P172.Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material

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Introduction: Gas Chromatography (GC) is the most commonly used method for the analysis of cannabis samples, e.g. in forensic toxicology. However, as this method is based on heating of the sample, the acidic forms of cannabinoids are converted into their decarboxylated counterparts. In order to determine neutral cannabinoids, a time-consuming derivatization step is mandatory. Conversely, High Performance Liquid Chromatography (HPLC) permits the determination of the authentic composition of the cannabinoids in the plant by direct analysis. In contrast to GC, no decomposition of the cannabinoids occurs during analysis by HPLC. Several HPLC methods are described in the literature, but most of them failed to separate efficiently all the cannabinoids or were not validated according to general guidelines.

Aims: The goal of the present study was to develop and to validate a simple HPLC/DAD method for the qualitative and quantitative determination of major neutral and acidic cannabinoids present in cannabis plant material. We have chosen to perform the determination of cannabinoids used for the classification of cannabis phenotypes, monitoring of the psychotropic potency and of potential interest for the medicinal cannabis research community: Δ9-tetrahydrocannabinol (THC), THC-acid, cannabidiol (CBD), CBD-acid, cannabigerol (CBG), CBG-acid, cannabinol (CBN) and Δ8-tetrahydrocannabinol (Δ8-THC). CBN and Δ8-THC are the main degradation products of THC; they have to be determined in order to evaluate the total THC content present in fresh plant material.

Methods: Our HPLC/DAD method was developed with an innovative methodology for modelling chromatographic responses. Following the practice of design of experiments, predictive multi linear models were developed and used in order to find optimal chromatographic analytical conditions.

The method was then validated following a novel approach using accuracy profiles based on β-expectation tolerance intervals for the total error measurement, and assessing the measurements uncertainty. The e.noval® software (Arlenda, Liège, Belgium) was used to compute all validation results. Δ8-THC was determined qualitatively but not included in the validation; the other cannabinoids above-mentioned were all validated.

Results: All compounds were well separated in the chromatograms obtained experimentally with the optimal conditions proposed. Even if the retention times of the cannabinoids were slightly longer than in the predicted chromatogram, the resolution was satisfactory.

THC, CBDA, CBD, CBGA, CBN were validated for concentration between 0.15% and 10% (% of weight of dry plant material). THCA was validated between 0.15% and 32%. The regression coefficients were always higher than 0.99. The Relative Standard Deviations (RSD) (precision) and the relative bias (trueness) values were lower than 11%. The relative expanded uncertainty was between 4 and 25%.

Conclusion: Using original tools, we have developed and validated a simple and accurate HPLC method for the quantification of major cannabinoids in cannabis plant material. This analytical method can be used for diverse applications, e.g. plant phenotype determination, evaluation of its psychoactive potency or also to control material quality.

P173.Two cases of confirmed ingestion of the novel designer compounds: 4-methylmethcathinone (Mephedrone) and 3-fluoromethcathinone.

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Introduction: Cathinone is a pharmacologically active alkaloid (stimulant) extracted from the leaves of the Khat plant (Catha edulis). Cathinone (Cath) and methcathinone (MC) are controlled under the UK Misuse of Drugs Act. However, derivatives such as ethcathinone (EC), 4-methylmethcathinone (4-MMC or mephedrone) and 3-fluoromethcathinone (3-FMC), which are not controlled under current law, have been produced and marketed to satisfy the drug dance scene culture. The dose, effects and safety of these products have not been evaluated, and are only known from user's discussion forums on drug chat room websites.

Aim: Two cases were admitted to an inner-city hospital Emergency Department (ED) on separate days within a 2 week period. Case 1, a 30 year old male, presented with drowsiness (GCS 6/15), respiratory rate 20 per minute, heart rate 47 per minute and BP 140/80mmHg. He was alert and orientated within 2 hours of presentation. He subsequently gave a history of ingestion of 1g of mephedrone, GBL, "neo-doves" and "neo-blues". Case 2, a 22 year old male, presented after oral ingestion of 200mg of mephedrone and subcutaneous injection of 3.8g of mephedrone. On arrival in the ED he was agitated with 7mm dilated pupils, heart rate 105 per minute and BP 177/111mmHg; these features settled within 6 hours of presentation. Serum and urine from both cases were sent for analysis at St George's - University of London.

Methods: A screening method was developed for eight methcathinone related compounds (Cath, MC, EC, 4-MMC, 2-FMC, 3-FMC, 4-FMC and dimethylcathinone (DMC)). Derivatives of Cath and MC were synthesised in-house as secondary standards by Kingston University and purity established by NMR. 500μL of urine or serum were extracted by liquid-liquid extraction and screened using gas chromatography with mass-spectrometric detection. Chromatographic separation of all derivatives was achieved over a 20min run. The principle fragment ion for 4-MMC and 3-FMC was *m/z* 58. Confirmation of methcathinone derivatives was by acetylation with acetic anhydride. Liquid chromatography with tandem mass spectrometric detection was used to confirm and quantitate 4-MMC and 3-FMC in urine and serum. Quantitative and confirmatory multiple reaction monitoring (MRM) transitions for 4-MMC and 3-FMC were m/z: 178.2/160.1, 145.1, 119.2 and 182.2/164.0, 149.0, 123.0 respectively. An additional compound was seen associated with the standard and urine sample containing 3-FMC. Preliminary investigation by NMR, MS and IR identified 3-fluoro*iso*methcathinone, a by-product of the synthesis of 3-FMC. Further investigation is required to establish its activity.

Results: Routine toxicological screening showed case 1 to be positive in urine for GBL, cyclizine (administered in ED) and 3-FMC. No drugs were found in the serum. Case 2 was positive for 4-MMC in urine and serum (0.15mg/L). No therapeutic or toxic reference ranges are available for 4-MMC and 3-FMC. The cathinone derivatives were not available to purchase as certified reference standards, so quantitative results should only be used as a guide.

Conclusion: The clinical features seen in case 1 were consistent with GBL toxicity, and the 3-FMC was consistent with the history. Case 2 is the first known case of lone use of mephedrone. Clinicians and analytical toxicologists should be aware of the potential for use of these compounds in patients presenting with signs of sympathomimetic toxicity.

P174.Analysis of ethanol in blood samples with a Micro-GC-Headspace

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Introduction: Ethanol is a substance which poses many problems in the field of road safety. For punishing drunk driving, it is important to have a method for robust and accurate analysis and a suitable apparatus. Currently, the analysis of ethanol in blood is usually performed by Headspace-GC in the laboratory to complete and validate the results of the breathalyzer at the police control. In order to obtain results more quickly and accurately, several series of tests were carried out on a transformed device, the Varian Micro-GC CP-4900. The injection system has undergone several transformations and a thermostated metal block was designed for equilibration of the vials. One advantage of this device is that it can inject the sample in two different modules: the same sample injection is analyzed on two different columns (with the same or a different polarity) and two detectors simultaneously.

Aim: The aim of this work is multiple. Firstly, tests are performed to check how modification of Headspace parameters influences the signal. Secondly, the accuracy of the method is tested by determining the relative standard deviation on ethanol solutions or samples by Micro-GC, as well as by doing a cross-analysis of blood samples tested positive for ethanol, in collaboration with ICHV (Central Institute of Valais Hospitals).

Methods: A mixture of 200 μl of calibration solution or sample, 200 μl n-propanol (internal standard) and 500 μl of an ionic solution is introduced in a