomes in NG108-15 neuroblastoma x glioma hybrid cells, following exposure for 24h. **Objectives:** To assess the effects of the 11 SCs that previously increased

autophagic flux, in the expression of proteins involved in autophagy, in the same neuronal cell model. **Methods:** The expression of ATG5, Beclin-1, Rab7A, LC3, and ubiquitin was analysed by Western blot, after incubation of NG108-15 cells with the 11 SCs (AMB-FUBINACA, AB-PINACA, MDMB-CHMICA, AB-CHMINACA, 5F-AMB, AB-FUBINACA, FUBIMINA, X-PB-22F, 5F-PB22, SDB-006 and JWH-122) at 1 nM and/or 1 µM (the concentrations eliciting autophagy), during 24h. Results from at least 4 independent experiments (in each one, 2 replicates per treatment) were normalised against β-actin and expressed as mean ± SD of the fold-change relative to the solvent control. Statistical analysis was performed

using one-way ANOVA, followed by a Dunnett's multiple comparisons test. **Results:** Despite the lack of statistical significance ($p > 0.05$), our preliminary data consistently showed an increase in ATG5 for all tested SCs, and in Beclin-1 for all except 1 nM AB-CHMINACA and 1 nM 5F-AMB. Furthermore, increases in Rab7A protein levels were observed for 1 μ M AB-FUBINACA, 1 nM AB-PINACA, 1 nM AB-CHMINACA and 1 μ M SDB-006; and in LC3 for

all SCs except 1 nM AMB-FUBINACA, 1 μ M AB-FUBINACA and 1 μ M JWH-122. **Conclusions:** Although further experiments are being conducted to confirm these results, the distinct patterns of expression of proteins involved in nucleation (as assessed by Beclin-1), elongation/maturation (ATG5 and LC3) and fusion (Rab7A) steps, indicate different mechanisms of autophagy triggered by the tested SCs.

Keywords: autophagy; cannabinoids; new psychoactive substances; drugs of abuse

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POSTER 105

Assessment of suspected cadaver desecration and the role of forensic anthropology – case report

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Resumo

Introduction: forensic anthropology focuses on the study of human skeletal remains, including the identification of human bone fragments and distinguishing different bones, investigation of skeletal injuries and its possible relation with the time of death, evaluation of morphological characteristics and other parameters [1]. Thus, in the presence of a fully skeletonized corpse, the forensic pathologist needs the collaboration of the forensic anthropologist to interpret autopsy findings [1,2]. Forensic autopsies after exhumations are becoming more common and a multidisciplinary assessment should be considered in each case [3]. **Case description:** a 27-year-old caucasian man died in a car accident during a police chase. The victim lost control of the car, falling into a river and becoming submerged. The forensic autopsy performed at that time concluded that the death was due to accidental drowning. Five years after being inhumated, an exhumation and new forensic autopsy was requested due to suspicion of manipulation and desecration of the remains. **Forensic investigation:** the corpse was transported to the North Delegation of the National Institute of Legal Medicine and Forensic Sciences after the exhumation. The remains were

completely skeletonized, requiring a forensic anthropology evaluation. After their observation and evaluation it was possible to infer that the complete skeleton was present; there were no peri-mortem traumatic injuries; and the only post-mortem alterations were incisions on the skull performed during former forensic autopsy. All the possible morphological characteristics (sex, age at the time of death, stature and ancestry) were estimated and no individualizing characteristics were identified. A bone fragment from the right femur was collected for genetic DNA comparative studies. **Conclusion:** all the features mentioned above were compatible with the remains belonging to the 27-year-old victim, which was later confirmed by DNA testing between the collected bone sample and a comparative DNA sample from his presumed mother. At autopsy, assisted by a thorough anthropologic evaluation, no signs of desecration or manipulation of the remains were identified. We conclude that when dealing with skeletonized remains, a multidisciplinary approach is needed, including a forensic anthropology evaluation, in order to properly answer all the questions that may arise during judiciary investigation [1,4,5].

Keywords: forensic anthropology; exhumation; skeleton; desecration of remains