Detection and quantification of 17 synthetic cannabinoids and one metabolite (JWH-018-COOH) in blood and urine, using a liquid chromatography-tandem mass spectrometry system.

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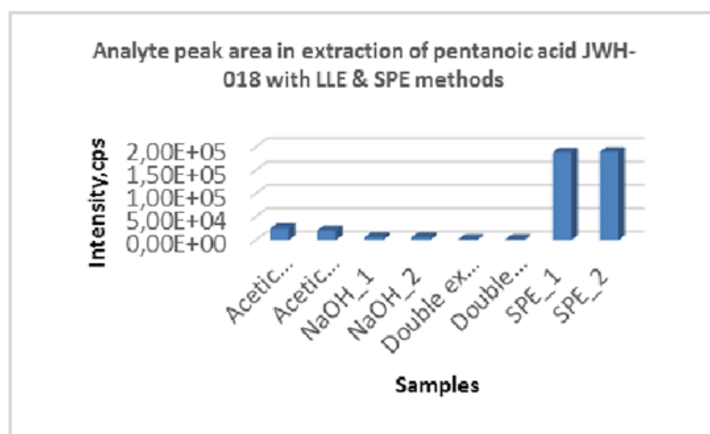
Introduction: In the UK, and other countries, cannabis is the most widely used illegal drug. Besides this, synthetic cannabinoids (SCs) have become an established part of the recreational drug scene all over the world. Designing assays that detect synthetic cannabinoids is a pitfall, as SCs are “moving targets” for the toxicologists. In order to avoid detection, illegal drug producers constantly change the structure of the SCs. In addition, because the commercially available urinary immunoassay for delta 9-tetrahydrocannabinoid (THC is the major active constituent of the drug) does not respond with SCs, laboratories usually develop their own mass- spectrometry-based assays. The purpose of this study was the development and the validation of a SC assay in blood and urine using liquid chromatography-mass spectrometry.

Method: An ABSciex, 3200 Q TRAP LC-MS/MS system, and column: Kinetex 2.6 µm C18 mm, 50 x 3.00 were used. The mobile phase was a 50:50 mixture of an aqueous phase (2.5 mL ammonium acetate 5mM (at pH: 4.8) + 500 mL distilled water) plus organic phase (250 mL acetonitrile + 250 mL methanol 50:50 + %0.1 acetic acid). 4 mL n-Hexane+ Ethyl acetate (99:1, 1 µg/L) as extraction solvent was used. Liquid-liquid extraction (LLE) was used with a range of concentrations of the mixture of SCs: 0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 µg/L in either (whole blood or urine). These were spiked with internal standard (IS) (which contained THC D3 and JWH D9 at 100 µg/L). This extraction was repeated under acidic conditions (by adding 200 µL of 20% acetic acid) and basic conditions (by adding 200 µL NaOH 0.25N). Solid phase extraction (SPE): the materials used in this method were matrix (i.e. blood or urine), internal standard, the mixture of SCs +1 metabolite (concentrations as describe as above) +2 mL acetonitrile. 4 mL of KH_2PO_4, 0.1 N, pH5 was used as a buffer. The SPE method was carried out by using cartridges (Oasis MCX) in four steps (condition, load sample, wash, elute).

Results and discussion: In this experiment two methods (LLE & SPE) in few environments were used and compared. The basic environment had the expected result especially accuracy, linearity and % of recovery were acceptable in most SCs except the metabolite (pentanoic acid JWH-018, fig.1). While the acidic environment all SCs had very low accuracy and recovery %. The combination of the double extraction of acidic and basic environment has been taken too long and also had poor recovery % in SCs metabolite. Upon completion, the results showed that solid-phase extraction had better results and acceptable recovery and accuracy % and have a linear graph on most of SCs especial the metabolite.



Conclusion: The introduction of a solid-phase extraction followed by LC coupled with electrospray ionization tandem mass spectrometry was applied for the development of robust

and specific analysis of SCs analogs and their metabolites. As a result the present method is likely to be applicable to the routine therapeutic and forensic toxicological monitoring of SCs. Further studies are needed for the extensive analyses to detect and identify novel SCs which drug designers continually create.

References:

1. Jean-Michel Gaulier. General unknown screening procedure for the characterization of human drug metabolites in forensic toxicology. InterScience.2009 ;(2):22.