assessed by measuring the differentiation ratios (i.e., percentage of primary neurites per total cell number) and the total length of neurites, after 72-h incubations in differentiation medium. Cell proliferation was evaluated by the sulforhodamine B assay (SRB) up to 72 h. **Results:** AMB-FUBINACA ($p<0.01$, at 1pM and 1µM) increased the differentiation ratios and the total length of primary neurites ($p<0.05$, at 1nM). On the other hand, neither AB-CHMINACA nor HU-308 significantly affected neuronal differentiation. Since NG108-15 cells do not express the CB2 receptor, data obtained with HU-308 suggest that SC-induced neurodifferentiation in these cells does not depend on CB2 activation, although this hypothesis still needs to be clarified. None of the drugs affected cell proliferation in this cell line at the concentrations tested. **Conclusion:** These results show that one of SCs most consumed worldwide (AMB-FUBINACA) impacts in vitro neuronal differentiation, suggesting that significant post-exposure effects, that depend on the abused SC, may also occur during human neurodevelopment.

Keywords: synthetic cannabinoids (SCs); neurodevelopmental disorders; cell differentiation; cell proliferation; sulforhodamine B assay (SRB).

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POSTER 103

Enantiomeric estimation of drugs consumption by gas chromatography – the role of suspended particulate matter in wastewater epidemiology

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Resumo

Introduction: The abusive consumption of licit and illicit psychoactive drugs (PADs) is ubiquitous all over the world and is a serious public health problem [1]. Wastewater based epidemiology (WBE) is a relatively recent approach that nowadays is used worldwide as a complementary tool to the traditional drug monitoring methods to estimate drug consumption at a community level. In this context, the suspended particulate matter (SPM) plays an important role concerning the determination of PADs by WBE approach, because PADs may be adsorbed to SPM, depending on their physico-chemical properties [2]. Moreover, the evaluation of enantiomeric fractions (EF) of chiral PADs, beyond the importance for environmental risk assessment, is unexpendable to discriminate between consumption, direct disposal and synthesis pathways for identification of manufacturing locations [3,4]. **Objectives:** The aim of this study is to develop and validate an indirect method by gas chromatography coupled to mass spectrometry (GC–MS) based on chiral derivatization using (R)-(–)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride, for enantiomeric quantification and estimation of community consumption of PADs including amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDMA), buphedrone (BPD), butylone, 3,4-dimethylmethcathinone (3,4-DMMC), 3-methylmethcathinone (3-MMC), as well as for a better understanding on the behaviour and distribution of PADs in SPM. **Material and Methods:** Raw sewage samples collected from the inlet of a wastewater treatment plant were filtered and the SPM was extracted

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 (R2 > 0.99 and R2>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